PhD DAY (II)

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Host–Microbiota Crosstalk in Ovarian Cancer

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Tumor-associated microbiota is increasingly recognized as an active component of the cancer microenvironment, yet its functional contribution to ovarian cancer progression and therapeutic response remains poorly defined. In this study, we experimentally investigated bidirectional host-microbe interactions in ovarian cancer and integrated whole-genome sequencing to identify the genetic basis underlying observed phenotypes. We isolated 45 bacterial strains from ovarian cancer and non-malignant ovarian tissues and performed extensive phenotypic characterization, including morphology, motility, biofilm formation, and antibiotic susceptibility testing. Chemotherapeutic drug-response assays showed that standard treatments (carboplatin, paclitaxel, and doxorubicin) reduced viability of ovarian cancer cell lines (SKOV3, OVCAR3, Kuramochi), the non-malignant fallopian tube epithelial model (FT190), and patient-derived ovarian organoids to ≤10–20%. In contrast, the majority of patient-derived bacterial isolates, including *Escherichia coli*, *Staphylococcus hominis, Streptococcus constellatus, Klebsiella michiganensis*, *Lactobacillus paracasei*, and *Lactobacillus zeae*, remained highly tolerant to these agents under both aerobic and anaerobic conditions. To directly assess functional host-microbe crosstalk, we performed soluble-factor interaction assays using bacterial and host-derived supernatants. Cell-free supernatants from all isolates (including tumor-associated, non-malignant tissue-derived, and reference strains) collected during logarithmic and stationary growth phases, induced divergent host responses ranging from approximately 70% growth inhibition to 45% proliferation enhancement, depending on bacterial species and growth state. Together, these findings demonstrate that tumor-associated bacteria engage in dynamic, context-dependent interactions with ovarian cancer cells through drug tolerance and soluble-factor–mediated effects, highlighting the importance of incorporating microbiological assays into ovarian cancer research and therapeutic evaluation.

INVOLVEMENT OF DURA MATER TISSUE IN RODENT MODELS OF PNEUMOCOCCAL MENINGITIS

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Background: Pneumococcal meningitis remains the most common cause of bacterial meningitis in children worldwide. However, the mechanisms underlying its pathogenesis remain poorly understood.
Methods: We investigated the spatial distribution of *S. pneumoniae* during meningitis in rodents and its interactions with vascular endothelium (CD31) and macrophages (CD169) in two distinct in vivo infection models. Using microscopy-based analyses, we quantified and localised bacteria within the brains of infected animals.
Results: Quantitative image analysis showed that, in our hematogenous meningitis model, a substantial proportion of bacteria in the dura mater were extravascular, consistent with early tissue invasion. At later time points, bacterial invasion was instead observed in the pia mater and the choroid plexus. In the intracranial meningitis model, dural macrophages displayed infection-associated morphological changes, and *S. pneumoniae* localized predominantly to macrophages at 18 hpi and to vessels at 42 hpi.
Conclusions: Our findings reveal that differences between the choroid plexus, the pia and dura mater reflect distinct tissue-specific dynamics. Overall, these results highlight the central role of perivascular macrophages in pneumococcal persistence and host–pathogen interactions during meningitis, providing new insights into disease pathogenesis and potential therapeutic targets.

Elucidating key steps of *Pseudomonas aeruginosa* infection by DEV lytic phage for phage therapy.

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Phage therapy is a promising strategy to combat infections caused by antibiotic-resistant bacteria, including *Pseudomonas aeruginosa (Pa*). Understanding the biology of therapeutic phages is essential to ensure their safety and efficacy. I am investigating the adsorption mechanism of DEV, a lytic phage killing *Pa*. DEV gp53 fibers are required to infect Pa strains exposing on their outer membrane (OM) a lipopolysaccharide (LPS) capped with the O-antigen moiety, which is DEV primary receptor, but are dispensable in *galU* mutants lacking the O-antigen, suggesting a secondary receptor and a distinct receptor-binding protein.[1,2] To identify DEV secondary receptor, we isolated a DEV-resistant *galU* double mutant. The mutant carried a 33-bp deletion in *lptD*, an essential gene encoding an OM protein. Complementation assays demonstrated that *lptD* expression restored phage adsorption, suggesting that LptD may be the secondary receptor. In agreement with this hypothesis, the expression of *P. aeruginosa lptD* in *E. coli*, normally not susceptible to DEV, enabled adsorption.To identify LptD receptor binding protein, UV mutagenesis of wt DEV or DEV Δ*gp53* mutant was performed. The phages were plated on the DEV resistant *galU lptD* strain and mutant phages forming plaques were genome sequenced. In both cases, mutations were identified only in the *gp54* gene, identifying gp54 as the LptD-specific receptor-binding protein. In collaboration with G. Cingolani group (UAB, USA), we demonstrated that gp54 forms a complex with gp56 and gp55 that regulates DEV tail unplugging.[3]Given the high conservation of gp54 among *Litunaviruses*, other phages in this genus may also exploit LptD as their receptor.

1) Forti, F., Bertoli, C., Cafora, M., Gilardi, S., Pistocchi, A., & Briani, F. (2023). Identification and impact on Pseudomonas aeruginosa virulence of mutations conferring resistance to a phage cocktail for phage therapy. Microbiology spectrum, 11(6), e0147723. https://doi.org/10.1128/spectrum.01477-23

2) Lokareddy RK, Hou C-FD, Forti F, Iglesias SM, Li F, Pavlenok M, Horner DS, 553 Niederweis M, Briani F, Cingolani G. 2024. Integrative structural analysis of 554 Pseudomonas phage DEV reveals a genome ejection motor. Nat Commun 15:8482.

3) Nieto Noblecia, J., Bellis, N. F., Antichi, C. A., Aminian, S., Forti, F., Falchi, F. A., Sposato, D., Imperi, F., Cingolani, G., & Briani, F. (2026). Pseudomonas aeruginosa DEV phage exploits the essential LptD outer membrane protein as receptor for adsorption. mBio, 17(2), e0356125. https://doi.org/10.1128/mbio.03561-25

From colistin persistence to resistance in carbapenem resistant *Acinetobacter baumannii*: evidence for a novel toxin–antitoxin mRNA–asRNA regulatory mechanism

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Background: Bacterial persistence is a transient, non-heritable state of metabolic dormancy that allows susceptible sub-populations to survive lethal antimicrobial concentrations and represents a critical evolutionary reservoir facilitating the transition towards stable genetic resistance. In high-risk pathogens, such as Carbapenem-Resistant *Acinetobacter baumannii* (CRAB), understanding the molecular mechanisms underlying this transient-persistent state is essential to elucidate how bacteria survive antibiotic exposure. Here, we investigated colistin persistence subpopulations and their Toxin/Antitoxin (T/A) omics in ST2 clinical colistin-susceptible (COL-S) CRAB that subsequently developed full and stable *in-vivo* COL-R.

Methods: High-dose colistin time-kill assays were performed to detect persisters in 10 clinical CRABs. Genomics and basal transcriptomics of chromosomal/plasmid toxin–antitoxin systems (T/As) were conducted in two representative ST2 COL-S CRAB to investigate the genomics and basal T/A transcriptional profiles.

Results: All strains showed a persistent subpopulation (~1% survival at 8h) under 5X COL-MIC exposure. Genomic analysis identified ten type-II and one type-IV T/A systems. Basal transcriptomic profiling revealed active expression patterns mainly involving GNAT superfamily T/A modules, with consistently low toxin mRNA levels associated with toxin- or antitoxin-directed antisense RNAs (asRNAs) in chromosomal loci. This regulatory architecture supports novel dual-combined models in which asRNAs act as primary modulators of T/A transcript balance, potentially influencing persistence-associated dormancy transitions mainly via a translational-termination mechanism. Conversely, the plasmid-encoded BrnT/A module showed a highly balanced expression profile.

Conclusion: Our findings highlighted the type-II GNAT T/A superfamily as molecular switchers of T/A transcript-balance by a novel asRNA–mediated regulatory mechanism in high-risk developing colistin persistence and resistance CRABs.

Decoding Drug Efficacy and Host–Pathogen Interactions in Tuberculosis Using a Standardized *In Vitro* Granuloma-Like Structures Model

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Tuberculosis remains a major global health challenge, largely due to the ability of *Mycobacterium tuberculosis* (*Mtb*) to evade immune responses and persist within granulomatous lesions. Replicating these complex host–pathogen interactions *in vitro* remain difficult, as widely used models fail to capture the multicellular organization and dynamic microenvironment of granulomas, ultimately limiting drug development.

In this study, we developed and refined a human PBMC-derived Granuloma-Like Structure (GLS) model and directly compared it with conventional macrophage monolayers to evaluate drug activity in a more representative setting. A panel of 11 antitubercular compounds was tested across a range of concentrations and experimental replicates. We also investigated drug combinations mimicking the clinically used BPaL regimen within the GLS system.

To improve robustness, we integrated CFU-based bacterial quantification with imaging-driven analysis of GLS formation and evolution, combining brightfield and time-lapse microscopy. This approach, supported by a hybrid human- and AI-assisted pipeline, enabled quantitative assessment of GLS size and number, highlighting how similar CFU values may arise from structurally distinct granuloma populations. Consistently, drug efficacy varied with GLS size distribution, a relationship further supported by a simple mathematical framework and *in silico* modeling.

Comparison with monolayer cultures revealed distinct drug response profiles, with GLS better capturing immune-related effects. Interestingly, early GLS conditions appeared to favor bacterial expansion. Altogether, this work supports GLS as a reproducible and scalable platform for studying tuberculosis and improving preclinical drug evaluation.

Linking air and leaves: disentangling the main drivers of microbial assembly and exchange in the urban phyllosphere

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Urban vegetation is known to support diverse microbial communities that play key roles in ecosystem functioning. However, the mechanisms governing their assembly and connectivity with the atmosphere remain incompletely understood. The present study investigates the relative contributions of different ecological drivers in shaping bacterial and fungal communities of the phyllosphere and their linkage with airborne microbiomes across an urban gradient in Milan (Italy). Leaves from four common plant species and particulate matter were sampled on a seasonal basis over one year, and microbial communities were characterised through 16S rRNA and ITS1 amplicon sequencing.

Multivariate analyses revealed that plant species, season, and sampling area (and their combinatory effects) are important drivers in shaping the microbial communities. Network-based indicator species analysis demonstrated substantial connectivity between air and phyllosphere microbiomes, with numerous taxa shared across habitats and plant species, suggesting active microbial exchange. This connectivity was particularly evident during transitional seasons, when overlap between airborne and leaf-associated communities changed actively.

Seasonal transition analyses further demonstrated dynamic shifts in microbial distribution, with alternating patterns of homogenization and diversification between habitats. Notably, bacterial communities exhibited stronger dispersal from shared to phyllosphere-specific assemblages, whereas fungal communities displayed different temporal dynamics.

The findings demonstrate that the composition of urban phyllosphere microbiomes is influenced by a complex interplay of deterministic host filtering and stochastic airborne dispersal. These have significant implications for our understanding of microbial contributions to ecosystem services in urban environments, thereby influencing the decisions of policy-makers and local authorities in the development of greener cities.