INSTITUT PASTEUR

International AMR symposium

Tackling antibacterial and antifungal resistant infections from disease to innovative therapies

December 9-10th, 2024
Institut Pasteur, Paris, France – Duclaux Amphitheatre

SCIENTIFIC & ORGANIZATION COMMITTEE

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INTRODUCTION & AIMS

In the frame of the AMR research priority at the Institut Pasteur, we are organizing together with the UPC Institut Hors-Murs Microb'UP a symposium on AntiMicrobial Resistance on December 9-10th, 2024 at the Pasteur Campus in Paris. During this meeting we aim to reposition the disease as the focal point to improve treatments of bacterial and fungal infections in a context of high antimicrobial resistance.

This one and a half day meeting will be organized around 4 sessions:

- Treatment escape and AMR in patients and animals
- Mechanisms of resistance and treatment escape
- Chemistry for new treatments
- Natural products and novel therapeutic strategies

The event will bridge biology, biotechnology, clinical studies, veterinary research and chemistry. We envision in addition to the presentations ample time for discussions, as well as short talks selected from submitted abstracts and poster presentations.

CONTACT & INFORMATION

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PROGRAM

Day 1 (afternoon) December 9th, 2024

1.15 pm Doors opening for the arrival of attendees

2:00 pm **Introduction by Christophe d'Enfert (Scientific Director, Institut Pasteur)**

2:10 pm **Session 1 – Treatment escape and AMR in patients and animals**

Chairs : Sarah Dellière & Philippe Glaser

2.10-2.40 **Laurence Armand-Lefèvre** – Hôpital Bichat, France
"Antimicrobial resistance realities in French hospitals and low- and middle-income countries"

2.40-3.10 **Thomas Van Boeckel** – University of Zürich, Switzerland
"Global mapping of antimicrobial resistance in animals"

3.10-3.30 **Thomas Maguire** – Airfinity, United Kingdom
"Simple models can be used to assess the impact of antibiotic consumption on antimicrobial resistance in critical priority bacteria and the potential role of interventions in its reduction"

3.30-3.50 **Juliette Guitard** – Hôpital Saint-Antoine, France
"New insights into the echinocandin resistance in Candida spp"

3.50 pm Coffee break

Chairs : Laurence Armand-Lefèvre & Thomas Van Boeckel

4.20-4.50 **Sarah Dellière** – Hôpital Saint Louis / Institut Pasteur, France
« Antifungal resistance in human pathogenic fungi: a growing threat"

4.50-5.10 **Lionel Rigottier-Gois** – INRAE, France
"Accelerate gut microbiota recovery to enhance resistance to colonisation against vancomycin-resistant Enterococci"

5.10-5.25 **Marie Desnos-Ollivier** – Institut Pasteur, France
"Yeast resistance in France from surveillance national programs"

5.25-5.40 **Short-talks selected from posters**
Farras Daffa Imtiyaz – Université de Poitiers, France
Armelle Leslie Megueya – Institut Pasteur du Cameroun, Cameroon
Camille Schneider – Institut Pasteur, France

5.45 pm – 7.30 pm Wine & Cheese

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Day 2 (Full day) December 10th, 2024

9 am — Session 2 - Mechanisms of resistance and treatment escape

Chairs : Marion Flipo & Christopher Schofield

9.00-9.30 **Iuliana Ene** – Institut Pasteur, France
"Beyond Drug Resistance: Alternative Drug Responses in Candida albicans"

9.30-10.00 **Athanasios Typas** – EMBL, Germany
"Strategies to control, delay or revert AMR"

10.00-10.20 **Zeynep Baharoglu** – IBPC and Institut Pasteur, France
"Uridine potentiates aminoglycoside lethality through activation of sugar transporters"

10.20-10.30 **Marie Royer** – Institut Pasteur, France
"Mecillinam resistome in Klebsiella pneumoniae: how resistance to a one-target antibiotic depends upon different stress responses"

10.30-10.40 **Erik Maikranz** – Institut Pasteur, France
"Measuring single-cell antibiotic susceptibility in droplets"

10.40-10.50 **Short-talks selected from posters**
Julie Plouhinec – Université de Caen, France
Elena Capuzzo – Institut Pasteur, France

10:50 am Coffee break

11:20 am — Session 3 - Chemistry for new treatments

Chairs : Iuliana V. Ene & Rolf Müller

11.20-11.50 **Christopher Schofield** – University of Oxford, United Kingdom
« Natural Product Inspired Antibiotics »

11.50-12.20 **Marion Flipo** – Institut Pasteur de Lille, France (*speaker Société Chimie Thérapeutique*)
« Novel efflux pump inhibitors: tackling antimicrobial resistance in Enterobacterales »

12.20-12.40 **Anna Hirsch** – Helmholtz Institute for Pharmaceutical Research Saarland, Germany
"Tackling underexplored drug targets as a pathway to antibiotics with a novel mode of action"

12.40-1.00 **Kevin Cariou** – Chimie ParisTech, France
"Organometallic Derivatization of Drugs for Promising Antifungal Treatments"

1.00-1.15 **Short-talks selected from posters**
Flore Miomandre – Université de Rennes, France
Enzo Eddebbarh – Université de Tours, France
Mylène Lang – Université de Strasbourg, France

1:15 pm Lunch break **& Poster session**

2:45 pm — Session 4 - Natural products and novel therapeutic strategies

Chairs : Paola B. Arimondo & Athanasios Typas

2.45-3.15 **Rolf Müller** – Helmholtz Institute for Pharmaceutical Research Saarland, Germany
"Approaches Towards Innovative Anti-Infectives From Microbes"

3.15-3.30 **Alexander Titz** - Helmholtz Institute for Pharmaceutical Research Saarland, Germany
"Antibiofilm & Antivirulence Therapeutics: antagonising lectins of P. aeruginosa"

3.30-3.45 **Eric Faudry** – IBS / CEA-CNRS-UGA, France
"Neutralizing human monoclonal antibodies that target the PcrV component of the Type III Secretion System of Pseudomonas aeruginosa act through distinct mechanisms"

3.45-4.15 **David Bikard** – Institut Pasteur / Eligo Bioscience, France
"In situ targeted mutagenesis of gut bacteria with base editors"

4.15-4.35 **Vincent Libis** – Learning Planet Institute / Inserm-Université Paris Cité, France
"Scalable discovery of novel antibiotics through synthetic biology"

4.35-4.55 **Solène Ecomard & Jérémy Seurat** – Institut Pasteur / ENS, France
« Macrophage-induced reduction of bacteriophage density limits the efficacy of in vivo pulmonary phage therapy »

4:55 pm — Conclusion & Awards ceremony for the best posters

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ABSTRACTS
PROGRAM

Antimicrobial resistance realities in French hospitals and low- and middle-income countries

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Global mapping of antimicrobial resistance in animals

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Simple models can be used to assess the impact of antibiotic consumption on antimicrobial resistance in critical priority bacteria and the potential role of interventions in its reduction

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Introduction: Antimicrobial resistance is a significant and growing global health emergency, directly causing an estimated 1.27 million deaths in 2019. This figure is expected to rise to around 10 million by 2050, mostly driven by the WHO critical priority bacteria: Acinetobacter baumannii, Klebsiella pneumoniae, Escherichia coli, and Pseudomonas aeruginosa. One of the major drivers of this increase is antimicrobial consumption, in both LMICs and HICs. Our model aims to assess the impact of antimicrobial consumption on resistance up to 2033 using a simple linear regression model, as well as show the utility of simple models such as these in assessing future antimicrobial resistance.

Methods: In our analysis, we have used a linear-regression model to assess the direct correlation between antimicrobial consumption and resistance in England. Monthly bloodstream infection (BSI) data from the UKHSA and annual data from ESPAUR (English Surveillance Programme for Antimicrobial Use and Resistance) between 2014 and 2022 was used to identify historical trends and model forward. The impact of changes in consumption of key first- and second-line antibiotics for four critical-priority bacteria was modelled, based on three consumption scenarios: current trends, 10% increase, and 10% decrease (e.g. if a new intervention was introduced).

Results: Our data identified a clear correlation between resistance and consumption in key bacteria in England. Our model shows that even a 10% increase in consumption of ciprofloxacin can increase resistance in K. pneumoniae to up to 65% in the UK by 2033, an increase from 19.5% in 2022. Based on current trends in infections and consumption, Acinetobacter baumannii resistance to piperacillin-tazobactam is forecasted to rise to around 18% by 2033, with a 10% increase in consumption increasing resistance to around 25%. A 10% decrease in consumption, however, could reduce resistance to as little as 3% by 2033. Interestingly, some drug-bug combinations appear to be driven less by consumption and more so by infection rates, with resistance to piperacillin-tazobactam in K. pneumoniae increasing to around 42% in all three scenarios.

Conclusions: Our data show that where a clear correlation is observed, a simple linear regression model can show utility in modelling antimicrobial resistance. While reduced consumption of current treatments, partly through use of novel antibiotics, would reduce overall resistance levels in the short-term in some bacteria, consumption is unlikely to decrease to the required degree. Additionally, bacteria seeing short-term decreases are likely to adapt rapidly to new selection pressures and continue developing resistance. Hence, prevention of these infections (and thereby antimicrobial consumption) via the use of novel interventions (e.g. vaccines or novel, non-traditional antimicrobials) may be the most appropriate strategy for reducing resistance. Novel vaccines against these bacteria could lead to infection rates and resistance levels dramatically reducing with increasing vaccine coverage.

Keywords: Resistance, Modelling, United Kingdom, Interventions, Vaccine Impact

New insights into the echinocandin resistance in *Candida* spp.

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Objectives: Despite the extensive use of echinocandins, the emergence of resistance in *Candida spp* has remained overall limited. In this study, we postulate a new hypothesis that could explain this intriguing shaping of echinocandin resistance in the clinical setting.

Methods: Series of consecutive isolates becoming echinocandin-resistant over time were genotyped using microsatellite length polymorphism analysis. Glucan synthase genes (*fks1, fks2)* were sequenced. The *in vitro* fitness was assessed comparatively for susceptible and resistant isolates. Finally, *Candida* FKS proteins were modelled *in silico*.

Results: In line with the literature, a limited emergence of echinocandin resistance was observed in our institution, with a relative predominance of *Nakaseomyces glabratus*. Isolates from the different series were genetically related, and mutated isolates all harboured *fks1* (*Candida albicans*, *Candida tropicalis*) or *fks2* (*N. glabratus*) mutations. For the latter, a conserved fitness was observed for resistant isolates, while resistant isolates from the former exhibited reduced fitness. Modelling the FKS1 dimer of mutated *C. albicans* revealed an alteration in a funnel structure responsible for the export of the glucan chain to the cell wall. In contrast, cross-complementation between FKS1 and FKS2 in *N. glabratus* offers the possibility of maintaining a normal export of the glucan.

Conclusions: Our results support a link between mutations in the species-specific glucan synthase complex and echinocandin resistance but also, with the export of the glucan chain to the fungal cell wall. This could explain why *N. glabratus* is more prone to the spread of echinocandin-resistant strains than *C. albicans* and related species.

Keywords: *Candida*, echinocandin resistance, (1,3)-β-D-Glucan synthase, fitness, molecular modelling

Antifungal resistance in human pathogenic fungi: a growing threat

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Antifungal resistance is an escalating global public health threat, impacting both immunocompetent and immunocompromised individuals. The widespread use of agricultural fungicides and the improper use of medical antifungals are driving the emergence of resistant fungal species. *Trichophyton indotineae* and *Candida auris*are prominent examples of these emerging threats. Additionally, pan-azole resistance in *Aspergillus fumigatus*, originating from the environment, is spreading and contributing to increased mortality rates in affected patients. While innovative antifungals are in development, their effectiveness may be short-lived, underscoring the urgent need for new therapeutic approaches, such as immunotherapy.

Accelerate gut microbiota recovery to enhance resistance to colonisation against vancomycin-resistant Enterococci (VRE)

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Our body is home to a wide diversity of bacteria that make-up the microbiota. While the microbiota provides a natural barrier effect against pathogenic microorganisms, it is also a reservoir and a portal of entry for ESKAPEs. Several factors, including antibiotic treatment, can destabilise the microbiota and allow ESKAPE pathogens to proliferate. These include vancomycin-resistant enterococci (VRE), whose intestinal proliferation in immunocompromised patients increases the risk of life-threatening infections. The use of consortia of harmless bacteria from the microbiota is one strategy being considered to combat ESKAPE pathogens. Five commensal species are known to contribute to resistance to VRE colonisation. Our aim was to identify additional commensal bacteria that contribute to the barrier effect to design a consortium able to enhance the natural resistance to colonisation against VRE.

Using an interdisciplinary approach combining longitudinal biological data and mathematical modelling, we have identified 15 molecular species that correlate negatively with VRE carriage in mice. We showed that supplementation with a mixture of 7 representative strains (Mix7) improves the barrier effect against VRE *in vivo* in a microbiota-dependent manner. We found that the microbiota influences the efficacy of supplementation. Differences were associated with variations in the composition during recovery and initial microbiota, and represent potential biomarkers for predicting response to Mix7. In a mouse model of an alternative stable state of dysbiosis, the response to Mix7 was associated with higher levels of short-chain fatty acids and a number of metabolites, reflecting the recovery of the microbiota back to baseline. This mix contains a Bacteroidetes strain required for the barrier effect in the presence of at least one of the 6 other strains. None of the supernatant of the 7 strains, alone or in combination, inhibits VRE growth *in vitro*, indicating an indirect inhibition effect against VRE. Interestingly, 5 of the 7 strains are shared between human and mouse and 2 have human functional equivalents.

This work may ultimately lead a personalised medicine by targeting patients at risk and susceptible to respond to the supplementation with commensal anti-VRE strains.

Keywords: vancomycin-resistant *Enterococcus* (VRE), antibiotic-induced dysbiosis, resistance to colonisation, consortium of commensal bacteria, microbiota modulation

Yeast resistance in France from surveillance national programs

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Introduction: Candidemia are invasive fungal diseases (IFD) representing public health threat worldwide. In recent years, the increase in the number of clinical isolates resistant to certain antifungal agents, notably due to the intensive use of azoles in agriculture, as well as the acquisition of resistance after treatment, has created new problems for patient management. On the other hand, global climate change is having an impact on IFD, and in particular the emergence of new species with intrinsic resistance to certain antifungals. Monitoring IFD and the antifungal susceptibility of the species involved is therefore a global health issue. Since 2002, the National Reference Center for invasive Mycoses and Antifungals (NRCMA) has set up several prospective multicenter surveillance studies of IFD in France. These programs not only collect clinical data, but also centralize isolates for standardized species identification, determination of antifungal susceptibility and detection of mutations leading to the monitoring of resistance among the clinical isolates responsible of IFD in France.

Materials & Methods: A program called YEASTS has been used to gather data on bloodstream infections due to yeasts between 2002 and 2022 in 26 hospitals from the Paris area. The RESSIF program has made it possible to monitor the epidemiology of IFD from 2012 to 2022 in France, by comprehensively centralizing episodes of IFD from 30 hospitals across the country. Finally, since 2023 the new program SINFONI started the centralization of IFD episodes from 60 hospitals. All episodes are declared by hospitals using online questionnaires and monitored. All clinical isolates received at the NRCMA are identified at the species level using polyphasic method (phenotypic analysis, MaldiToF, sequencing), antifungal susceptibility profiles are determined using the standardized EUCAST microdilution method and protein mutations associated to antifungal resistance are determined using sequencing of the target genes.

Results: In France, fungemia correspond to the major type of IFD (50%) reported, with *C. albicans* as the major species (49%) followed by *C. glabrata* (19%) and *C. parapsilosis* (16.6%). Sporadic cases of echinocandins acquired resistance were observed since 2003, mainly for *C. albicans* (0.22%) and *C. glabrata* (1.2%) but also for other 5 yeast species. There is no evidence of an increasing number of resistant isolates in 2023, with no C.*albicans* resistant isolate among the 895 cases declared and only 1.4% *C. glabrata* among the 365 cases reported. Many different mutations in *Fks* can be involved in resistance, since 2003 we found 8 different mutations among the 52 *C. albicans* resistant isolates and 18 among the 72 *C. glabrata*.

Fluconazole-resistant isolates of *C. parapsilosis* responsible for candidemia in France were observed since 2004. Proportion of resistant isolates was very variable according to the year (0-27%), with only few hospitals concerned by potential genetic cluster of isolates. In 2023, among the 339 candidemia due to *C. parapsilosis* reported, only 1.18% isolates were resistant to fluconazole, mainly due to the Y132F amino acid modification in the *ERG11* coding region.

Conclusion: Since 2003 we only observe few sporadic cases of echinocandin acquired resistance with many diverse Fks mutations, but no increase in the proportion of resistant isolates in 2023 among the 2046 cases of candidemia declared in France.

Actually, isolates of *C. parapsilosis* involved in bloodstream infection and resistant to fluconazole remain relatively rare in France and correspond to clusters specifically found in few hospitals. Those isolates mainly have the Y132F mutation in the Erg11 protein, which is the major mutation reported worldwide.

Based on all the data obtained by the different surveillance programs in France, we can conclude that cases of acquired resistance due to antifungal treatment such as echinocandins remain rare, but the increase in the number of isolates with a reduced sensitivity profile, particularly to azoles in patients who have not been pre-exposed, suggests a change in the susceptibility of environmental isolates.

In addition, we regularly observe the emergence of new species involved in IFDs, some with intrinsic resistance to one or more classes of antifungal agents. The modification of ecosystems, particularly with climate change, is likely to increase this emergence. Correct species identification and monitoring of the antifungal susceptibility are necessary to optimize patients management.

Keywords invasive fungal infections, yeasts, antifungal resistance, surveillance program

Beyond Drug Resistance: Alternative Drug Responses in *Candida albicans*

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Candida albicans is a prominent opportunistic fungal pathogen responsible for a wide spectrum of infections, particularly in immunocompromised individuals. The clinical effectiveness of azole antifungals, such as fluconazole, is increasingly threatened by the emergence of azole-tolerant strains, which contribute to persistent infections and treatment failures. This presentation will delve into the diverse mechanisms by which *C. albicans*—a major human fungal pathogen—responds and adapts to antifungal therapies. Population heterogeneity within *C. albicans* drives fascinating phenomena such as tolerance and heteroresistance, enabling subsets of cells to evade the action of azoles, the most widely used class of antifungal drugs.

Strategies to control, delay or revert AMR

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Uridine potentiates aminoglycoside lethality through activation of sugar transporters

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Aminoglycosides are broad-spectrum antibiotics effective against Gram-negative bacteria. Aminoglycoside uptake depends on membrane potential, but the precise mechanisms are incompletely understood. We report a new mechanism of aminoglycoside uptake through carbohydrate transporters in *E. coli*. To assess antibiotic susceptibility, we deleted and over-expressed 26 carbohydrate transporters. We observed that AGs act as substrates for 18 over 26 tested carbohydrate transporters, and that this mechanism appears to be shared among several Gram-negative ESKAPEE pathogens. Differential aminoglycoside entry was measured using a fluorescent-labeled aminoglycoside and flow cytometry. To further exploit this mechanism in order to enhance aminoglycoside lethality in Gram-negative pathogens, we screened for molecules that induce transporters' expression using a transcriptional *gfp* fusion with the *cmtA* transporter gene with 198 different carbon sources. We identified uridine as an activator of *cmtA* as well as additional 12 sugar transporters among the 18 involved in aminoglycoside entry. Co-administration of uridine and aminoglycosides significantly improved aminoglycoside treatment efficiency against clinical *E. coli* strains, including resistant ones, due to enhanced bacterial uptake. Furthermore, combination of uridine with aminoglycosides also demonstrated improved treatment outcomes *ex vivo* in human blood, and *in vivo* on a murine urinary tract infection model. Given the safety profile of the use of uridine in humans, we propose that uridine can be a potentiating adjuvant to aminoglycoside treatment of infectious diseases in a clinical setting, and could be used against strains with multiple resistances.

Keywords: antibiotic resistance, potentiation, aminoglycoside transport

Mecillinam resistome in *Klebsiella pneumoniae*: how resistance to a one-target antibiotic depends upon different stress responses.

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Klebsiella pneumoniae (Kp) is one of the main actors of the antibiotic resistance burden. In 2019, this species was responsible for 113,730 out of 462,500 deaths attributable to β-lactam resistance (1). β-lactams, the most frequently prescribed antibiotic class in humans, target different penicillin-binding proteins (PBP) at different affinities, depending on the molecule. To get insight into the killing mechanisms by these antibiotics in Kp, and into the associated resistance mechanisms, I focused on mecillinam (MEC), a β-lactam targeting only PBP2.

Firstly, spontaneous MEC^R Kp mutants could grow at higher MEC concentrations than *Escherichia coli* MEC^R mutants, despite the parental strains showing the same Minimal Inhibitory Concentration (MIC=0.25µg/mL). This growth at high MEC concentrations was due to the chromosomal β-lactamase SHV, present in almost every Kp strains.

Secondly, we Illumina-sequenced 101 Kp colonies that grew on high MEC concentrations. Candidate causative mutations of resistance were detected in a broad range of functional categories including the cell envelope, amino acid metabolism, central carbon metabolism and translation. I selected six MEC^R mutants representative of these categories for in-depth characterization. All had an MIC > 256 µg/mL. Even though those mutants were growing at high MEC concentrations, they all showed growth defects in the presence of MEC.

In *E. coli*, the main MEC resistance mechanism is the overexpression of the bacterial division protein FtsZ to bypass PBP2 inhibition (2). This overexpression was shown to be triggered by the stringent response via an increased (p)ppGpp level. Thus, we compared targeted genes in KpΔ*relA* MEC^R mutants to those selected in the WT by sequencing 72 colonies. No mutants for the amino acid metabolism category and a single one for the translation category was identified, suggesting that MEC resistance was RelA-dependent in these two categories.

Therefore, Kp MEC^R mutants involved multiple pathways and harbored very diverse phenotypes. In most cases, MEC resistance was RelA-independent. Finally, the very high MICs reached were due to Kp SHV β-lactamase.

Keywords: mecillinam, antibiotic resistance, metabolism, SHV β-lactamase

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Measuring single-cell antibiotic susceptibility in droplets

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While the occurrence of antibiotic resistance is becoming a major concern for public health, studies investigating how the susceptibility of single cells and their lineages is related to the susceptibility of entire populations remain scarce. This is due to technical difficulties in performing large amounts of experiments and observing single cells and their progeny. We use microfluidic droplets as micro-reactors to overcome these difficulties and obtain thousands of individual growth curves for colonies starting from single cells. From this, we study how bacteria escape antibiotics for various pharmacological modes of action and antibiotic concentrations. We combine these measurements with stochastic modeling to relate the single-cell susceptibility with the individual birth, and death rates. This allows us to link dynamic to digital end-point measurements and clarifies what digital antibiotic susceptibility measurements are really measuring.

Keywords: single-cell, stochastic growth, microfluidic droplets.

Natural Product Inspired Antibiotics

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Novel efflux pump inhibitors: tackling antimicrobial resistance in Enterobacterales

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Infections caused by multidrug resistant Gram-negative bacteria are a major threat to human healthcare. In 2021, an estimated 4.71 million deaths were associated with bacterial antimicrobial resistance, with *Escherichia coli* and *Klebsiella pneumoniae* being among the deadliest pathogens. One of the most worrying resistance mechanisms in Gram-negative bacteria is due to the active transport of several classes of antibiotics outside the bacteria via efflux pumps. The tripartite AcrAB-TolC efflux pump, which is very similar in *E. coli* and *K. pneumoniae*, is the predominantly active system responsible for the extrusion of many antibiotics causing both basal and acquired broad-spectrum antibiotic resistance.

The aim of the project is to develop efflux pump inhibitors (EPIs) capable of potentiating the activity of existing antibiotics *in vitro* against Enterobacterales strains and *in vivo* in a mouse lung infection model.

A novel chemical family of allosteric inhibitors of the AcrAB-TolC efflux pump was discovered by a phenotypic screening on *E. coli*. These inhibitors potentiate the activity of all AcrAB/TolC substrate antibiotics (fluoroquinolones, macrolides, β-lactams, tetracyclines, etc) tested on *E. coli* and *K. pneumoniae*. Co-crystallisations of some inhibitors with the AcrB protein were obtained, enabling determination of the binding mode of the compounds to their target.[1]
Our recent medicinal chemistry efforts have allowed to identify BDM91288, a compound with an improved potency, favourable *in vitro* plasmatic and microsomal stabilities, and an accumulation in the lungs of mice. In a mouse model of lung infection with hypervirulent *K. pneumoniae*, BDM91288 (30 mg/kg) showed no antibacterial activity alone, but when combined with levofloxacin (10 mg/kg), a significant reduction of the bacterial load was observed compared to levofloxacin alone.[2]

In conclusion, we have discovered a novel chemical series of EPIs that, by allosterically inhibiting AcrB, is able to boost a large panel of AcrAB-TolC efflux pump substrates on *E. coli* and *K. pneumoniae* wild-type and resistant strains, and to potentiate the activity of levofloxacin in a mouse model of pulmonary infection with *K. pneumoniae*.

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Tackling underexplored drug targets as a pathway to antibiotics with a novel mode of action

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To find new hits and lead compounds for anti-infective drug-discovery, we combine various hit-identification strategies with phenotypic antibacterial screening on underexplored drug targets.[1] This approach will be illustrated with a selection of un(der)explored targets.

Firstly, we succeeded in fragment merging and linking, affording highly selective and potent inhibitors of the extracellular metalloprotease and virulence factor of *Pseudomonas aeruginosa*, the elastase LasB.[2,3] Multiparameter optimisation based on extensive biological profiling, including the establishment of complex biological assays led to chemically diverse lead compounds with good lung exposure and *in vivo* efficacy in lung and eye infection models. Our approach promises to deliver the urgently needed anti-infective agents featuring both new chemical scaffolds and unprecedented modes of action.

Secondly, we succeeded in the identification of synthetic small-molecule inhibitors of the b-sliding clamp DnaN,[4] showing good affinity, functional inhibition, broad-spectrum antibacterial activity and a balanced *in vitro* ADMET profile. We are currently advancing this promising chemical class in a CARB-X-funded hit-to lead optimisation campaign in parallel to the optimisation of an equally promising class of natural products, the mycoplanecins, as novel agents for the treatment of tuberculosis.

Keywords: Anti-infective drug discovery, Target-based drug design, Antivirulence agents, Underexplored targets, Multiparameter optimisation

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Organometallic Derivatization of Drugs for Promising Antifungal Treatments

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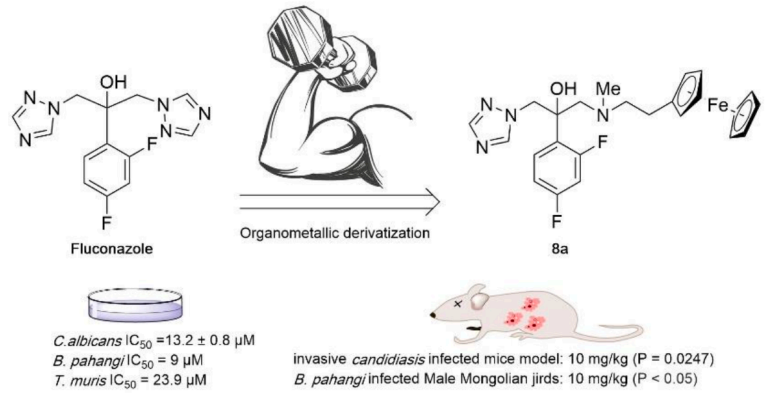
The advent and globalization of modern anti-infectious therapies in the XXth century has led to the emergence of resistance phenomena, which have become increasingly difficult to overcome. After rising the alarm about antibiotics, the WHO has recently published a list of critical fungal pathogenes against which therapeutic dead-ends need to be overcome[1]. Metal-based drugs[2] constitute an ever-growing class of therapeutic agents, with a wide array of modes of actions, that can allow to overcome such therapeutic dead ends[3]. A sub-category of these drugs consists of metallocene compounds, which have many applications in the fields of chemical biology and medicinal chemistry. A very successful example is the ferrocene-containing compound ferroquine, which is a close derivative of the antimalarial drug chloroquine. Ferroquine is currently in clinical phase II trials as an antimalarial drug candidate.

In this context we pursue two lines of research: the incorporation of organometallic-moities into anti-infectious drug-frameworks to improve their therapeutic profile and the development of new chemical methods to access original metallocenyl synthons. During this talk we will first present our latest results on several series antimycotic compounds based on fluconazole (FCZ) that incorporate a metallocene moiety and have given extremely promising results on critical strains such as FCZ-resistant Candida sp. including in in vivo murine models[4–6].



Keywords: drug design – resistance – antifungal – organometallic

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Approaches Towards Innovative Anti-Infectives From Microbes

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The global rise of antimicrobial resistance, mainly due to the mis- and overuse of antibiotics, is one of the most pressing issues of our time. To counteract this development, novel resistance-breaking antibiotics are urgently needed. Historically, the vast majority of antibiotics have been derived from microbial natural products. As compared to traditional bacterial producers, such as actinomycetes and bacilii, myxobacteria have been studied less extensively and thus harbor a large potential for the discovery of entirely new natural product scaffolds exhibiting promising bioactivities. Comparisons of myxobacterial metabolite profiles with the number of underlying biosynthetic gene clusters encoded in their large genomes show, that many compounds still remain unknown. Further, recent studies indicate that the order of myxobacteria likely comprises many more biodiverse representatives than previously assumed. According to metagenomics analyses, myxobacteria (including many underexplored representatives) are highly abundant in the soil microbiome, where they play a crucial role in soil nutrient and carbon cycling. Taken together with our recent genomic analyses, these findings suggest that the biosynthetic potential of myxobacteria is a long way from being exhausted.

We recently demonstrated that chemical diversity correlates with taxonomic distance in myxobacteria. Accordingly, we are more likely to isolate novel compound classes from strains which are phylogenetically distant from previously characterized strains as compared to closely related strains. This knowledge is applied to prioritize strains for natural product discovery, thus increasing the chance of discovering compound classes with yet unknown chemical structures and biological activities.

I will discuss recent results from our laboratory regarding the identification of novel bioactive NPs from myxobacteria based on different approaches, and show recent advances in the preclinical development of selected compound classes.

Antibiofilm & Antivirulence Therapeutics: antagonising lectins of *P. aeruginosa*

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Bacterial biofilms are a severe problem for therapy. The Gram-negative bacterium P. aeruginosa is currently the most critical bacterial pathogen as defined by the WHO priority pathogen list. This bacterium is difficult to treat due to excessive development of resistance to antibiotics and its abundant biofilm formation. The latter is a major resistance determinant of this pathogen since the biofilm shields embedded bacteria from chemotherapy and host defence. Therefore, several approaches to identify new anti-infectives against this bacterium aim to block biofilm formation.

P. aeruginosa utilises the two lectins LecA (PA-IL) and LecB (PA-IIL) for initial adhesion to the host, for biofilm formation and as virulence factors. These are promising drug targets that are addressed in our research for therapeutics, diagnostics and conjugates. We could demonstrate that drug-like lectin LecA and LecB inhibitors are potent inhibitors of bacterial virulence in vitro and in vivo. Our LecB inhibitor DH181a synergizes with tobramycin in murine chronic lung infection models and lowers bacterial colonization of the lung (unpublished). These data are highly promising and will provide the basis for future exploration of this strategy. Furthermore, various other ESKPAE pathogens have lectins that may serve as drug targets in the future.

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Neutralizing human monoclonal antibodies that target the PcrV component of the Type III Secretion System of *Pseudomonas aeruginosa* act through distinct mechanisms

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Pseudomonas aeruginosa is a major human opportunistic pathogen associated with a high incidence of multi-drug resistance. The antibody-based blockade of *P. aeruginosa* virulence factors represents a promising alternative strategy to mitigate its infectivity. We used a pipeline based on single specific B cell sorting to isolate, from cystic fibrosis patients, human monoclonal antibodies (mAbs) targeting proteins from the *P. aeruginosa* Type 3 Secretion System (T3SS). A panel of mAbs directed at PscF and PcrV was characterized. Among those, two mAbs, P5B3 and P3D6, that bind to the injectisome tip protein PcrV, exhibited T3SS blocking activity. We solved the crystal structure of the P3D6 Fab-PcrV complex, which revealed that this mAb binds to the C-terminal region of PcrV. Further, we compared the T3SS-blocking activity of three PcrV-targeting mAbs, including two from previous independent studies, using two distinct assays to evaluate pore formation and toxin injection. We conducted a mechanistic and structural analysis of their modes of action through modeling based on the known structure of a functional homolog, SipD from *Salmonella typhimurium*. The analysis suggests that anti-PcrV mAbs may act through different mechanisms, ranging from preventing PcrV oligomerization to disrupting PcrV's scaffolding function, thereby inhibiting the assembly and function of the translocon pore. Our findings provide additional evidence that T3SS-targeting Abs, some capable of inhibiting virulence, are elicited in *P. aeruginosa*-infected patients. The results offer deeper insights into PcrV recognition by mAbs and their associated mechanisms of action, helping to identify which Abs are more likely to be therapeutically useful based on their mode of action and potency.

Keywords: Anti-virulence; Type 3 Secretion System; monoclonal antibodies; structure-activity relationship, modes of action.

In situ targeted mutagenesis of gut bacteria with base editors

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Microbiome research is now demonstrating a growing number of bacterial strains and genes that affect our health. Although CRISPR-derived tools have shown great success in editing disease-driving genes in human cells, we currently lack the tools to achieve comparable success for bacterial targets in situ. Here we engineer a phage-derived particle to deliver a base editor and modify *Escherichia coli*colonizing the mouse gut. Editing of a β-lactamase gene in a model *E. coli* strain resulted in a median editing efficiency of 93% of the target bacterial population with a single dose. Edited bacteria were stably maintained in the mouse gut for at least 42 days following treatment. This was achieved using a non-replicative DNA vector, preventing maintenance and dissemination of the payload. We then leveraged this approach to edit several genes of therapeutic relevance in *E. coli*and *Klebsiella pneumoniae* strains in vitro and demonstrate in situ editing of a gene involved in the production of curli in a pathogenic *E. coli* strain. Our work demonstrates the feasibility of modifying bacteria directly in the gut, offering a new avenue to investigate the function of bacterial genes and opening the door to the design of new microbiome-targeted therapies.

Scalable discovery of novel antibiotics through synthetic biology

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Discovery of bioactive secondary metabolites of microbial origin have declined over the past decades, depriving clinical pipelines from a key source of novel antibiotics. Encouragingly, the natural repertoire of microbial secondary metabolites remains vastly underexplored, and recent developments in genome mining technologies offer ways to accelerate the pace of discoveries. Sequencing and bioinformatics allow prioritization of biosynthetic genes predicted to encode new metabolites, and cloning and heterologous expression of such genes can speed up the discovery of therapeutically relevant molecules. Here, an approach allowing to massively parallelize these processes will be presented. The streamlined interrogation of a large number of biosynthetic genes contained in a strain collection led us to discover several previously uncharacterized natural products, including a novel antibiotic. We will showcase a viable route to scalable natural product discovery through heterologous expression, on the condition of leveraging economies of scales along the process.

Macrophage-induced reduction of bacteriophage density limits the efficacy of in vivo pulmonary phage

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The rise of antimicrobial resistance has led to renewed interest in evaluating phage therapy. In murine models highly effective treatment of acute pneumonia caused by Pseudomonas aeruginosa relies on the synergistic antibacterial activity of bacteriophages with neutrophils. Here, we show that depletion of alveolar macrophages (AM) shortens the survival of mice without boosting the P. aeruginosa load in the lungs. Unexpectedly, upon bacteriophage treatment, pulmonary levels of P. aeruginosa were significantly lower in AM-depleted than in immunocompetent mice. To explore potential mechanisms underlying the benefit of AM-depletion in treated mice, we developed a mathematical model of phage, bacteria, and innate immune system dynamics. Simulations from the model fitted to data suggest that AM reduce bacteriophage density in the lungs. We experimentally confirmed that the in vivo decay of bacteriophage is faster in immunocompetent compared to AM-depleted animals. These findings demonstrate the involvement of feedback between bacteriophage, bacteria, and the immune system in shaping the outcomes of phage therapy in clinical settings.

Keywords: Antimicrobial resistance, Bacterial infection, Pneumonia, Innate immunity, Mathematical model

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2	**ANCUTA Diana**	Therapeutic management of experimental *Borrelia Bavariensis* infection in a murine model	
3	**BENKHALED Anissa**	Apramycin-Gallium combination for the treatment of Acinetobacter baumannii pulmonary infections	
4	**BERTEAU Olivier**	Radically new approach for antimicrobial discovery	
5	**BIRKELBACH Joy & KIRSCH Susanne H.**	Advancing the myxobacterial compound discovery pipeline	
6	**BOURGUET Marie-Lise**	Restoring drug activity against resistant bacteria with insights from marine natural product analogues	
7	**BRANDT Philipp**	Unraveling aneuploidy control mechanisms and their impact on antifungal drug resistance in the fungal pathogen Candida albicans	
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1

Antifungal persistence of *Cryptococcus neoformans* against Amphotericin B.

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Cryptococcus neoformans can persist inside the host during antifungal treatments, which can cause treatment failure and disease recurrence. The current understanding of antifungal persister cells in *C. neoformans* is sparse. This project aims to generate and characterize persister cryptococci, which survive through antifungal treatment. Using time-kill assays and flow cytometry, we show that Amphotericin B (AMB) causes oxidative stress and cell death in most of the population. However, a small subpopulation — the persister population—stays refractory to AMB exposure. We find that antifungal persister cells tolerate >16 times the minimum inhibitory concentration (MIC) of AMB and continue to divide in the presence of the drug. Time-kill assays reveal only a fraction of persister cells (0.01-10%) form colonies after AMB removal, suggesting the loss of culturability in many of these cells. Growth curve analyses show a longer lag phase in persister cells, consistent with the slower appearance of the colonies on YPD-agar plates in time-kill assays. Persister cells have significantly larger vacuoles and smaller cell sizes than untreated cells. Furthermore, after recovery from the AMB stress, persister cells show the same characteristics as the original population— culturability, growth curve, and MIC—signifying reversible phenotypic changes. Our results indicate that persister cells have physiological changes—that probably preexist or are triggered in the presence of the drug—which help them to persist during antifungal stress. Further, transcriptomics and single-cell tracking by microfluidics will allow us to determine the molecular mechanisms for the phenotypes observed and the pre-existing/triggered nature of persistence. In conclusion, our study generated in vitro persister cells and identified some of the mechanisms used by *C. neoformans* to survive during antifungal stress.

Keywords: *Cryptococcus neoformans*, Antifungal persistence, Amphotericin B, Time-kill assay, Minimum inhibitory concentration.

2

Therapeutic management of experimental *Borrelia Bavariensis* infection in a murine model

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The mouse model is commonly used for the study of many human diseases, both from a pathogenetic point of view and especially for testing new drugs. The C_3H/HeJ mouse is well suited for the study of Lyme disease and, for this reason, the aim of our study was to evaluate the efficacy of antibiotic therapy on a model of borreliosis induced by intradermal and intracerebral inoculation with GFP producing *Borrelia bavariensis* (Bb) modified strain, isolated from the CSF of a human patient.

A total of 50 SPF C_3H/HeJ mice, 11-13 weeks old, 27±2 grams, were included in the study, divided into groups of 5 animals named as follows: PBS 7, PBS 14, PBS 21, Ceftriaxone (CEF) and Doxycycline (DOX). Each group was inoculated intracerebrally (n = 25) with 10 µL suspension of Bb 10^7 spirochete/mL and intradermally (n = 25) with 100 µL Bb, of the same concentration.

In the DOX group, 50 mg/kg Doxycycline was injected starting on the first day post Bb inoculation, twice daily for 5 days, then once daily for another 10 days. Ceftriaxone was administered similarly but at a dose of 30 mg/kg. To confirm the induction of borreliosis, animals were euthanized on day 7, 14 and 21 (PBS groups), which 5 days before the euthanasia date were intraperitoneally injected with Gentamycin, 1mg/kg (to visualize Bb by fluorescence microscopy) and Cortisone acetate, 3mg/mouse (for mice immunosuppression and better visualization of Bb). DOX and CEF groups, after the end of treatment, were rested for 7 days (to avoid carry-over of antibiotics into the culture medium where the biological samples were collected) and also received Gentamycin and Cortisone acetate 5 days before the euthanasia date.

Ear, dura mater, brain, bladder and knee joint samples were collected for cultural examinations, hematological examination, quantitative PCR and immunohistochemistry.

Cultural examination showed positivity of all samples collected up to day 14 in the intracerebrally and intradermally inoculated PBS groups. PCR analysis confirmed the previous examination, but detected Bb also in samples collected on day 21, both in the PBS group and in the CEF and DOX groups. The systemic immunoinflammatory index showed a more pronounced inflammation in the intracerebrally inoculated group of animals, and in the hematological examination we noted a strong leukocytosis, especially in the PBS groups, compared to the antibiotic-treated groups.

Translational research and application to humans of current therapeutic protocols developed in the mouse model will require further adjustments since, through the examinations performed, we were able to demonstrate the remission of borreliosis in groups treated with specific antibiotics for Lyme disease, especially if it is localized in the cerebral level. Moreover, this boreliosis model is a great opportunity to test other novel treatments.

Keywords: borreliosis, mouse, ceftriaxone, doxycycline, *Borrelia bavariensis*

3

Apramycin-Gallium combination for the treatment of *Acinetobacter baumannii* pulmonary infections

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Acinetobacter baumannii is an opportunistic pathogen commonly linked to pulmonary infections in intensive care unit patients. Due to its extensive antibiotic resistance mechanisms, the WHO has identified *A. baumannii* as a critical priority for the development of new antibiotics. Apramycin (APR), an aminoglycoside with a unique chemical structure, has shown an ability to evade most resistance mechanisms encountered by other aminoglycosides used clinically. In 2023, APR completed two Phase I clinical trials in the U.S. to assess its safety and pharmacokinetics following IV administration. Gallium, in its Ga(III) salt or complex form, exhibits bactericidal effects against various Gram-negative bacteria by mimicking ferric iron (Fe(III)) and disrupting essential iron-dependent bacterial pathways. Clinical trials have shown Ga(III) to be safe, well-tolerated, and effective for improving lung function in patients with chronic *P. aeruginosa* infections. However, its poor water solubility at physiological pH due to insoluble hydroxide formation limits its efficacy at infection sites. Given that aminoglycosides can form soluble complexes with metals, we hypothesized that APR and Ga(III) could form a synergistic complex suitable for inhaled administration, enhancing efficacy against Gram-negative bacteria such as *A. baumannii*.

To test this, we determined the MICs of APR, Ga(III) as nitrate salt, and a 1:1 molar ratio of APR-Ga(III) combination against 24 clinical strains of *A. baumannii*. The combination reduced MICs for APR and Ga(III) by 32- to 4096-fold and 1257- to 20,000-fold, respectively. Notably, this synergy was not observed against other Gram-negative bacteria, including *Escherichia coli*, *Klebsiella pneumoniae*, and *P. aeruginosa*. Additionally, combinations of Ga(III) with other aminoglycosides (tobramycin and amikacin) showed no enhanced efficacy. Time-kill curves on four *A. baumannii* isolates (with APR MICs ranging from 8 to 64 mg/L and Ga(III) MICs of 64 mg/L. For combination same molar ratio as MIC were tested. Sampling were made at T0, T2h, T4h, T6h, T24h and T32h) were performed using an Emax model, which quantified bacterial killing dynamics. For all isolates, the APR-Ga(III) combination exhibited twice the potency of APR alone, as shown by a 50% reduction in EC50 (half maximal effective concentration) . To assess the combination's ability to suppress resistance development, we conducted serial MIC assays over 15 days on two *A. baumannii* isolates (APR MICs of 4 and 16 mg/L). In isolates treated with APR alone, MICs doubled by day 2 and gradually rose to 512 mg/L by day 15. In contrast, MICs for the APR-Ga(III) combination remained stable for seven days, only increasing to 16-32 mg/L by day 15.

This study highlights the APR-Ga(III) combination as a promising therapeutic approach to enhance efficacy and potentially limit resistance development in *A. baumannii*.

Keywords: *Acinetobacter baumannii,* Antibiotic resistance, Apramycin-Gallium combination

4

Radically new approach for antimicrobial discovery

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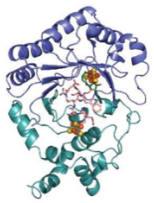
To face the current antibiotic resistance crisis, novel strategies are urgently required. Indeed, despite considerable efforts, in the last 30 years only few antibiotics have been launched to the market. Natural products have markedly contributed to the discovery of novel antibiotics and drug leads, with more than half anti-infective and anticancer drugs approved by the FDA, being of natural origin. Among them, thanks to their modular structure and simple biosynthetic logic, ribosomally synthesized and posttranslationally modified peptides (RiPPs) are promising compounds[1].

In the last years, we and other groups have shown the pivotal role of an emerging class of biocatalysts called radical SAM enzymes, in the biosynthesis of RiPPs1. Thanks to recent advances in structural biology[2,3], in silico analysis and recombinant expression systems, we have demonstrated that radical SAM enzymes produce RiPPs with unprecedented molecular architectures and properties[2,4,5]. These enzymes are also key players within the human microbiota for the biosynthesis of novel RiPP antibiotics[6,7]. Overall, radical SAM enzymes are attractive biocatalysts for the discovery and engineering of alternatives to conventional antibiotics.



Keywords: Antibiotics, RiPP, microbiota, peptide,

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5

Advancing the myxobacterial compound discovery pipeline

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For years, our group has dedicated itself to the cultivation and evaluation of myxobacterial extracts in the search for novel anti-infective compounds. This approach has been instrumental in identifying a range of natural products from our extensive myxobacterial strain collection, showcasing activities against bacterial, fungal, viral, and parasitic pathogens. In light of the escalating global threat of antimicrobial resistance (AMR), these efforts have become increasingly urgent. To strengthen our capacity to contribute to this pressing global need, we have undertaken significant advancements to reinvigorate and optimize our discovery pipeline. These enhancements include improvements at every stage of the workflow: maximizing the chemical diversity and potential of each strain, increasing the scale of material available for screening, enhancing the complexity of the samples analyzed, and refining our ability to predict active components from primary screening data. Furthermore, we have coupled these improvements with strategic investments in automation and high-throughput technologies. Key upgrades include LC-MS analytics, advanced fractionation methods, liquid handling systems, the development of robust bioactivity assays, and enhanced sample storage and data management capabilities. These innovations collectively enable us to streamline the discovery of bioactive compounds, with an emphasis on identifying candidates that could address the AMR crisis. Here, we present an overview of our recent efforts, highlighting selected examples of active compounds discovered, alongside the implementation of our enhanced library pipeline. By harnessing these advancements, we aim to contribute new solutions to the growing challenge of AMR and expand the arsenal of effective therapeutics for combating infectious diseases.

Keywords: Natural products libraries, myxobacterial extracts, anti-infectives discovery pipeline, automation, LC-MS analytics

6

Restoring drug activity against resistant bacteria with insights from marine natural product analogues

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The widespread incidence of antimicrobial resistance necessitates the discovery of new classes of antimicrobials as well as adjuvant molecules that can restore the action of ineffective antibiotics. A strategy for overcoming Gram-negative bacterial resistance is to identify compounds that can circumvent drug resistance by enhancing the activity of antibiotics that are currently ineffective. Our project aimed to demonstrate that marine natural products and synthetic analogues are a source of novel molecules that can restore the activity of antibiotics towards difficult to treat Gram-negative bacteria. Such drug 'rehabilitation' could help halt the spread of antibiotic resistance.

Ten new series of analogues related to our original hit antibiotic-enhancing compound were performed. Modifying the molecules' 'head group' produced analogues with antibiotic- enhancing properties but no detectable cytotoxicity or haemolytic properties.

One series, the indole-carboxamide-polyamines has been particularly studied and is now the focus of ongoing development. Derivatives have been shown to be able to restore the activity of unused antibiotics against resistant bacteria at levels of activity to those observed against non-resistant bacteria. We have been able to investigate their mechanism of action involved against both Gram-negative and positive bacteria.

The results confirm that the marine environment has emerged as a promising source for the discovery of new classes of antimicrobials.

Keywords: antibiotic enhancement; antimicrobial activities; marine natural products, polyamine conjugates.

7

Unraveling aneuploidy control mechanisms and their impact on antifungal drug resistance in the fungal pathogen *Candida albicans*

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Candida albicans causes >1.5 million fungal infections and ~1 million deaths each year. The fungus is predominantly diploid, but aneuploid forms have also been described. Aneuploidy, defined as an imbalance in the number of chromosomes in a cell, is associated with responses to stress or antifungals. However, the mechanisms regulating aneuploidy are poorly understood.

When exposed to high concentrations of fluconazole, a subpopulation of a susceptible C. albicans population can become resistant to the antifungal drug. This subpopulation was associated with the gain of a particular chromosome, indicating that aneuploidy and antifungal drug resistance are linked. Using a BFP/GFP fluorescence reporter system integrated on a chromosome of interest, flow cytometry can track and quantify cells that become aneuploid within a C. albicans population. This approach will shed light on how aneuploidy is related to fluconazole resistance, how many cells within a diploid cell population become aneuploid when exposed to fluconazole, and at which timepoint aneuploidy occurs.

To identify genes that regulate aneuploidy, a C. albicans overexpression collection is being screened for ploidy shifts using DNA staining and flow cytometry. Preliminary screening of a subset of mutants revealed 3 categories of mutants: (1) near-diploid (similar to the parent isolate, ~66 %), (2) decreased chromosome copy number (~22 %), and (3) increased chromosome copy number (~12 %). Mutants displaying increased chromosome numbers include the transcription factor TEA1, the protein kinase KSP1, metabolic genes, as well as several uncharacterized ORFs. These mutants are being evaluated to determine whether they impact antifungal drug resistance.

The project aims to identify novel regulators of aneuploidy and their effects on azole responses in C. albicans. The results will provide insights into the mechanisms of aneuploidy in pathogenic fungi and could help combat acquisition of antifungal resistance.

Keywords: *C. albicans*, antifungal drug resistance, aneuploidy

8

Identification from a Rwandan propolis of linoleic fatty acid as an antimicrobial agent against *Cutibacterium acnes* bacteria

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Acne is a very common skin condition that causes pimples to approximately 80% of adolescents during puberty despite the many effective treatments developed. In our research, we identified a molecule namely linoleic acid from a propolis collected in Rwanda and demonstrating significant inhibition of *C. acnes* growth at a concentration of 16 µg/mL. According to our data results, linoleic acid can effectively treat *C. acnes* and could be a possible alternative to formulation of an active propolis ointment.

Keywords: Fatty acids, Linoleic acid, Acne, *Cutibacterium acnes*, Propolis, Antibacterial activity.

9

Identification from a Rwandan propolis of 2,4-Ditert-butyl phenol as an antimicrobial agent against *Cutibacterium acnes* bacteria

Florent Rouvier[1,#], Lydia Abou[2], Emmanuel Wafo[1], Perrine André[3], Julien Cheyrol[3], Mohamed-Mohsen Khacef[3], Claude Nappez[3], Hubert Lepidi[4] and Jean Michel Brunel[*,1]

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Acne is the most prevalent dermatological condition among humans, affecting approximately 80% of adolescents during puberty. To date, numerous compounds have been used for acne treatment, including erythromycin ointments and antiseptics, with varying degrees of success. The emergence of erythromycin-resistant *C. acnes* strains has spurred the search for new antimicrobial agents, particularly from natural sources. In our research, we identified a molecule, 2,4-Di-tert-butylphenol (2,4-DTBP), from propolis collected in Rwanda. This compound demonstrated significant inhibition of *C. acnes* growth at a concentration of 16 µg/mL. Our *in vitro* and *in vivo* results suggest that 2,4-DTBP could effectively eradicate *C. acnes* and may serve as a potent alternative for the formulation of an active propolis ointment for acne treatment.

Keywords: 2,4-Di-*Tert*-Butyl-Phenol, Acne, *Cutibacterium acnes*, Propolis, Antibacterial activity.

10

SHORT TALK

Control of *Staphylococcus aureus* infection by physical plasma

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Wound infection by bacteria such as Staphylococcus aureus is one of the main causes of death in burn victims. *S. aureus* is an opportunistic pathogen characterized by a biofilm-forming capacity that makes infections more di<icult to eradicate. Moreover, the spread of S. aureus strains resistant to first-line antibiotics represents an additional threat, leading to an urgent need to identify new antibacterial approaches. The use of cold atmospheric plasma (CAP) could be such an approach and is particularly interesting in cases of antibiotic resistance. We have recently shown that CAP has powerful antibacterial e<ects and healing properties in a mouse model of infected burn wounds. However, CAP action against biofilm and the underlying molecular mechanisms have yet to be deciphered. Here, we treated methicillin-resistant S. aureus (MRSA) biofilm with CAP in two di<erent set-ups: biofilm grown in liquid culture and on agar plates. In both conditions, CAP showed antibiofilm activity. Next, we investigated the e<ect of CAP at the molecular level. RNA was extracted from CAP treated MRSA biofilm grown in liquid medium to characterize the transcriptome, which could point to molecular pathways regulated by CAP. CAP treatment of MRSA on agar plates allowed the isolation of resistant colonies whose genome was sequenced. The genomic information is currently being combined with the expression data to decipher the molecular mechanisms driven by CAP. Moreover, the antibiofilm activity of CAP was tested on 3D-bioprinted human skin, a more complex model made up of keratinocytes, fibroblasts, and macrophages. The models and the e<ect of CAP were characterized using histochemistry and immunofluorescence techniques.
In summary, our findings demonstrate that CAP exhibits promising antibiofilm e<ects against MRSA in various models, including 3D-bioprinted human skin. Further, transcriptomic and genomic analyses provide initial insights into the molecular pathways modulated by CAP, potentially revealing new targets for combating antibiotic-resistant biofilms. These results underscore CAP's potential as an innovative antibacterial strategy, particularly in the context of challenging infections like MRSA associated wound biofilms.

Keywords: *S. aureus* – MRSA – Biofilm - Cold Atmospheric Plasma

11

SHORT TALK

Design and synthesis of natural lactone derivatives for antibacterial treatment

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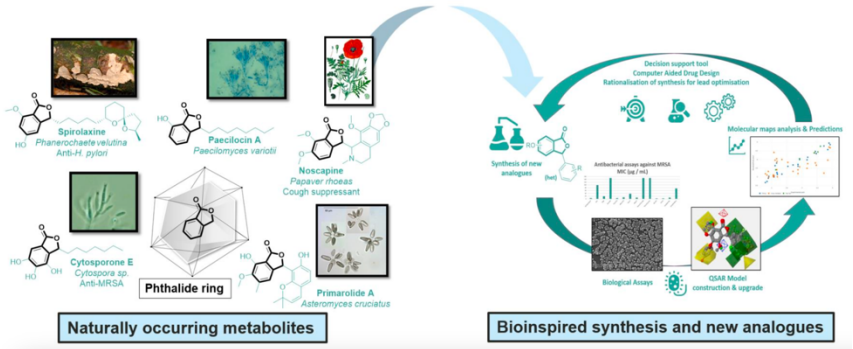
In the last decade, the emergence of multi-resistant bacteria has resulted in approximately 5 million deaths per year. To address this growing issue, it is necessary to discover new molecules that possess antibiotic properties and innovative mechanisms. Among the potential solutions, molecular diversity found in nature can help identify new therapeutic compounds.

Taking inspiration from natural compounds, we are directing our research towards small oxygenated heterocycles called phthalides. These lactones possess a diverse range of biological properties, such as antineoplastic, anti-inflammatory, anti-ischemic, and antibacterial activity; These molecules have shown promising results in combating resistant strains. Notably, paecilocin A, spirolaxine, and cytosporone E are exemplary molecules that exhibit antibacterial activity. Although effective against many strains, including methicillin-resistant *Staphylococcus aureus* (MRSA), the mechanism of action of thesephthalides remains to be elucidated, and their synthetic development needs to be pursued. Our team has previously demonstrated the extensive activity of a series of paecilocin A and cytosporone E analogues on MRSA, showcasing our competence and expertise in this area. Efficient new synthetic methodologies have been developed for rapid access to arylated analogues of cytosporone E. The ease of synthetic access and the biological properties of phthalides mentioned above encourage further research efforts in the development of these molecules.

The aim of the study is to develop libraries of phthalide antibiotics bioinspired by cytosporone E. It should be noted that it is not possible to access resistance mutants of *Staphylococcus aureus*, but only mutants with reduced sensitivity. The research aims to comprehensively construct a rational structure-activity relationship (SAR) model, which will serve as a decision support tool to optimize biologically active compounds. Our team has successfully developed new synthesis protocol that is not only easy to implement, but also enables the addition of new chemical functions. This is a crucial step towards achieving the desired diversity in constructing the quantitative RSA model. We constructed the model using the 3D QSAR method, with a panel of biologically evaluated molecules and tools from the Rome Center for Molecular Design laboratory (University of Rome). The model was built through a series of steps, including dataset construction, conformational search, alignment, and comparative molecular field analysis (CoMFA).

Efficient and rapid access to phthalides inspired by cytosporone E has been made possible through the development of a new synthesis methodology. The extensive library of aryl phthalides, which is based on a high degree of molecular diversity in the substituents, provides ample information about their biological activities, allowing for the construction of a rational three-dimensional structure-activity relationship model. The application of diverse chemoinformatic techniques has facilitated the creation of a variety of CoMFA models that can be used to generate molecular maps and predict biological activities against MRSA. The generated model's results have significantly improved our synthetic development and guided our functional choices enhancing biological activities, while offering us the possibility of generating models of higher quality than the previous ones in an iterative manner.



Keywords: MRSA, heterocycles, metabolites, lactones, drug design

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The ABRomics platform — a One Health Antimicrobial resistance analysis service

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Antibiotic resistance (ABR) is a major global public health issue designated for urgent action by international institutions, especially regarding the emergence and the global dissemination of multidrug resistant bacteria (MDRB) and antibiotic resistance genes (ARGs) carried by mobile genetic elements. They are widely transmitted between humans, animals, and the environmental domains (One Health context). Tracking transmissions of outbreaks and identifying sources of contamination is achievable with Whole Genome Sequencing (WGS) data combined with epidemiological information. Ensuring the sharing of high-quality sequence data alongside interoperable curated metadata are key requirements for understanding the spatiotemporal patterns of dissemination of MDRB and ARGs.

The ABRomics platform is developed to address such major issues by facilitating systematic bioinformatics analyses for ABR experts and the wider research community. Users can deposit biological sequences, manage projects, and launch various types of analyses to answer generic and specific questions related to AMR. Associated with all deposited sequences, minimal metadata compatible with ENA submission are requested to ensure traceability, and backward and forward compatibility in our data repository.

ABRomics takes as input genomics WGS sequencing data (FASTQ files, and in some cases FASTA files) and does the following analyses: quality checks, genome assembly and annotation, taxonomic assignation (species identification), MLST typing (strain identification), plasmid typing, and finally detection of ARGs genes and virulence factors. The platform relies on the usegalaxy.fr Galaxy [1] server to perform the bioinformatics analyses allowing complex scientific computation on the IFB Core Cluster. ABRomics workflows are published on the Intergalactic Workflow Commission and tools are available on the Galaxy ToolShed to ensure reproducibility and accessibility.

The interface enables the users to search and cross-reference results of previous analyses stored in our database. Among the different features provided, we focused on highlighting search results based on the presence of genes conferring resistance or a particular predicted phenotype. Furthermore, fine-tuned permissions management and data encryption ensure the protection and confidentiality of user data as well as data exchanges within a lab consortium.

A demonstration of ABRomics features will be presented

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Innovative inhibitors of the bacterial transferase MraY, towards a new generation of antibiotics

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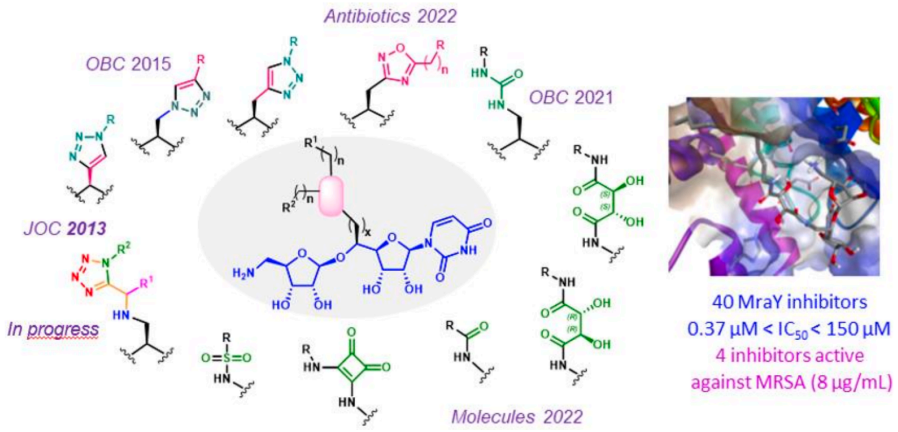
Infectious diseases are one of the main causes of human mortality worldwide, and combating the emergence of nosocomial infections involving multi-resistant bacterial strains is one of the World Health Organization's ten research priorities. One way to fight bacterial AMR is to focus on biological targets displaying a new mode of action compared to those of the approved antibiotics. Considering their high specificity and their unique occurrence in bacteria, enzymes involved in peptidoglycan biosynthesis are promising targets, since each of them is essential for the bacterial growth. In this context, our goal is to focus on inhibiting the MraY transferase[1], an unexploited target catalysing the first membrane step of peptidoglycan biosynthesis, in order to delay the emergence of bacterial resistance.

The synthesis and the in vitro and in cellulo biological evaluation of several families of MraY inhibitors[2] based on an aminoribosyl uridine scaffold, important for the biological activity of these compounds, will be presented. The diversity-oriented synthesis of these inhibitors notably relies on multi- component reactions. The SAR results will be rationalized by molecular modeling studies.

Key words: Nucleosidic Antibiotics, CuAAC, Ugi-azide, biological evaluations, docking studies.



Structure and activity of the synthetized MraY inhibitors

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Enhancing Colistin Efficacy Against Multi-Drug Resistant Gram-Negative Bacteria Using Farnesol- Loaded Lipid Nanoparticles

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Our previous work demonstrated that farnesol-loaded lipid nanoparticles (F-LNPs) enhance colistin (CST) efficacy, reducing its MIC by up to 64-fold against *Acinetobacter baumannii* and *Escherichia coli*. Here, we investigated the broader applicability of F-LNPs by testing them against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, examining mechanisms of action and the potential reduction of CST resistance. While F-LNPs showed a weak effect against *P. aeruginosa* isolates (n=6), they significantly enhanced CST efficacy against all *K. pneumoniae* strains tested (n=18) in a concentration- and strain-dependent manner, with a remarkable 1024-fold MIC reduction in a *mcr-1* strain. CST's primary action is outer membrane (OM) destabilisation followed by inner membrane (IM) disruption and co-administration with F-LNPs may mediate CST's action.
Once measured with propidium iodide and SYTOX™ blue uptakes, F-LNPs alone enhanced IM permeability, further amplified when combined with CST. We further investigated OM destabilization by testing the combination on OM-modified, CST-resistant *K. pneumoniae* isolates with LPS modifications: phosphoethanolamine (pEtN), 4-amino-4-deoxy-L-arabinose (L-Ara4N), or both. F-LNPs enhanced CST efficacy by 8- to 1024-fold across all isolates, with no clear correlation between LPS modification type/extent and efficacy enhancement. Electron microscopy showed no visible OM alterations with F-LNPs alone, and they did not lower the MICs of antibiotics with OM permeability limitations (vancomycin and linezolid), contrasting with a sub- MIC CST positive control. These findings indicate that F-LNPs primarily enhance CST efficacy by amplifying IM destabilisation but not OM permeability. To assess resistance prevention, we conducted 11 consecutive MIC measurements at the highest CST concentration permitting bacterial growth. In pEtN *K. pneumoniae*, F-LNPs with CST prevented resistance development; CST alone led to a MIC increase from 4 to ≥1024 mg/L, while the combination maintained a MIC of 0.03 mg/L. High CST resistance was associated with a zeta potential (a measure of charge density on the bacterial surface) shift from -25.5±3.1 mV to -14.4±2.9 mV, while it remained at approximately-21.2±1.9 mV with CST and F-LNPs. Similar effects were noted in L-Ara4N and pEtN+L-Ara4N modified strains. In an *in vivo* experiment, F-LNPs with CST improved survival in larvae infected with 106 CFU/larva, achieving 43% survival ($p < 0.05$) at day 7 with the CST-F-LNP combination (75 mg/kg) compared to 0% with CST alone (10 mg/kg). Overall, our *in vitro* and *in vivo* results showed that this combination has the potency to control resistant *K. pneumoniae* by permeabilizing its inner membrane while minimising the chance of resistance development to CST.

Keywords: Gram-negative bacteria, lipid nanoparticle, farnesol, colistin efficacy, resistance.

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Re-exploring the salicylanilide scaffold for the inhibition of mycolic acid synthesis in *Mycobacterium tuberculosis*

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Tuberculosis (TB) remains a global health crisis, further exacerbated by the slow pace of progress in developing new treatment options, and the emergence of extreme and total drug resistance to existing drugs. Infection cases keep increasing yearly, with a global number of 7.5 million of people diagnosed in 2022[1]. As for other bacterial bugs, the most pressing challenge lies in the urgent need of finding new active compounds, acting through novel modes of action to tackle resistances[2]. A unique feature of Mycobacterium tuberculosis (Mtb), the causative agent of TB, is its complex and lipid-rich cell envelope. Therefore, targeting the biosynthesis pathway of cell envelope mycolic acids is a historical strategy against Mycobacterium tuberculosis, yet the enzyme FadD32 is still an underexplored although highly relevant (and druggable) target[3]. We have previously developed a miniaturized automatized enzymatic assay[4], which allowed the high-throughput screening of a drug repurposing library containing 1280 approved human or veterinary drugs (Prestwick Chemical Library)[5]. We obtained 36 hits that we further validated for their phenotypic activity on M. tuberculosis (MIC 0.08 – 10 µM). This led to identification of one promising pharmacophore, presenting a salicylanilide scaffold that we are now studying further by synthesizing and evaluating derivatives. Exploring SARs, we develop a hit to a lead medicinal chemistry project, searching for a novel anti-tubercular candidate targeting FadD32. The preliminary results of this approach will be presented.

Keywords: tuberculosis, salicylanilide, assay development, SAR, FadD32

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SHORT TALK

FUNGIPEP: Synthetic lipopeptides to fight multiresistant fungal infections

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As invasive fungal infections (IFI) are increasingly common in the nosocomial setting (>1.6 million patients die annually of IFI[1]), the WHO published in October 2022 a fungal priority pathogen list, counting as a first step to prioritize fungal pathogens and promote research which until then was somehow left behind.

Echinocandins (EC), as part of the more recent antifungals, are cyclic lipopeptides which non-competitively inhibit the β-(1,3)-D-glucan synthase (GS)[2] (found exclusively in fungi), leading to the loss of the fungal wall integrity [3]. Their specificity gives them the advantage of being well-tolerated but despite their evident potential only 4 EC are currently on the market and some examples of resistant strains have already been identified [4].

The aim of the project *Fungipep*, is to identify new drug candidates inhibiting the GS with the objective of skirting emerging resistance encountered with currently available EC. Another important aspect of this project is to elucidate the inhibition mechanisms used by these original lipopeptides as the recognition site of EC on the FKS1 catalytic unit of GS has never been totally confirmed.

To reach these high goals, a first part of the project focuses on the synthesis of unprecedented EC-analogues by SPPS (associated or not with click-like bioconjugate chemistry) to identify some structure/activity relationships. All the synthesized lipopeptides are tested on various strains of fungi isolated from hospitalized patients, making it possible to quickly evaluate their bioactivity and adjust their structure accordingly.

The synthetic routes developed during this project are also exploited to synthesize innovative molecular tools like lipopeptides carrying a fluorophore. These ones will later be used in fluorescence microscopy to try to confirm the binding site of EC by FRET experiments (in association with an FKS1-iLOV fusion protein that we have designed and are currently bioproducing).

Keywords: echinocandins, lipopeptides, antifungals, fungal infections, glucan synthase

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Exploring the mechanism of β-lactam resistance in corynebacteria

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Introduction and objectives

The genus *Corynebacterium* includes species that are usually highly sensitive to β-lactamines (*C. diphtheriae*) and species that are usually resistant (*C. jeikeium*) (1). The aim of this work was to identify proteins that may be involved in the mechanism of β-lactam resistance.

Materials and methods

The strains studied came from the Institut Pasteur collection or were clinical isolates. Antibiotic susceptibility was determined by liquid microdilution, E-TEST or MTS, and solid-state diffusion susceptibility testing following CA-SFM V1.2023 recommendations. Proteins were produced in *Escherichia coli* using the pET-2818 and PET-TEV vectors, then purified by chromatography (2-3). Heterologous expression of *C. jeikeium* Pbp2c in *C. glutamicum* RES167 was achieved using the pMM36 shuttle vector (3). Purified Pbp2c was used to immunize mice, and the immune sera were used to study its production by western blot (3).

Results, discussion and conclusion

Comparison of Corynebacterial genomes identified a locus (jk0410-jk0412) present only in β-lactam-resistant strains (2-4). Jk0411 possesses the conserved motifs found in β-lactamases, however, no β-lactamase activity was detected either from colonies, crude extracts or the purified jk0411 protein. *Jk0412* encodes a class B PLP (Pbp2C). Its heterologous expression in *C. glutamicum* confers resistance to all β-lactam classes, including carbapenems and C5G. The presence of this locus correlates with the resistance phenotype, but in some strains requires prolonged incubation or induction by clavulanic acid or amoxicillin to be detected. This resistance locus has recently been described in a strain of *C. diphtheriae* (5). Taking this mechanism of inducible resistance into account should enable new recommendations for the interpretation of antibiotic susceptibility in corynebacteria.

Key words: Corynebacteria - resistance - β-lactams.

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Rapid emergence of daptomycin resistance in a strain of *C. striatum* causing severe infection

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Introduction and objectives
Usually a commensal of the skin and mucous membranes, *Corynebacterium striatum* can be responsible for severe infections such as endocarditis, meningitis, pneumopathy and osteoarticular infections[1]. This species is usually susceptible to β-lactam antibiotics, but multi-resistant strains have been described. Here we describe the case of a lethal infection caused by an amoxicillin-resistant strain of *C. striatum* that rapidly developed resistance to daptomycin.
Materials and methods
Clinical isolates were obtained from joint fluid and blood cultures from an 85-year-old patient. Antibiotic susceptibility was determined by solid-state diffusion antibiotic susceptibility testing (SSDAT) and macro-testing (E-TEST) following CA-SFM V1.2022 recommendations. The gene encoding the low affinity Plp2c was screened by PCR on a DNA extract obtained using the Ingenius (Elitech) apparatus[2].
Results, discussion and conclusion
The 85-year-old patient was admitted with fever and left shoulder pain. He had received 11 days of antibiotics (tazocillin from D0 to D3, followed by ofloxacin) for a *M. morganii* urinary tract infection. Peripheral blood culture and joint fluid samples revealed an amoxicillin-resistant *C. striatum* infection (MIC = 12 mg/L). This strain harbors the *plp2c* gene encoding a low-affinity penicillin-binding protein (Plp2c) responsible for high levels of resistance to β-lactam antibiotics, including carbapenems and 5th generation cephalosporins[2]. Daptomycin was prescribed at a dosage of 10 mg/kg for 21 days. The MIC of daptomycin in the control blood culture strain at D8 had risen from 0.032 mg/L to > 256 mg/L. The increasing number of publications describing corynebacterial strains highly resistant to daptomycin indicates that this is probably not a reliable treatment option, while the emergence of resistance to glycopeptides and lipoglycopetides has not been described to date[3-5].

Key words: Corynebacteria - resistance - β-lactam-Daptomycin

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In-host evolution of *Yersinia enterocolitica* during a chronic human infection

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Bacteria exhibit remarkable evolutionary adaptability in response to the selective pressure encountered during infection and antibiotic treatment in humans. We characterized four *Yersinia enterocolitica* clonal isolates from successive bacteremia episodes that evolved within a 75-years-old patient in the span of 14 years. Pan-genome analysis and genome comparison showed that their common evolution was characterized by a genome size reduction resulting in the loss of a hundred of genes. Quinolone resistance in the last two isolates was acquired through a so far undescribed deletion in the DNA gyrase gene *gyrA*. A phylogenetic analysis by maximum likelihood identified two genes, *tesB* and *pitA*, presenting a positive selection signal, suggesting that these mutations leading to the product inactivation provided a survival advantage to bacteria during chronic infection. Mass-spectrometry analysis revealed a strong proteome remodeling in these two isolates which was correlated with a truncation leading to inactivation of the stringent response regulator DksA. Impaired carbon, energy and purine metabolisms substantiate their severe growth defects *in vitro*, accounting for antibiotics tolerance and possibly to therapeutic failure. 3rd-generation cephalosporin resistance of the last isolate was correlated with a truncation of OmpF, the main porin translocating antibiotics through the outer-membrane, as well as an increased production of BlaA and AmpC β-lactamases.

This is the first report of genetic and phenotypic changes associated to within-host adaptation of a pathogenic *Yersinia* species under antibiotic pressure.

Keywords: Bacteremia, Genomics, Proteomics, Within-host evolution, Yersiniosis

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Scalable gene-to-structure discovery of bioactive secondary metabolites from *Streptomyces* spp.

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Secondary metabolites (SMs) produced by microbes are essential for ecological functions and serve as critical medicines for humans (e.g. antibiotics, antifungals, antitumors). However, traditional drug discovery from microbes has been hindered by frequent rediscovery of known compounds and difficulties in accessing silent, unexpressed SMs in laboratory conditions. Recent advancements in genomics have transformed the identification of SMs by allowing the detection of biosynthetic gene clusters (BGCs) in microbial genomes, revealing that the majority of SMs remain undiscovered. To accelerate discovery of underexplored chemical space and combat high rediscovery rates related to horizontal gene transfer, a novel discovery platform has been developed to capture and dereplicate thousands of BGCs from microbial libraries.1 This platform enables the transfer of untapped BGCs into model hosts for heterologous expression, simplifying product detection and facilitating transcriptional activation at scale. A high throughput sequencing and multiplexed cloning approach allows for the simultaneous mobilization of hundreds of BGCs, offering significant scalability and tackling all problems that hinder microbial SMs discovery. Alongside bioactivity-guided approaches, structure-based exploration holds a tremendous premise for drug discovery by leveraging structure-activity relationship (SAR) knowledge for guided exploration. The current research aims to demonstrate a scalable, gene centric, cross-species exploration of SMs, their biosynthesis and antimicrobial activity. By classifying BGCs into subgroups based on extensive comparative genomics, this method facilitates deeper exploration of SMs possessing shared structural building blocks and biosynthetic pathways, ultimately leading to the discovery of novel microbial SMs with potential therapeutic applications and deepening the understanding of their biosynthesis. We will illustrate this gene-to-structure approach by demonstrating the prediction and facilitated exploration of potentially bioactive SMs from a library of Streptomyces strains that contain a non-proteinogenic amino acid, piperazic acid.

Keywords: high-throughput heterologous expression, drug discovery, gene-to-structure, piperazic acid

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How to unravel the mystery of B13, a promising imipenem adjuvant against MRSA?

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Introduction: In recent decades, multi-drug resistant strains have emerged among Gram-positive bacteria. To combat the spreading of antimicrobial resistance, one strategy is to identify new druggable targets that will restore antibiotic efficiency when hit. Many studies highlight the lipoteichoic acid (LTA) D-alanylation pathway – a cell wall modification pathway - as a promising target. In this context, Pasquina *et al*. described in 2016 amsacrine as an inhibitor of DltB, a membrane protein involved in translocating D-alanyl moieties onto LTA. Despite its promising ability to resensitize *Staphylococcus aureus* to several antibiotics, the molecule also inhibits eukaryotic topoisomerase, making amsacrine clinically irrelevant. To bypass this issue, a collection of chemicals derived from amsacrine and/or identified *in silico* as potential DltB inhibitors was created. Surprisingly, one of these compounds (named B13) demonstrated anti-resistance effects without inhibiting LTA D-alanylation pathway.

This study aims to (i) better characterize B13 and (ii) develop and validate a proteomic approach that will allow the identification of the target and mechanism of action of this novel adjuvant.

Methods: Thermal Proteome Profiling (TPP) is based on the fact that a molecule binding on its protein target will significantly increase protein thermal stability. This increased stability is defined by a shifted melting temperature that can be monitored with mass spectrometry. Here, a previously described inhibitor of DltA (May *et al.,* 2005) is used to validate the method. In parallel, the MIC of Imipenem against a methicillin-resistant *Staphylococcus aureus* (MRSA) clinical strain, cell wall D-alanylation, and cytotoxicity were assessed in the presence of B13.

Results: B13 efficiently resensitized the MRSA clinical isolate to imipenem without demonstrating any inhibition of the LTA D-alanylation pathway, nor cytotoxicity on HaCaT cells. TPP performed on *S. aureus* raw protein extracts demonstrated a significant thermal shift regarding DltA. Other proteins related to DltA such as DltC or AcpS were also stabilized in the presence of DltA inhibitor.

Conclusion: As expected, TPP analysis identified DltA as the major target candidate of the DltA inhibitor. In addition, the method provided information on the thermal stabilization of proteins known to interact with DltA, suggesting that TPP can detect whole interaction pathways. Taken together, these results validate the TPP protocol as a new approach to identify the unknown target(s) of B13, a promising imipenem adjuvant.

Keywords: Thermal proteome profiling, target, MRSA, adjuvant, DltB, teichoic acid D-alanylation, antibiotic resistance, *Staphylococcus aureus*, cytotoxicity.

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COVID-19 Pandemic Influence on Group B *Streptococcus* Colonization, Serotype Dynamics and Antimicrobial Resistance Patterns in Pregnant Women from the Brazilian Amazon

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Streptococcus agalactiae (Group B *Streptococcus*, GBS) is a leading cause of infection during pregnancy, preterm birth, and neonatal infection, particularly in low- and middle-income countries (LMICs). The coronavirus disease 2019 (COVID-19) pandemic has globally contributed to accelerating the development of antimicrobial resistance (AMR), as well as altering the dispersion and prevalence of multidrug-resistant bacteria (MDRs). This study evaluates and compares the prevalence of colonization, serotypes, and antimicrobial susceptibility of GBS isolates from pregnant women in Porto Velho, Brazil, before, during, and after the COVID-19 pandemic. A total of 1,449 pregnant women between 35 and 37 weeks of gestation, attended at Public Health Units between February 2018 and November 2023, were included in the study. The pregnant women were grouped into three periods: pre-pandemic (Feb 2018 to Feb 2020, n=443), during the pandemic (Mar 2020 to Apr 2023, n=774), and post-pandemic (May 2023 to Nov 2023, n=232). GBS was identified in rectovaginal samples using microbiological and genotypic methods. The phenotypic profile of antimicrobial susceptibility was evaluated through the disk-diffusion test. Capsular typing was determined by multiplex PCR. GBS was detected in 20.7% (300/1449) of the pregnant women. A decrease in the colonization rate was observed over the periods: from 22.6% before the pandemic to 20.7% during, and 17.2% after the pandemic. Serotype Ia was the most prevalent (39%), followed by serotypes V (15.8%), II (14.1%), Ib (11.9%), III (10.2%), IV (5.1%), and VI (4%). The prevalence of serotypes varied across the studied periods. Serotype Ia remained the most prevalent, while serotype V showed the greatest increase during the pandemic, and serotype Ib continuously declined. Regarding antimicrobial susceptibility, all samples were sensitive to penicillin, ampicillin, cefazolin, and vancomycin. Resistance was observed to tetracycline (85.4%), azithromycin (32.2%), erythromycin (28.8%), levofloxacin (9.6%), clindamycin (6.2%), and chloramphenicol (3.4%). Erythromycin, azithromycin, and levofloxacin experienced an increase in non-susceptibility rates of 13.7%, 18.5%, and 20.8% between the pre- and post-pandemic periods. Multidrug resistance (MDR) significantly increased post-pandemic (5.7% vs. 15.9%), while the rate of samples susceptible to all tested antimicrobials decreased (16.7% vs. 6.8%). The study suggests that the excessive use of antibiotics during the pandemic, associated with contingency strategies, may have imposed selective pressure, resulting in a decrease in GBS colonization rates and the selection of strains with resistance and MDR profiles. Therefore, monitoring antimicrobial resistance in GBS is crucial for tracking the evolution of resistance rates, and continuous surveillance is necessary in the post-pandemic era.

Keywords: *Streptococcus agalactiae*, pregnant women, antimicrobial resistance, Multidrug resistance, COVID-19 pandemic.

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SHORT TALK

Hospital wastewater as a reservoir and spreader of environmental antimicrobial resistance in Cameroon

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Background: Antimicrobial resistance (AMR) among bacteria is a growing problem worldwide, and wastewater treatment plants (WWTP) have been considered as one of the major contributors to the dissemination of AMR to the environment. Despite the fact that the discharge of hospital effluent without prior treatment is a cause for concern, little data is available on the impact of wastewater on the environmental ecosystem and on the health of populations in Cameroon. This study aims to assess the occurrence of antimicrobial resistance genes (AGRs) in hospital waste water.

Methods: Sixteen wastewater samples from four of the largest healthcare facilities in the cities of Yaounde and Douala in Cameroon were colleted from September to October 2023, both at the first manhole of the hospital WWTP and at the point of discharge of hospital effluent into the environment. Illumina next-generation sequencing was performed on the Biomics platform for each sample and Fastq sequences were assembled using Megahit v1.2.9 software. Metagenomics analysis were carried out to assess diversity and relative abundance of AGR using the Abricate v1.0.1 tool with the CARD database.

Results and discussion: A total of 416 ARGs coding for resistance to more than a dozen antimicrobial families were recorded. We observed a predominance in terms of diversity and abundance of ARGs coding for resistance to beta-lactams (138 AGRs) and aminoglycosides (60 AGRs). ARGs coding for last-resort antimicrobial (carbapenems, oxazolidinones, etc.) used for human and animal infections were found in 100% of the samples. The number of ARGs per sample varied from 103 to 252, with an average of 174
ARGs per sample, generally showing higher diversity for sampling points located before the WWTP. However, a great abundance and diversity of AGRs was observed at the discharge point of hospital effluents into the environment, with between 103 and 203 ARGs coding for resistance to most families. This observation reflects the inadequacy or inefficiency in the treatment method of hospital effluents and the potential risk to human health and the environment posed by the discharge of these effluents into surface waters used by surrounding populations.

Conclusion: Cameroon's hospital effluents constitute both a hotspot of AMR determinants and a threat to the environment and human health in Cameroon. Particular attention should be given to strengthening the treatment of these effluents before their discharge into the environment in order to limit the spread of AMR.

Keywords: Hospital wastewater, antimicrobial resistance gene, Cameroon.

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Development of a Microfluidic-Biosensor System for Evaluating Polymicrobial Biofilm Inhibition Using Niobium Nanoparticles

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This poster will introduce the development of an advanced microfluidic-biosensor system designed for real-time evaluation of polymicrobial biofilm infections (*Candida albicans, Sthaphylococcus aures and Pseudomonas aeruginosa*) on a chip. Biofilms, particularly polymicrobial ones, pose a significant challenge in healthcare due to their resilience and resistance to conventional treatment methods. Our innovative system combines microfluidics and biosensing technologies to create a controlled environment for biofilm growth and assessment, facilitating precise control of flow and nutrient conditions for more accurate simulation of physiological environments. Central to our study is the exploration of a novel metal nanoparticle, niobium, hypothesized to possess unique antimicrobial properties that may inhibit and prevent biofilm formation. By integrating niobium nanoparticles within our microfluidic-biosensor platform, we aim to quantitatively analyze its effects on biofilm attachment, growth, and structural integrity.

The findings from this system could offer valuable insights into niobium's potential as an antimicrobial agent, informing new approaches for biofilm management in clinical and industrial applications. Through this platform, we anticipate advancing our understanding of biofilm-nanoparticle interactions and contributing to the development of more effective biofilm prevention and eradication strategies.

Keywords: Polymicrobial biofilm, Microfluidic-biosensor, Niobium nanoparticles, Bacteria and fungus inhibition

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PBP2:MreC an ideal target to identify new antibacterial agents

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Antibiotic resistance has been increasing dramatically due to the rapid dissemination of resistance genes. The reduction in antimicrobial therapeutic options is an urgent global health threat and as such, identifying novel drug targets is critical for developing innovative therapies. Most of the existing antibacterial treatments are based on the inhibition of enzymatic activities involved in various biological processes. However, a promising strategy relies on targeting Protein-Protein Interaction that are essential in the assembly of critical protein complexes. Since the discovery of penicillin, the assembly of the bacterial peptidoglycan (PGN) has been recognized as a major target for antibiotics against bacterial infections. In rod-shaped bacteria, the enzyme PBP2 and the structural protein MreC are known to form a key core component of the Rod complex that control the PGN assembly during elongation. Previously, it was demonstrated that disrupting the PBP2:Mrec complex in H. pylori, a gram-negative bacteria for which antimicrobials are in high demand, leads to defects in cell shape, impaired growth and cell death (1). Given the conservation of the PBP2:MreC interaction across monoderm and diderm bacteria, this protein-protein interaction represents an ideal target for new antibacterial therapeutic strategies. Structural information on the PBP2:MreC core complex identified a 110 amino acids Specific Interaction Domain (SID) sequence on MreC essential for the binding to PBP2 (1). Successively, the PBP2:MreC complex crystal structure was solved, demonstrating that the formation of the complex requires PBP2 to open up in a "V" shape, accommodating the β-domain of MreC (2). This protein-protein interaction involves a hydrophobic zipper generated by aromatic residues on both sides. With this information we are designing various peptides containing the key amino acids found at the PBP2:MreC interface, preventing the formation of the protein complex and ultimately inhibiting bacterial growth.

First, we identified functional binding sites within PBP2 and MreC using InDeep, a machine learning tool able to predict the intractability and druggability of proteins. Using the insight gathered with this informatic tool, diverse peptides were designed with the aim to inhibit the hydrophobic interaction between the two proteins. Alternatively, de novo sequences targeting both PBP2 and MreC were generated using the software FoldX. The peptides were then synthesized and their inhibitor activity was tested using an in vitro FRET based assay named HTRF (Homogeneous Time-Resolved Fluorescence). At the same time, libraries of small molecules were also tested *in vitro* using the same FRET assay, leading to the identification of 6 potential hits.

Keywords: antibiotic research, screening, peptides, protein protein interaction

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Development of new peptide-based antimicrobial agents targeting trans-translation in bacteria

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Several issues during transcription/translation can lead to the stalling of ribosomes at the 3' end of a mRNA without a stop codon. As a result, when a bacterial ribosome reaches the end of the nonstop mRNA, it cannot elongate or terminate and the complex gets stuck on the problematic mRNA. The protein synthesis capacity of the bacteria is then severely compromised and it is critical for cell survival to resolve these nonstop situations. In bacteria, a rescue mechanism known as trans-translation can release stalled ribosome in the absence of stop codon. Required for bacterial stress tolerance, growth and virulence, trans-translation ensures bacterial survival. During this process, a small protein called SmpB recognizes stalled ribosomes by inserting its helical C-terminal tail in the vacant ribosomal mRNA path and allows the correct positioning of its partner, transfer-messenger RNA1,2. In the search for alternative antibiotic strategies, the rational design of SmpB C-ter tail decoy peptides would provide highly specific trans-translation inhibitors with potentially broad-spectrum bactericide activity and limited side-effects on host cells.

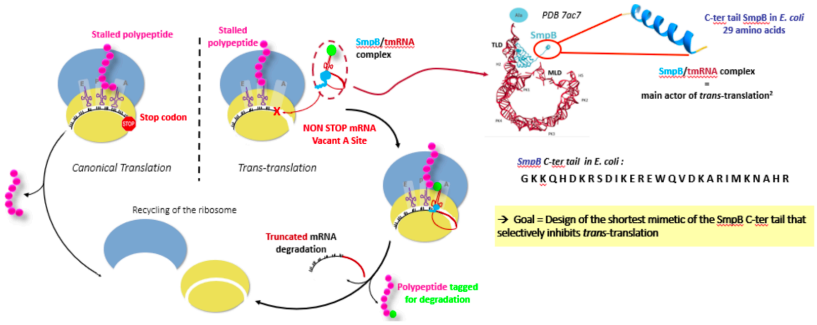

Figure 1: Translation and trans-translation mechanism

Developed in E. coli model, the peptide mimicking the SmpB C-ter tail selectively inhibits trans-translation over translation. This C-ter tail peptide was studied in silico to identify the most energetic contributions with the ribosome. An alanine scan was performed on these contributing positions, guiding the truncation of the peptide. These truncated peptides showed also inhibition of trans- translation, validating thus a proof-of-concept and opening ways to potent innovative drugs with an unmet antibiotic pathway.

Keywords: antibioresistance, peptide, trans-translation, SmpB, in silico and in vitro studies

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Identification of the bacterial target of phthalides with antibiotic activity and characterization of associated phenotypes

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In the face of the emergence of multi-resistant bacterial strains to conventional antibiotics, the development of new antibacterial therapies is becoming a public health priority. Phthalides are secondary metabolites, some of which possess antibacterial activity, such as cytosporone E. The synthesis of bio-inspired pharmacomodulations of this compound has improved antibacterial activity against *Staphylococcus aureus*, a pathogen highlighted by the WHO as a priority. To enhance antibacterial activity and reduce cytotoxicity, identifying the target(s) is essential to develop more relevant pharmacomodulations. This study aims to characterize this target and work on the phenotypes associated with phthalide activity.

Regarding the first part, we demonstrated that a sub-inhibitory concentration of phthalide caused a defect in the separation/aggregation of bacterial cells. A bioinformatics analysis predicted that these molecules would target the MurG protein, responsible for a key step in peptidoglycan synthesis. However, we showed that phthalides didn't induce any transcriptional changes in the gene encoding this protein. The functional study of phthalides on this protein began with the construction of protein with substitutions of amino acids predicted to be important in the phthalide/MurG interaction. Future interactomics analyses should confirm the hypothesis that MurG is the target of this family of antibiotics.

The other part of this work focused on the phenotypic activity of phthalides. It was demonstrated that these molecules have a relatively narrow spectrum of action, inhibit biofilm formation and have a synergistic effect when combined with a beta-lactam antibiotic. This new family of antibiotics represents a promising breakthrough in the therapeutic field. In the face of growing resistance to existing treatments, it opens news perspectives for effectively combating bacterial infections, including those caused by multi-resistant strains.

Keywords: phthalides, new antibiotics, anti-infectious therapy, target characterization

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Bothrops moojeni Lectin effects on the Production of Inflammatory Cytokines by Human Neutrophils infected with *Streptococcus agalactiae*

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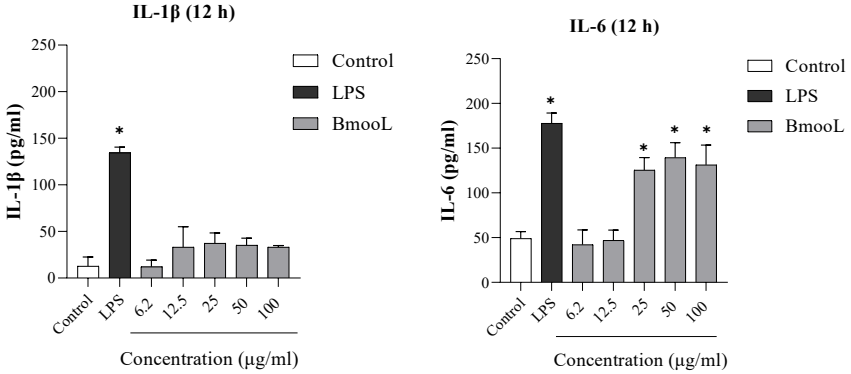
Bioactive molecules derived from snake venoms have been widely studied for their therapeutic and pharmacological potential. Lectins, carbohydrate-binding proteins present in various venoms, play a crucial role in modulating immune responses by interacting with specific receptors on leukocytes. *B. leucurus* lectin induced antibacterial activity against Gram-positive bacteria, but not against Gram-negative bacteria, suggesting that the difference in susceptibility would be associated with the interaction of the lectin with the peptidoglycan present in the cell wall of Gram-positive bacteria. Group B Streptococcus (GBS) are Gram-positive bacteria that are one of the main causes of serious infections in newborns. In neutrophils, GBS can be recognized by a variety of endosomal TLRs and produce significant amounts of pro-inflammatory cytokines through the activity of the NLRP3 inflammasome complex. In this study, firstly we explored the effects of a lectin isolated from *Bothrops moojeni* venom (BmooL) on the production of inflammatory cytokines by human neutrophils, specifically focusing on the interleukins IL-1β, IL-6, and TNF. Human neutrophils were isolated from peripheral blood and incubated with different concentrations of BmooL (6 – 100 μg.mL^{-1}) for 2, 4, and 12h. Lipopolysaccharide (LPS) was used as a positive control, while RPMI medium served as the negative control. After the incubation periods, the supernatants were collected and analyzed for IL-1β, IL-6, and TNF levels via enzyme immunoassay. At the 2- and 4h time points, there was no detectable release of IL-1β, IL-6, or TNF. After 12h, LPS significantly induced IL-1β production compared to the control, while BmooL did not stimulate detectable levels of this cytokine at any concentration tested. In contrast, BmooL significantly increased IL-6 production at 25, 50, and 100 μg.mL^{-1}, demonstrating a response similar to that induced by LPS. These results suggest that BmooL induces IL-6 production in human neutrophils without significantly affecting IL-1β and TNF production after 12h of stimulation. Our findings underscore the immunomodulatory potential of snake venom lectins and suggest their possible role in IL-6-mediated inflammatory processes.

Keywords: *Bothrops moojeni,* lectin, inflammation, neutrophils, cytokines, Streptococcus agalactiae



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Design and synthesis of bisubstrate inhibitors of epigenetic methyltransferases for human diseases: from cancer to infectious diseases.

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Epigenetics deals with mechanisms implied with genes expression and regulation access to genetic information without altering the underlying DNA sequence. This access is controlled by the compaction or decompaction of the chromatin regulated by epigenetic modifications. DNA methylation patterns, post-translational histones modifications, expression profile of microRNAs and nucleosome positioning are some of the epigenetic modifications that modulate gene expression. Among these, DNA methylation and histone post-transcriptional modifications are the most studied ones. It is clear today that the epigenetic regulation is involved in many diseases, such as cancer or infectious diseases, and aberrant epigenetic patterns are found participate to the sick phenotype. These chemical modifications are reversible and, thus, constitute promising therapeutic targets.

Today, it is well established that the enzymes involved in modifying DNA and histone (writers/erasers) and proteins that bind to these modifications (readers) are established anticancer targets. While their application in treating infectious diseases is constrained by a lack of knowledge regarding the epigenetic enzymes involved and the discovery of specific compounds. Here we describe the development of specific chemical tools to target the aberrant DNA and histone methylation in cancer, with the long-term aim of applying them to infectious diseases and fight antimicrobial resistance.

DNA and histone methylation are catalysed by the methyltransferases, DNMT (DNA methyltransferases) and HMT (histone methyltransferases). DNA methylation occurs on the C5 position of the cytosine base in the DNA (5mC), while histones methylation mainly occurs on lysine and arginine, which can be, respectively, mono, bi and tri methylated by histones lysine methyltransferases (HKMTs) or mono, bi (symmetrical/asymmetrical) methylated by histones arginine methyltransferases (PRMTs). All methyltransferases are constituted of two distinct pockets: one for the substrate that will be methylated and one for the binding of the cofactor. These two pockets are linked by a narrow catalytic channel where the methyl group is transferred. Moreover, all methyltransferases are using the same cofactor, *S*-Adenosyl-$_L$-methionine (SAM), as donor of the methyl group. To increase the specificity of inhibitors, instead of targeting one of the pockets, a promising strategy is to target both pockets at the same time by synthesising bisubstrates derivatives formed of SAM and substrate analogues covalently linked together. We will describe here the synthesis of a specific chemical library of bisubstrate inhibitors designed to target histone and DNA methyltransferases and their evaluation in cells.

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SHORT TALK

Metabolism of oxidases and inhibition of NADH peroxidase improve tigecycline activity against *Enterococcus faecium*

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Since the 70s-80s, enterococci and especially *E. faecium* are considered a major concern for public health because of their ability to cause hospital acquired infection (HAI). This multidrug resistant opportunistic pathogen is categorized by the WHO as a high priority for the development of new therapeutic treatments. Tigecycline was approved in 2005 as a new antibiotic treatment against *E. faecium* infections. This semi-synthetic molecule targets the 30S subunit of ribosome and possesses a bacteriostatic activity. Wasselin and collaborators demonstrated a link between activity of tigecycline and oxidative stress in *E. faecium* AUS0004. When the NADH peroxidase (Npr) is deleted, an accumulation of hydrogen peroxide (H_2O_2) is observed and tigecycline becomes lethal in this context. *E. faecium* accumulates high level of H_2O_2 during growth. Two lactate oxidases (Lox1 and Lox2) and one pyruvate oxidase (Pox) are present in this bacterium whose activity in metabolism is classically associated with H_2O_2 production.

Using these results, we want to find way to avoid H_2O_2 degradation or increasing level of metabolism-derived H_2O_2 could be a way to improve tigecycline activity.

The aim of our study was first to investigate the role of these 3 oxidases in the production of hydrogen peroxide, and then to evaluate if increasing their activity can cause an accumulation of H_2O_2 that added to tigecycline's activity could lead to *E. faecium* death. We first determined survival of oxidase's mutants deficient in one or several oxidases after 24h in presence or absence of tigecycline. In parallel, H_2O_2 was quantified. We also semi-quantified acetate and lactate to look at the role of oxidases in metabolism. These experiments allowed us to conclude that Lox1 has an important role in metabolism (degradation of lactate to pyruvate) and production of H_2O_2 compared to Lox2 and Pox.

The second goal was to find a molecule that binds to and inhibits Npr. A preliminary *in silico* molecular docking analysis highlighted several potential inhibitor molecules. We selected fourteen of them according to their binding affinity and tested their inhibitory capabilities *in vitro* by following degradation of NADH (Npr cofactor). Six of these fourteen molecules showed an inhibitor activity on Npr.

These results constitute a promising first step in finding strategies to potentiate tigecycline activity.

Keywords: tigecycline, NADH peroxidase, oxidative stress, metabolism, *Enterococcus faecium*

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An anti-virulence drug targeting the evolvability protein Mfd protects against infections with antimicrobial resistant ESKAPE pathogens

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Antibiotic resistance is a dramatic health challenge and development of new antibiotics efficient
against Gram-negative bacteria of the ESKAPE group is an emergency. Here, we propose the development of effective first-in-class antibiotics to tackle bacterial resistance by acting on a novel bacterial target, the Mutation Frequency Decline protein (Mfd), and by promoting its inhibition by novel therapeutic molecules.

Mfd is a non-essential transcription repair coupling factor conserved in bacteria and absent in eukaryotes. Mfd enables the bacteria to overcome the host defense responses, by conferring resistance to nitric oxide, a major toxic component of the innate immune system. We have identified selective Mfd inhibitors and demonstrated their efficacy against Gram-negative bacteria. We have tested their efficacy against bacteria of the ESKAPE group. We have also tackled their pharmacologically challenging properties and developed optimal nanoparticle formulations to ensure their efficient delivery. The originality of our project from a therapeutic perspective is the development of compounds that, instead of killing the bacteria responsible for the infection, will block their pathogenic pathways. Targeting Mfd's function allows to boost the immune system efficiency and to only focus on bacteria restricted to the inflammation site, thus reducing resistance pressure. This translational project aims to deliver a drug candidate with a solid pre-clinical proof of concept of innocuity to the host and broad range efficacy. As result, a strong therapeutic innovation able to bring an effective healthcare solution to the out-of-control rise of antibiotic resistance will be provided.

Highlight

- NM102 is a "first in class" molecule specifically targeting the active site of the bacterial Mfd protein
- NM102 has a new mode of action: it inhibits Mfd function during immune stress response
- NM102 also inhibits Mfd evolvability function and thereby decreases bacterial resistance to known antibiotics
- NM102 effectively treats Gram-negative infections in animal models
- NM102 is efficient against clinically relevant resistant bacteria and provides an increased efficacy in combination with the b-lactam meropenem

Keywords: Mutation Frequency Decline; new target; high-throughput screening; in silico modeling; antimicrobials; clinical pathogens; immune stress; in vivo efficacy; antimicrobial resistance; antivirulence drug.

Selenium as a versatile player in anti-TB drug synthesis

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Tuberculosis (TB) is an infectious disease caused by the airborne transmission of the bacteria Mycobacterium tuberculosis (Mtb). TB remains a disease of global significance, causing 1.3 million deaths and 10.8 million cases of active disease worldwide (2024). The World Health Organization (WHO) has identified a global case detection gap of 3.1 million patients with undiagnosed cases predominantly occurring in high-TB-burden countries becoming the leading cause of death by infection worldwide[1]. The impact in public health is a reflection of several facts: lack of available drugs with anti-tuberculosis activity; the development of resistance towards the available antibiotics leading to multi- drug resistance; poor tolerability or misuse of the therapeutic regimen and the etiology of the disease, which translates into the alternation of its active and latent states[2].

Some recent strategies against tuberculosis focus on identifying new therapeutic targets and in developing multi-target molecules. Our research group has developed a small library of hybrid compounds with proven anti-tuberculosis activity, specifically targeting Mtb electron transport chain (ETC).

Despite the growing body of research exploring selenium (Se) as an anti-TB strategy, most of studies have focused on its utility in form of seleno-nanoparticles. Se-containing molecules present interesting oxidation-reduction properties that can be relevant in their biological activity. Therefore, exploring bioisosterism strategies, in our project we developed the synthesis of Se-analogs of the antitubercular hybrid compounds, since they may offer a better performance regarding molecular properties and antitubercular activity compared to its chalcogen analogues[3].

In this work, selenium containing heterocycles and other seleno-organic functionalities like selenoamides compounds were synthesised (Figure 1) and characterised by nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry.
Future work will focus on stability studies and anti-tuberculosis assessment, while comparing the activity with the already stablished anti-tuberculosis compounds.

Keywords: Tuberculosis; multi-target; bioisosterism and selenium.

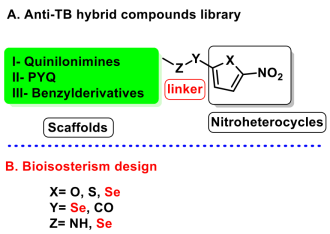

Figure 1. Development of novel seleno-organic structures with possible anti-TB activity.

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Dual AmpC and AmpD Mutations in *Pseudomonas aeruginosa*: Unraveling the Mechanisms of Resistance to Ceftazidime and Avibactam

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Background: Ceftazidime/avibactam (CZA) constitute a combination therapy comprising a beta-lactam (BL) and a beta-lactamase inhibitor (BLI), recognized as an effective therapy for Gram-negative bacterial infections due to multidrug-resistant (MDR) *Pseudomonas aeruginosa (Pa)*. However, resistance to this BL/BLI combination has been commonly reported subsequent to antibiotic treatment. Two isogenic clinical isolates were collected: one susceptible to CZA and ceftolozane/tazobactam and one resistant to both combinations, which exhibited mutation in the chromosomic beta-lactamase *ampC* (AmpC^{G183D}) and its negative regulator *ampD* (AmpD^{H157Y}). Here, we explore the role of each of the two mutations on resistance to ceftazidime (CAZ) and avibactam (AVI).

Methods: Single mutants *ampC* (AmpC^{G183D}) and *ampD* (AmpD^{H157Y}) and a double mutant *ampC* (AmpC^{G183D})/*ampD* (AmpD^{H157Y}) were generated in a PAO1 reference strain as documented in previous article[1] and were used in this study. Minimum inhibitory concentrations (MICs) of CAZ, AVI and CZA were assessed by broth microdilution according to EUCAST recommendation[2]. Colony-forming units (CFU) were monitored under static conditions over time during time-kill curves (TKC) against CAZ and AVI in PAO1 and its mutant, in monotherapy and in combination with variable concentrations of both molecules. Relative expression of the ampC gene was evaluated in each strain using quantitative PCR (qPCR).

Results: qPCR analysis confirms that there is no significant change in *ampC* expression levels in both PAO1 and the AmpC^{G183D} mutant. However, strains harboring the AmpD^{H157Y} mutation displayed overexpression of *ampC*. MICs of the reference and mutant strains are presented in Table 1. For all strains, the MIC of avibactam exceeded 256 mg/L. No difference was observed in CAZ and CZA MIC of AmpC^{G183D} mutant compared to PAO1. The presence of the AmpD^{H157Y} mutation alone resulted in an increase in the MIC of CAZ and a moderate increase in CZA. Elevated MICs for CZA were observed only with concurrent mutations of AmpC^{G183D} and AmpD^{H157Y}. In TKC experiments with varying ratios of both CAZ and AVI, the PAO1 and AmpC^{G183D} mutant displaying basal expression of *ampC,* exhibited similar profiles with an initial decay of CFU followed by regrowth at CAZ MIC (2 mg/L) for all AVI concentrations tested (from 0.125 mg/L to 64 mg/L). The AmpD^{H157Y} mutant, overexpressing *ampC*, showed bacterial decay without regrowth at CAZ MIC (16 mg/L) for weak concentrations of AVI (from 0.5 mg/L). The double mutant, overexpressing a mutated *ampC,* showed a reduction in CFU without regrowth at CAZ MIC (32 mg/L) for medium concentrations of AVI (from 2 mg/L).

Conclusions: Understanding the effects of mutations that confer resistance to the combination of ceftazidime and avibactam combination is challenging. Our results demonstrated that the presence of the AmpC^{G183D} mutation alone is not sufficient to confer resistance to ceftazidime and ceftazidime-avibactam in *Pseudomonas aeruginosa*. Moreover, the inhibitory effect of avibactam is significant only when wild-type *ampC* is overexpressed, whereas it is reduced when mutated *ampC* (AmpC^{G183D}) is overexpressed. A semi-mechanistic PK/PD model is being developed to precisely characterize the respective contributions of these mutations on the efficacy of both CAZ and AVI.

Keywords: *Pseudomonas aeruginosa*, ceftazidime, avibactam, *ampC*, *ampD*

Table 1 : Minimal inhibitory concentrations (mg/L) of PAO1 and mutant-derived strains. EUCAST breakpoint is established at 8 mg/L.

Strain	MIC (mg/L)		
	CAZ	AVI	CZA[a]
PAO1	2	>256	2
PAO1 - AmpC^{G183D}	2	>256	2
PAO1 - AmpD^{H157Y}	16	>256	4
PAO1 - AmpC^{G183D} / AmpD^{H157Y}	32	>256	16

[a] Avibactam concentration is fixed at 4 mg/L.

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Depletion of Lgt, a broadly conserved lipoprotein modification enzyme, leads to reduced cell size and loss of viability in *Helicobacter pylori*.

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Bacterial lipoproteins are key elements for bacterial physiology and virulence and are characterized by fatty acids by which they are anchored into phospholipid membranes. The first step of post-translational modification of lipoproteins is catalyzed by integral membrane enzyme Lgt that adds a diacylglyceryl group from phosphatidylglycerol to the sulfhydryl group of the invariant cysteine in pre-prolipoprotein resulting in prolipoprotein and glycerol-1-posphate. Lgt is conserved in all bacterial species, and essential for growth and viability in proteobacteria including *Helicobacter pylori*[1]. *H. pylori* is a high priority pathogen colonizing the human stomach thereby causing gastritis and gastric ulcers that may lead to the development of stomach cancer. Cell shape plays an important role in the pathogenicity of *H. pylori* and the gastric colonization is facilitated by its characteristic spiral shape, which allows it to move through the mucus layer to reach the epithelium. *H. pylori* undergoes a cell shape transition from spiral rod to coccoid during late stationary phase growth in which peptidoglycan remodeling likely plays an important role. As coccoids, *H. pylori* is able to evade the innate immune system[2]. Little is known about the role of lipoproteins in cell shape transition and pathogenicity in *H. pylori*.

We constructed two Lgt depletion strains in *H. pylori*, in one strain *lgt*is expressed from an inducible promoter encoded on a plasmid and in the second strain *lgt* is expressed from a regulated *cag*UT promoter from a neutral locus on the chromosome[1]. We showed that depletion of Lgt in *H. pylori* leads to growth arrest and reduced viability in both strains. We observed short rod-shape bacteria while DNA and membrane staining patterns were similar to non-depleted cells. Lgt depletion in *E. coli* greatly impacts morphology[3], however no such aberrant cell shape was observed in *H. pylori*. Upon extended growth under Lgt depletion conditions we observed the appearance of revertants that adapted to sustain growth either through genetic or metabolic adaptation. These revertants also transitioned into coccoids. We described a similar phenomenon in *E. coli* upon depletion of Lgt, illustrating the need for Lgt for bacteria to survive. We compared the X-ray crystal structure of Lgt from *E. coli*[4] with an AlphaFold2 model of Lgt of *H. pylori* and showed that they are very similar despite their sequences being only 20% identical. Furthermore, we showed by complementation experiments that Lgt of*H. pylori* is functional in *E. coli*. We are currently performing cross-complementation analysis with Lgt of *E. coli* in *H. pylori* and are determining the basis of the revertant phenotypes. Our studies will contribute to understanding functional conservation of Lgt in bacteria and the impact of lipoprotein modification by Lgt on physiology and pathogenicity of *H. pylori*.

Keywords: *Helicobacter pylori*, lipoprotein, Lgt, target, cell wall

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Phenotypical and molecular characterization of antimicrobial resistance in *Klebsiella* spp. isolates from intensive care units (ICUs) in Porto Velho, Rondônia, Brazilian Amazon.

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Healthcare-related infections (HAIs) are considered the adverse event that frequently affects patient safety, with a significantly higher risk in Intensive Care Units (ICUs). Among the pathogens isolated is *Klebsiella* spp., a Gram-negative bacterium that has developed resistance to antimicrobials through various mechanisms. The aim of this study was to perform phenotypic and molecular characterization of antimicrobial resistance in isolates of *Klebsiella* spp. from clinical samples of patients, healthcare professionals and ICU hospital surfaces in Porto Velho, Rondônia, Brazil. The samples were collected from December 2017 to February 2018, December 2018 to January 2019 and November 2020 in the ICUs of three public hospitals in Porto Velho. Bacterial identification was done by PCR and sequencing of the 16S (rRNA) gene. The antimicrobial susceptibility profile was assessed by disk diffusion and broth microdilution. Resistance genes were tested by conventional PCR. Genetic polymorphisms were analyzed by pulsed-field agarose gel electrophoresis (PFGE) and phylogenetic analysis by MLST (Multilocus Sequencing Typing). In both cases, multidrug-resistant (MDR) *K. pneumoniae* isolates positive for $bla_{\text{CTX-M}}$ were selected. Of the total of 1,511 bacteria, 14.9% (226/1,511) were identified as *Klebsiella* spp., of which 62.4% (141/226) were obtained from patient samples, 23.9% (54/226) from healthcare professionals and 13.7% (31/226) from hospital surfaces. *K. pneumoniae* was the species predominantly found in samples from patients (78%) and hospital surfaces (90.3%) and *K. aerogenes* in intensive care professionals (38.9%). The collection sites with the highest frequency of bacterial isolation included axillae (43.3%) and the oral cavity (42.6%) in clinical specimens from hospitalized patients; the nasal cavity (70.4%) in samples from healthcare professionals; and beds (54.8%) and mechanical ventilation devices (19.4%) on hospital surfaces. *Klebsiella* spp. obtained from hospitalized patients and hospital surfaces showed high percentages of resistance (>50%) to the antibiotics cefuroxime, cefotaxime, ceftriaxone, ciprofloxacin and aztreonam and a high MDR rate. Bacteria from healthcare workers showed low percentages of resistance. Positivity for ESBL-encoding genes was 95%, 75% and 70% for bla_{TEM}, bla_{SHV} and $bla_{\text{CTX-M}}$, respectively. There was no evidence of the presence of the bla_{NDM}, $bla_{\text{OXA-48}}$, bla_{IMP}, bla_{VIIM}, bla_{GES}, bla_{SPM} e *mcr-1* genes, with amplification only for bla_{KPC} in 17% of the samples. PFGE identified 33 clonal groups (A-G1), with groups F and G having the highest number of isolates. MLST revealed the occurrence of 18 Sequence Types (ST), with ST629 being the most prevalent (32.1%), followed by ST11 (12.1%), ST15 (7.1%), ST307(7.1%) and ST101 (7.1%). Four new STs were determined (ST6840, ST6841, ST6842 and ST6843). The PFGE and MLST results demonstrated the clonal spread of MDR bacteria between patients, between patients and intensive care professionals, between patients and hospital surfaces, and even between hospitals, indicating the cross-transmission of *K. pneumoniae* in ICUs. These findings emphasize the need to adopt measures to prevent the spread of these microorganisms, including rigorous hygiene practices and infection control protocols, to prevent the emergence of infections that may become untreatable, given the low availability of new antibiotics.

Keywords: Antimicrobial Resistance; *Klebsiella* spp.; β-lactamases; Intensive Care Units.

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SHORT TALK

Understanding antibiotic resistance transmission within and between humans in *Klebsiella pneumonia* and *Escherichia coli* – a theoretical modelling study

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The rise of multidrug resistance in bacteria has become a critical public health concern, particularly within healthcare settings. By precisely formalizing the mechanisms and factors involved, modelling can help understanding the processes underlying resistance emergence, selection, and transmission across multiple scales between and within-individual. Here, we use mathematical modelling to explore resistance transmission mechanisms in Enterobacterales in healthcare environments.

A mechanistic and multi-scale model was developed to formalize bacterial transmission, plasmid exchange and gene dynamic between and within individuals in a hospital ward. Clones and resistance genes are modelled individually and can be carried and transmitted by humans (patients or staff) simultaneously. Inter-individual transmissions occur through contacts and account for bacterial population bottlenecks. The model also considers within-individual diversity and evolution of bacterial population, enabling horizontal plasmid transfer between strains, gene transfer between plasmids and gene integration to chromosome. Assuming a typical hospital ward, with patients under antibiotic treatment, we explore the joint dynamics of E. coli and a K. pneumoniae strains together with plasmids carrying resistance genes through a simulation study. The aim is to assess the impact of within-individual mechanisms included in the model on the global dynamics and epidemiology in the ward.

Simulations were run assuming different sets of theoretical parameters for each scenario of within-individual mechanisms combination. In case of bacteria with low transmission rate, for a general case for E. coli, horizontal transfer from other species such as K. pneumoniae canrepresent an important factor of maintained prevalence in hospitals. Our modelling results also highlight important new parameters of epidemic success, such as the bacterial population bottleneck at transmission, in addition to more classical epidemiological parameters defining R0, the clearance and transmission rates.

This novel multi-scale model, by integrating more precise evolutionary and ecological phenomena involved in resistance, provides new opportunities to analyse data collected in hospitals and explore mechanisms at the origin of resistance acquisition in healthcare settings.

Keywords: Mathematical model; Hospital; Transmission; Gene; Resistance

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Synthesis and optimization of novel peptidomimetics derivatives inhibiting the efflux pumps of *Pseudomonas aeruginosa*

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The rise of multidrug-resistant *Pseudomonas aeruginosa* (PA) is a critical challenge in the treatment of cystic fibrosis (CF), as this pathogen is a leading cause of morbidity and mortality in CF patients. PA's resistance is often attributed to its efficient efflux pumps, particularly the MexAB-OprM system, which expels antibiotics before they can act. Addressing this mechanism is crucial to overcome treatment failures and to improve patient outcomes.

In this study, funded by the French association "Vaincre la Mucoviscidose," we present an innovative approach aimed at inhibiting PA's efflux pump system. We focus on the synthesis of novel peptidomimetics designed to block the MexB transporter, a key player in the recognition and expulsion of antibiotics. Unlike traditional inhibitors, our peptidomimetics are built on a newly developed scaffold that could enhance their affinity and specificity for MexB, potentially leading to more effective inhibition of efflux activity. Docking analysis and initial biological test have shown promising results.

This strategy represents an encouraging new avenue in combating antibiotic resistance in CF patients. By targeting the efflux pump mechanism, we hope to restore the efficacy of existing antibiotics and offer a much-needed therapeutic solution for this vulnerable patient group.

Keywords: Multidrug-resistant Pseudomonas aeruginosa, Efflux pump inhibition, peptidomimetics, cystic fibrosis, antibiotic resistance.

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Potentiating azoles against *Candida albicans*

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Candida albicans is a human commensal fungus, which in conditions of reduced host immunity can cause life-threatening infections. The rise of drug resistance to commonly used antifungals underscore the need for the development of novel and effective antifungal therapies. The lifespan of current antimicrobials can be extended by using drug combinations, as has been shown with other anti-infectives. Here, we aimed to develop and characterize a drug combination using novel molecules from the class of 1,4-benzodiazepines (1,4-BZDs) and a highly used antifungal against *C. albicans* strains.

We found that 1,4-BZDs significantly increased the susceptibility to ergosterol-targeting azole drugs and showed synergistic activity in combination. 1,4-BZDs did not affect the growth dynamics of *C. albicans* on their own and appeared fungistatic in combination with fluconazole. The drug combination also inhibited filamentation and biofilm formation, properties that are essential for the pathogenicity of this fungus. Surprisingly, the drug combination did not significantly impact known mechanisms of azole resistance, including changes in the expression of efflux pumps or ergosterol genes. However, transcriptomic and microscopy analyses indicated that the drug combination acts by disturbing lipid homeostasis and vesicular transport. Through screens of barcoded collections of *S. cerevisiae* mutants we found additional evidence of 1,4-BZDs targeting lipid homeostasis.

To determine the potential for using the 1,4-BZDs/fluconazole combination *in vivo*, we also examined the toxicity of the compounds. We found that the drug combination showed minimal toxicity levels, indicating that this class of molecule could be further developed for use in antifungal treatment. Additional studies will be necessary to establish the precise mechanism of azole potentiation by 1,4-BZDs and to optimize these molecules for increased efficacy.

Keywords: Fungal pathogens, drug development, novel therapeutics

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Small molecule antibiotic against *A. baumannii* without cross-resistance and potential new MoA

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The rise of drug-resistant pathogens is a ticking time bomb, threatening to set back decades of progress in global health. In the fight against these superbugs, carbapenem-resistant *Acinetobacter baumannii* emerges as one of the most critical bacteria, as classified by the World Health Organization.

In 2018, CDRI identified the thiourea derivative SRI-12742 as an antibiotic against AB (Chopra et al, Int. J. Antimicrob. Agents (2018) 22–27). The compound's MIC is 4 µg/mL against the MDR AB isolate BAA-1605 and activity for clinical strains was assessed (MICs 4 mg/L to >64 µg/mL). SRI-12742 exhibited concentration-dependent bactericidal activity (1.6 log10 CFU/mL reduction at 10×MIC in 24h), comparable with minocycline. In a murine neutropenic thigh infection model of AB infection, SRI-12742 reduced CFU counts by ca. 0.9 log10 CFU, comparable to polymyxin B. In addition, SRI-12742 synergised with all classes of antibiotics tested.

In a 2020-2023 cooperation, the hit was expanded with approx 150 synthetic derivatives. Highly active derivatives were identified with MICs down to 0.125-0.5 µg/mL against 25 clinical isolates. No cross-resistance has been observed, the target is currently unknown, activities for target ID are ongoing.

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Combining machine learning with high-content imaging to infer ciprofloxacin susceptibility in isolates of *Salmonella Typhimurium*

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Antimicrobial resistance (AMR) is a growing public health crisis that requires innovative solutions. Current susceptibility testing approaches limit our ability to rapidly distinguish between antimicrobial-susceptible and -resistant organisms. *Salmonella* Typhimurium (*S.* Typhimurium) is an enteric pathogen responsible for severe gastrointestinal illness and invasive disease. Despite widespread resistance, ciprofloxacin remains a common treatment for *Salmonella* infections, particularly in lower-resource settings, where the drug is given empirically. Here, we exploit high-content imaging to generate deep phenotyping of *S.* Typhimurium isolates longitudinally exposed to increasing concentrations of ciprofloxacin. We apply machine learning algorithms to the imaging data and demonstrate that individual isolates display distinct growth and morphological characteristics that cluster by time point and susceptibility to ciprofloxacin, which occur independently of ciprofloxacin exposure. Using a further set of *S.* Typhimurium clinical isolates, we find that machine learning classifiers can accurately predict ciprofloxacin susceptibility without exposure to it or any prior knowledge of resistance phenotype. These results demonstrate the principle of using high-content imaging with machine learning algorithms to predict drug susceptibility of clinical bacterial isolates. This technique may be an important tool in understanding the morphological impact of antimicrobials on the bacterial cell to identify drugs with new modes of action.

Keywords: antimicrobial resistance, high-content imaging, machine learning, *Salmonella* Typhimurium, ciprofloxacin

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Addressing antimicrobial resistance in agriculture: Development of antibiotics for animal health

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Antimicrobial resistance (AMR) is a growing threat to global health, driven in part by the overuse of antibiotics in agriculture and aquaculture [1,2]. To address this, our research aims to develop novel antibiotics and combinations that specifically target animal pathogens without contributing to cross-resistance with critical human antibiotics. Our approach seeks to ensure that new antimicrobials are effective, do not confer resistance to major classes of human antibiotics, are safe and importantly are an economically viable alternative for farmers, thus offering a potential sustainable solution to AMR in animal health.

We used high-throughput phenotypic screening methods to identify the most active compounds against key animal pathogens under varied growth conditions (aerobic, microaerophilic and anaerobic environments and different temperatures), focussing on poultry in the first instance. Selected hits were further analyzed for their frequency of resistance development, followed by the fitness cost of the mutants, their stability and cross-resistance potential. Promising lead compounds from this in vitro work are being pursued as potential candidates for bacterial infection prevention/treatment in poultry farming.

Our strategy aligns with global regulatory efforts to reduce the routine use of human antibiotic use in animal agriculture, ensuring new antimicrobials are effective and sustainable solutions for AMR in animal health.

Keywords: antimicrobial resistance, drug discovery and development, drug screening, agriculture.

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The *Salmonella* effector SifA facilitates nutrient access to prevent bacterial dormancy

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Salmonella can invade cells using bacterial effectors, leading to various intracellular niches. Recently, our team developed a novel fluorescent reporter, the *Salmonella* Intracellular Analyzer (SINA), which precisely differentiates *Salmonella* subpopulations at the single-bacterium level. This tool enabled us to identify a novel dormant intracellular *Salmonella* subpopulation within epithelial cells that can survive for up to 7 days in vitro. We hypothesized that the intracellular fate of *Salmonella* is influenced by specific T3SS effectors. To test this, we constructed a collection of mutants with deletions of genes encoding bacterial effectors and analyzed their intracellular lifestyles using the SINA reporter at 6 and 24 hours post-infection (hpi). Our results revealed that the deletion of the T3SS effector SifA significantly increased the dormant subpopulation at later infection time points, making SifA the first identified effector that regulates bacterial dormancy in epithelial cells. SifA is essential for the formation of *Salmonella*-induced filaments (SIFs) within the mature *Salmonella*-containing vacuole (SCV). When infecting HeLa cells with wild-type *Salmonella*, we observed SIFs in cells containing vacuolar *Salmonella*, but not in those harboring dormant bacteria. Moreover, SIFs are important for nutrient supply to the mature SCV; we found that inhibiting SIF formation resulted in a higher percentage of dormant bacteria, similar to the effects of lowering glucose concentration during infection. We also explored the roles of bacterial and host glycolysis pathways, both of which appear necessary for *Salmonella* to avoid dormancy. Together, our results suggest that nutrient access, facilitated by proper regulation of the endocytic pathway, is crucial for *Salmonella* to establish a replicative vacuole and prevent a dormant metabolic state.

Keywords: *Salmonella*, T3SS, SifA, dormancy, epithelial cells

The *Pendulisporaceae* and their potential to produce biologically active natural products

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Natural products and their semi-synthetic derivatives are one of the mainstays of small-molecule drugs in modern medicine, in particular in the field of anti-infectives[1]. Over the past decades, Gram-negative myxobacteria have demonstrated their capability to produce biologically active and chemically diverse natural products[2].

In our efforts to expand the scope of cultivated myxobacteria, we have found the *Pendulisporaceae*, a novel myxobacterial family with a distinct morphology. In addition to this unusual sporulation behaviour, members of the *Pendulisporaceae* have large genomes with up to 60 biosynthetic gene clusters (BGCs). The biosynthetic machinery in the four known representatives of this novel family shows only a small overlap with BGCs from other myxobacteria, highlighting the distinct biosynthetic potential of *Pendulisporaceae*. Investigation of their secondary metabolism resulted in the discovery and purification of several natural product families, two of which stood out due to their biological activities. Representatives of the nonribosomal peptide-polyketide-terpene hybrid myxoquaterines were found to display antifungal, potent antiviral and cytotoxic activities. Furthermore, a new derivative of the sorangicin scaffold with potent activity against multi-drug resistant *Staphylococcus aureus* was isolated from a *Pendulispora* strain[3].

Keywords: natural products, myxobacteria, anti-infectives

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Structure-Guided Rational Approach in the Design of Peptidomimetics Inhibiting Efflux Pumps in *Pseudomonas aeruginosa*.

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Inhibiting efflux pumps in the pathogen *Pseudomonas aeruginosa* is currently one of the most promising strategies to combat multiple antibiotic resistance. My project focuses on developing new peptide inhibitors specifically targeting RND-type efflux pumps, such as MexXY-OprM and MexAB-OprM, which play a crucial role in the resistance of *P. aeruginosa*.

By leveraging our laboratory's combined expertise in peptidomimetic design and structural studies of efflux pumps through X-ray crystallography and electron microscopy, we are adopting a rational approach guided by high-resolution structures to design these inhibitors. These compounds will then be evaluated using biophysical interaction techniques, as well as Minimum Inhibitory Concentration (MIC) tests and efflux assays, to determine their effectiveness in blocking the pumps and restoring antibiotic sensitivity.

Moreover, considering the devastating impact of *Pseudomonas aeruginosa* infections in patients with cystic fibrosis, our work could pave the way for new therapies capable of overcoming antibiotic resistance. In this way, we hope to offer new treatment options and improve the quality of life for these patients.

Keywords: Efflux pumps, Multidrug resistance, Peptidomimetics, Structural biology, Efflux pump inhibitors.

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Antibiotic-phytochemical conjugates: a feasible approach to combat AMR?

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Various types of antibiotics have been identified/developed to treat bacterial infections since Sir Alexander Fleming discovered penicillin in 1928. However, due to overuse and/or misuse of antibiotics in last century, many bacteria have developed resistances. Antimicrobial resistance (AMR) has emerged to be a big threat to human and animal health, promoting people to find new antimicrobial drugs and therapies. Previous studies have shown that co-administration of synergistic antibiotics can delay/prevent AMR development, and many phytochemicals possess potent antimicrobial activity and exhibit synergistic effect with antibiotics. Thus, we initiated a novel approach of developing hybrid antimicrobial compounds by chemically linking synergistic antibiotics and phytochemicals. Our first hybrid compound, sulfamethoxazole-gallic acid (Hybrid 1), exhibited potent activities towards susceptible Streptococcus and Enterococcus stains and a multidrug-resistance E. faecalis clinical isolate. However, it is less effective against other bacterial species. To improve antimicrobial activity and bacterial susceptibility, we designed and synthesized four new conjugates, sulfamethoxazole-eugenol, sulfamethoxazole-protocatechuic acid, sulfamethoxazole-vanillic acid and sulfamethoxazole-caffeic acid. Compared to Hybrid 1, these four conjugates exhibited stronger antimicrobial activities towards all six tested Gram-positive and one tested Gram-negative bacteria, implicating that synthesis of antibiotic-phytochemical conjugates is a promising strategy in developing novel antimicrobials.

Keywords: AMR, combination therapy and antimicrobial conjugate

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