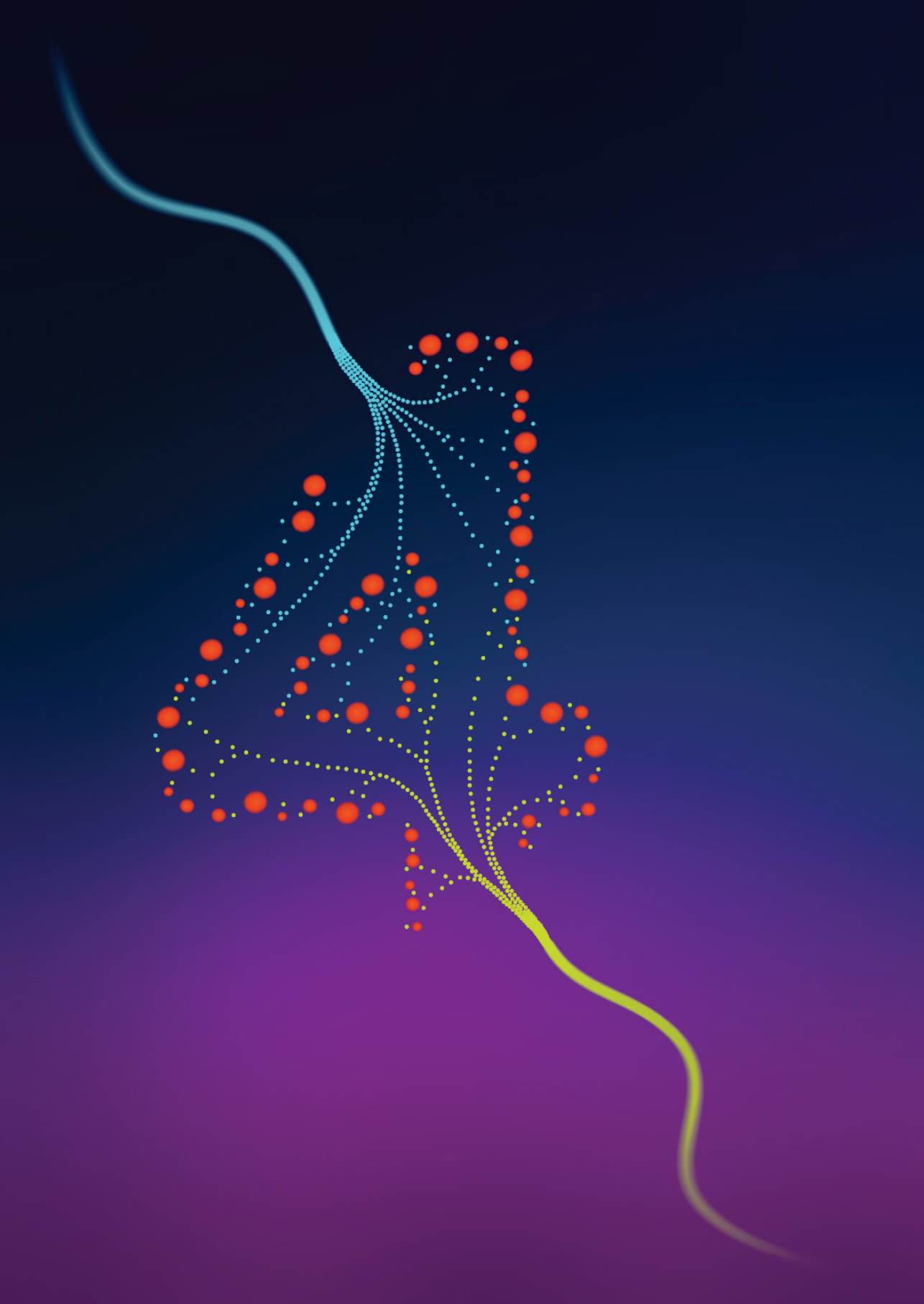
How nutrients shape antibiotic sensitivity of Pseudomonas aeruginosa: food for thought

Kok, M.



Section IV

Discussion and summary

How nutrients shape antibiotic sensitivity in *Pseudomonas aeruginosa*

Food for thought

Chapter 7

General discussion, future perspectives, and and overall conclusion

7.1. Summary and general discussion

Pseudomonas aeruginosa is classified by the World Health Organization as a top-priority pathogen, owing to its capacity for severe infections and its resistance to many antimicrobials. This threat is particularly acute for immunocompromised patients, such as those with cystic fibrosis (CF), who often acquire chronic P. aeruginosa infections early in life. Prolonged antimicrobial exposure in these infections drives the development of multidrug-resistant strains. To combat this rise in resistance, it is increasingly recognized that understanding how P. aeruginosa survives within the CF lung is crucial. In this context, a key element lies in the infectious microenvironment, which shapes bacterial physiology and influences antibiotic efficacy1. In an effort to capture the influence of the infectious microenvironment, recent studies have employed innovative in vitro culture media to better replicate the infectious phenotype. However, the diverse microenvironments in the CF lung, coupled with the biological versatility of P. aeruginosa, call for a more comprehensive approach to elucidate its triangular relationship with antibiotic pharmacodynamics (PD). This thesis hypothesized that nutrient conditions in the environment play a central role as pharmacodynamic drivers and key determinants in the evolution of antibiotic resistance, ultimately affecting therapeutic outcomes. To address this, foundational work was conducted to elucidate how altered nutrient and oxygen conditions influenced the antibiotic pharmacodynamics of P. aeruginosa.

In Section I, a comprehensive overview of the diverse microenvironments within the CF lung was provided and the ways in which *P. aeruginosa* adapts to the available nutrients were described (**Chapter 2**). This thesis highlights that these microenvironments can vary among patients and even within the same lung, potentially affecting *P. aeruginosa* adaptation in distinct ways. To explore this in more detail in Section II, the impact of specific nutrients (**Chapter 3**) and oxygen gradients (**Chapter 4**) relevant to the CF lung on antibiotic sensitivity was investigated. In Section III, the role of microenvironmental interactions in driving evolutionary processes by influencing the selection of mutants was examined. It was discussed how exploiting metabolic adaptations during resistance evolution may offer therapeutic advantages (**Chapter 5**), and *P.*

aeruginosa adaptation during antibiotic resistance development was reported under single-nutrient conditions (**Chapter 6**).

Adaptation to heterogeneous cystic fibrosis lung

In **Chapter 2**, the nutrient environments present in the CF lung were reviewed for its role in shaping the metabolic adaptation of *P. aeruginosa*, as these adaptations play a key role in the failure of antibiotic therapies. The bacterial metabolic state determines bacterial growth, redox balance, and energy levels, all factors that influence antibiotic susceptibility. In this context, the thick, dehydrated mucus in the CF lung creates diverse microenvironments characterized by nutrient-rich but oxygen-limited conditions. These gradients support the formation of biofilm aggregates by *P. aeruginosa*, which further compartmentalize nutrients and oxygen, shaping metabolic heterogeneity. The metabolic versatility of *P. aeruginosa* is central to its survival and adaptation within the CF lung environments. *P. aeruginosa* can utilize a wide range of substrates, including amino acids and short-chain fatty acids, and flexibly switch between aerobic respiration, fermentation, and denitrification to maintain energy production under varying conditions[2].

Metabolic adaptations strongly influence antibiotic sensitivity. Antibiotics such as aminoglycosides, fluoroquinolones, β-lactams, and polymyxins all depend on specific metabolic states for their effectiveness[3]. *P. aeruginosa* can rewire its metabolism by suppressing respiration, activating metabolic shunts, or producing protective extracellular matrix components, thereby reducing the impact of these drugs.

Importantly, the CF lung microenvironment is not uniform. Intra- and inter-patient variability shaped by factors like lung compartmentalization[4], CF genotype[5], comorbidities[6] leads to significant differences in nutrient availability and thus in antibiotic response. Understanding these metabolic responses in physiologically relevant contexts is a critical step toward more effective, personalized antibiotic interventions. Progress depends on integrating clinical insights with advanced *in vitro* models and multi-omics approaches to accurately reflect the complex CF lung environment[7]. Overall, this chapter outlined the current understanding on this topic and identified important gaps to guide future research.

Nutrients shape antibiotic treatment response

Nutrients and oxygen are among the most evident drivers of metabolic processes, which have increasingly been linked to the mechanisms of action of multiple antibiotic classes[8,9]. As a result, reduced antibiotic sensitivity is often associated with nutrient-scarce or oxygen-deprived environments, and supplementation is sometimes used to sensitize pathogens[10]. Although these observations highlight the regulatory role of the nutrient environment in shaping microbial responses to antibiotics, the impact of specific conditions relevant to the CF lung remains poorly understood.

In **Chapter 3**, the impact of specific nutrients antibiotic sensitivity was investigated. To this end, a basal culture medium was modified by alternately adding individual nutrients, and changes in antibiotic response were measured using time-kill assays with a bioluminescent *P. aeruginosa* strain, allowing real-time monitoring of bacterial population dynamics.

Using mathematical PD modeling, changes in antibiotic responses across different nutrient conditions were assessed. By focusing on quantification of changes in the half-maximal effective concentration (EC_{50}), nutrient-induced, antibiotic class-specific changes in antibiotic PD were revealed, indicating distinct underlying biochemical mechanisms. This finding expands beyond the traditional view of reduced metabolic activity in nutrient-poor conditions typically correlated with antibiotic tolerance or persistence mechanisms[3]. The absence of significant fitness differences among bacterial populations in our media formulations further suggests that biochemical, rather than purely growth-rate dependent adaptations, underly the observed sensitivity shifts. This aligns with a previous mathematical model, showing a stronger correlation between antibiotic lethality and metabolic states rather than growth rates alone[11].

Investigating the impact of single-nutrient alterations provides mechanistic clarity by establishing a direct relationship between specific metabolic pathways and antibiotic responses. These single-nutrient effects were shown to have clinical relevance, as illustrated by our in vitro pharmacokinetic-pharmacodynamic (PK-PD) simulations showing nutrient-induced changes in treatment outcomes. Previous studies have similarly shown the potential for leveraging nutrient-based findings to identify adjuvant therapies, combining

antibiotics with nutrient modulation to enhance bacterial killing10. While it is important to acknowledge the inherent limitations of our in vitro models, since antibiotic responses in clinical environments involve a broader and more complex range of interactive factors, our results nonetheless represent meaningful progress. Specifically, these findings advance our understanding of the personalized nature of treating P. aeruginosa infections, which are heavily influenced by microenvironmental interactions.

In addition to nutrient availability, oxygen levels represent another key environmental factor that is often overlooked in conventional in vitro antimicrobial activity assays. In **Chapter 4**, the specific impact of oxygen availability on antibiotic responses in P. aeruginosa was investigated. Instead of focusing solely on acute responses to anoxia, the study also assessed how prolonged anoxic exposure (<1% oxygen for 22 days) impacts antibiotic sensitivity. To this end, time-kill assays were performed, comparing a laboratory strain of P. aeruginosa to lineages adapted through prolonged growth under anoxic conditions.

The results indicated that antibiotic effects under anoxic conditions are antibiotic-specific, consistent with known differences in oxygen dependency among antibiotic mechanisms of action reported previously[12–14]. The antibiotic sensitivity profiles changed profoundly following prolonged anoxic conditioning, suggesting sustained modifications in cellular processes related to antibiotic sensitivity. Importantly, these differences sustained when anoxically adapted strains were subsequently cultured under atmospheric conditions, underscoring stable physiological adaptations.

The distinct antibiotic sensitivity profiles observed following anoxic adaptation highlight the potential limitations of standard *in vitro* antimicrobial activity assays, which do not accurately replicate conditions of anoxic infection sites. This emphasizes the urgent need to develop culture conditions that better mimic *in vivo* infection environments. Additionally, the findings suggest that brief exposure to clinically relevant conditions is insufficient. Instead, a comprehensive characterization encompassing multiple, sustained environmental conditions is required to reflect the true complexity of clinical infections. Although this study initially focused on conditions relevant to cystic fibrosis (CF), similar considerations likely apply to other *P. aeruginosa* infection

sites, such as urinary tract infections[15], mucus plugs in chronic obstructive pulmonary disease (COPD) or bronchiectasis[16].

Overall, the findings in Section II highlight the critical role of metabolic regulation in determining antibiotic sensitivity of *P. aeruginosa* under diverse nutrient and oxygen conditions. Nutrients and oxygen form the foundational components for cellular biosynthesis and energy metabolism. Thus, their influence on antibiotic effectiveness might logically follow, since antibiotics often target actively dividing cells. However, the results demonstrate that antibiotic sensitivity can be substantially altered by even single-nutrient changes, with specific outcomes dependent on the precise combination of nutrient conditions and antibiotic class.

The observations across various antibiotic classes support the concept that increased nutrient availability does not necessarily translate into enhanced antibiotic efficacy. Research on the metabolic-targeting natural product promysalin, which can exhibit both synergistic and antagonistic effects when combined with standard antibiotics in *P. aeruginosa*[17], underscores that nutrient metabolism can activate protective cellular pathways that counteract antibiotic actions. This indicates that nutrient metabolism can indeed trigger protective cellular pathways dependent on the antibiotic pressure[18]. It is also important to acknowledge that *P. aeruginosa* harbors an efficient hierarchical nutrient-utilization regulatory systems in nutrient-rich conditions, which means that combining separate nutrient media formulations will likely not produce additive antibiotic sensitivity effects. For example, *P. aeruginosa* can produce redox-active metabolites that suppress respiration and induce a low-energy cellular state even in oxygen-rich conditions[19]. These nutrient-induced metabolic changes result in phenotypic adapted strains over long-term colonization of the CF lung[20]. The findings in this section demonstrated that these adaptation processes profoundly impact antibiotic sensitivity profiles. Such insights underscore the importance of elucidating the biochemical basis of nutrient-driven antibiotic sensitivity and highlight the need for integrative, phenotype-focused -omics approaches. Metabolomics and proteomics are powerful techniques that can capture subtle shifts in intracellular metabolite concentrations, enzyme levels, and metabolic flux patterns, thereby providing a high-resolution view of the metabolic reconfigurations during antibiotic treatment[21,22].

If we are to adopt a more biochemically informed approach, it is crucial that future studies be conducted in environments representative of both the antibiotic's mechanism of action and the conditions of actual infections. In addition to the incremental approach of distinguishing the effects of individual nutrients, detailed investigation into the nutrient composition of the infectious environment is essential. The infectious microenvironment is dynamically shaped by interactions with host cells and microbial communities, generating metabolic byproducts and oxidative stress that modulate bacterial metabolism. Consequently, the use of co-culture systems that model host-pathogen[23] or polymicrobial interactions[24] is essential for fully capturing the intricate interplay between nutrient environments and antibiotic sensitivity. By building on this experimental data, future research can more accurately account for the complex nutritional and metabolic landscapes which *P. aeruginosa* exploits to influence antibiotic susceptibility.

Nutrients shape antibiotic resistance evolution

It is well established that phenotypic changes play a central role in the adaptive capacity of populations, driving natural selection. In this context, populations that adapt to environmental perturbations with higher fitness levels come to dominate. Under antibiotic pressure, heritable changes, such as chromosomal mutations that confer resistance through target modifications, reduced uptake, or increased active efflux, rapidly prevail. These changes are often integral to essential cellular mechanisms and, consequently, frequently incur a fitness cost. Beyond the fact that alterations in fitness and resistance mechanisms induce metabolic changes, it is increasingly recognized that mutations affecting metabolism represent a key category of antibiotic resistance mechanisms[25].

In **Chapter 5**, metabolic adaptations during antibiotic resistance evolution were reviewed, and it was discussed how metabolomics can be applied to understand and exploit these adaptations. Although metabolomics is the comprehensive study of all small molecules in a biological sample, there is no single analytical method that can measure them all due to their extensive physicochemical diversity. Therefore, effective metabolomics studies require thoughtful selection of analytical approaches and sample preparation methods that are tailored to the specific biological questions at hand. For example, the role of metabolic quenching was discussed to counter the high turnover rate of energy

metabolism; apolar extraction procedures were highlighted for retrieving membrane profiles for studying cell envelope changes; and spatially oriented ionization techniques were noted for their utility in examining cell-to-cell communication within biofilm structures. Although 23 metabolomics-focused papers were included in the review, these studies were distributed across various bacterial species and antibiotic classes. It can therefore be concluded that a more systematic application of metabolomics is needed to further elucidate the role of metabolism in antimicrobial resistance mechanisms.

In **Chapter 6**, the role of metabolism in antibiotic resistance evolution was further explored by challenging the metabolic versatility of *P. aeruginosa* through antibiotic adaptive laboratory evolution experiments conducted under single-nutrient conditions. This work was built on the concept that the metabolic constraints imposed by antibiotic resistance mutations, together with the regulatory influence of nutrient supply, shape the evolutionary response[26,27]. Phenotypic and genotypic changes in *P. aeruginosa* were investigated following a 10 day evolution period under antibiotic pressure, using high concentrations of arginine, glucose, glutamate, and lactate as single-nutrient conditions.

The evolved lineages displayed significant differences in minimal inhibitory concentrations (MIC) across the single-nutrient evolution conditions. Earlier work also reported nutrient-dependent MIC shifts in *P. aeruginosa*, but primarily when comparing nutrient-rich with nutrient-poor media[28]. Although that study found only modest MIC divergence, it still identified medium-specific resistance mutations. In our experiment, mutations unique to specific evolution conditions were also detected, but only six across the 15 distinct conditions that were sequenced. An additional partial least-squares discriminant analysis (PLS-DA) revealed a small set of mutational patterns that reliably distinguished our evolution conditions. The narrow range of mutational change parallels the almost identical post-evolution growth-rate shifts observed within each antibiotic class. This limited fitness variation steers the populations toward a restricted set of genotypes[29].

These findings demonstrated that even in single-nutrient media *P. aeruginosa* can evolve along multiple trajectories. However, this diversity manifests primarily as shifts in MIC rather than as distinct mutational signatures. Because *P. aeruginosa* can persist in the CF lung for years, the phenotypic changes observed may eventually solidify into stable genotypic differences. Continuous,

automated *in vitro* systems are well suited to study such long-term evolution[30], but they are technically demanding, time-consuming, and resource intensive. A more practical first step would be to investigate in more detail how infection-relevant nutrient conditions shape antibiotic resistance development, bearing in mind that altering even a single nutrient can tip the evolutionary balance.

In summary, Section III highlighted that metabolic processes play a crucial role in antibiotic resistance development and demonstrated that, despite its metabolic versatility, nutrients exert a regulatory influence on *P. aeruginosa* resistance development. Although our findings delve into how the nutrient environment contributes to the complex evolutionary trajectories observed in the CF lung, they represent only a small piece of the overall picture, and further research in this area remains essential. Nevertheless, the results indicate that the influence of the nutrient environment is antibiotic-dependent, underscoring the need for more in-depth investigation of the biochemical responses and specialization processes that occur during antibiotic exposure.

7.2. Future perspectives

Antibiotics are indispensable for everyday healthcare, yet immunocompromised patients bear a disproportionate burden of infectious disease and also serve as a reservoir for antibiotic-resistant strains. Examining infections in these individuals provides an unique window into the various ways antibiotic therapies can fail. The CF lung represents such an unique environment, where a typically commensal bacterium like *P. aeruginosa* becomes one of the most challenging infections to treat. Understanding the mechanisms that make the combination of the CF lung and *P. aeruginosa* so burdensome may also offer critical insights into other bacterial infections. A key factor shaping *P. aeruginosa* infection in the CF lung is the unique nutrient environment, yet this aspect is often overlooked. The nutrient environment can profoundly influence bacterial behavior and can lead to misinterpretation of antibiotic susceptibility testing performed using standard culture media. Therefore, in the quest to better understand the *P. aeruginosa* pathophysiology, it is essential to focus on: I) mapping physiologically relevant infection sites, II) examining the influence of nutrients on antibiotic

susceptibility testing, III) working on capturing nutrient-induced antibiotic sensitivity changes, and IV) elucidating the underlying biological mechanisms responsible for the antibiotic effect changes.

Mapping of physiologically relevant infection sites

The respiratory tract is a spatially organized system comprising diverse microenvironments, each of which may support distinct infection dynamics[31]. The findings presented in this thesis, demonstrating the strong influence of nutrient availability on antibiotic pharmacodynamics, underscore the critical need to improve our understanding of the chemical composition of these distinct niches. Accurate mapping of these environments is therefore an essential next step in this line of research.

It is crucial to recognize that not all samples taken from the CF lung are created equal and each sample carries inherent biases and limitations that influence interpretation. For instance, expectorated sputum is the most accessible and commonly used sample type, but it represents a heterogeneous blend of secretions from multiple regions of the lung. This pooling of distinct microenvironments reduces spatial resolution and contributes to a substantial disconnect between the chemical profiles observed in sputum and those derived from *in vitro P. aeruginosa* cultures[32]. Sampling epithelial lining fluid via microsamplers offers more region-specific insights but is limited by its invasiveness and the need for saline instillation, which dilutes the sample and complicates direct metabolite comparisons across patients or timepoints[33,34]. Another layer of complexity arises from the heterogeneous biofilms. Spatially resolved analytical techniques have revealed intricate metabolic structuring within biofilms, including mathematical models informed by -omics data that demonstrate their metabolic heterogeneity[35].

The field of oncology has pioneered efforts to address such complex biological heterogeneity through network-based frameworks. Over the past decade, researchers have begun constructing multiscale “tumor atlases” that integrate cellular phenotypes, bulk -omic alterations, and interactions with the tumor microenvironment[36]. These atlases are built by combining conventional pathology with spatial biology and multi-omic datasets, offering a comprehensive view of tumor evolution shaped by local conditions[37]. Beyond advancing

fundamental understanding, these tools now support stratification of patient populations and guide personalized treatment strategies.

Inspired by these advances in oncology, future efforts to characterize the infectious microenvironment of the CF lung must similarly embrace integrative, spatially resolved frameworks. This means not only aligning molecular, microbial, and pathological data layers, but also actively distinguishing between different CF patient phenotypes. Stratifying infections based on microenvironmental features will be essential for translating nutrient-induced pharmacodynamic effects into meaningful, individualized treatment decisions.

In vitro nutrient composition in antimicrobial activity assays

While our understanding of the spatial and chemical complexity of the infectious environment advances, accurately measuring its clinical impact requires that *in vitro* antimicrobial activity assays be adapted accordingly. The physicochemical composition of the culture medium represents a critical and tractable factor for more accurately mimicking infectious conditions[38]. Prior studies have shown that medium composition can significantly alter antibiotic activity[39], and findings presented in **Chapter 3** demonstrate that even a single nutrient modification can markedly shift antibiotic sensitivity. Combined with the environmental heterogeneity outlined previously, these results underscore the need to adapt *in vitro* antimicrobial assays to infection-specific microenvironments to better predict clinical outcomes and guide therapy.

Nutrient conditions are also likely to influence other *in vitro* antimicrobial activity assays, including combination antibiotic testing and biofilm susceptibility testing. For instance, the synergy of antibiotic combinations depends on drug-specific mechanisms of action[40], which can be sensitive to medium composition[41]. In biofilm models, nutrients critically impact maturation. In the CF lung, *P. aeruginosa* grows in aggregates that can be mimicked using alginate beads, but mature biofilms represented by full bead coverage require supplementation with alternative electron acceptors[42]. The reduced antibiotic susceptibility of biofilm populations are closely linked to the metabolic adaptations associated with their heterogeneous structure[43]. Incorporating infection-relevant nutrient environments into these alternative *in vitro* assays may be essential to overcome the inconsistent clinical outcomes they currently produce[44,45].

Completely changing the *in vitro* experimental methodologies for clinical antimicrobial susceptibility testing (AST) is challenging, as current standardized protocols ensure interlaboratory comparability and are far less time-consuming than alternative approaches. Another potential step in improving AST is refining isolate selection by extracting bacterial populations from different lung regions of the same patient, as regional adaptations may significantly influence antibiotic susceptibility[46]. For example, **Chapter 4** shows that oxygen levels affect antibiotic sensitivity, highlighting how microenvironmental specialization drives distinct colony phenotypes. A well-known CF-relevant subpopulation, small colony variants (SCVs), can survive otherwise lethal antibiotic concentrations but are often underrepresented in conventional AST due to their difficult-to-culture nature[47]. Moreover, as demonstrated in **Chapter 4**, specialization to anoxic conditions can lead to different treatment outcomes during aerobic *in vitro* experiments. To accurately assess antibiotic susceptibility in such specialized populations, AST must replicate the infection conditions under which isolates evolved. In this context, incorporating anaerobic AST may be particularly valuable for chronic *P. aeruginosa* infections.

In summary, accurately predicting antibiotic efficacy in the heterogeneous CF lung environment will require antimicrobial assays that incorporate relevant nutrient conditions, assay parameters, and isolate selection.

Nutrient induced changes to antibiotic pharmacodynamics

To map how nutrient availability modulates antibiotic PD, this thesis moved beyond static MIC testing and applied dynamic time-kill assays combined with mathematical modeling. Although MIC values remain the clinical standard, they cannot resolve the differences in growth and kill kinetics that emerge immediately after antibiotic addition. Recording full time-kill curves in different media conditions and fitting them with PD models revealed that identical MICs can mask substantial variation in growth and kill rates[48]. These lessons underline the need to complement standard AST with kinetic assays that explicitly incorporate nutrient context.

Both the nutrient and oxygen environments exert a significant influence on these growth and killing dynamics, as they are often associated with slow growth, tolerance, and persistence effects. In **Chapter 3**, a sigmoidal concentration–effect model was employed to more accurately describe the

antibiotic PD changes under varying nutrient conditions. Although this approach enabled the study of a wide range of antibiotic–nutrient combinations, it also presents clear disadvantages when moving towards more antibiotic-specific responses. The response data used to design our one-compartment PD model were obtained from a static time-kill assay, which fails to accurately capture biphasic killing curves of heterogeneous populations, exhibits variability depending on bacterial population size, and fails to track bacterial adaptation during antibiotic exposure. These limitations highlight the need for dynamic *in vitro* culturing systems, which could provide deeper insight into time-dependent PD characteristics like shifting growth and killing kinetics and resistance development[49].

Chapters 3 and 4 demonstrated that nutrient availability strongly influences antibiotic sensitivity, and in **Chapter 6** genetic outcomes of resistance evolution under these varied conditions were observed. To bridge these findings, PD models capturing the full time course of antibiotic effects can reveal differences in antibiotic selection pressure. These PD differences can subsequently inform population genetic models, linking observed antibiotic sensitivity shifts directly to genetic variation[50]. The complex interplay between nutrient conditions, bacterial growth rates, and antibiotic susceptibility highlights the difficulty of accurately replicating selective pressures *in vitro*. Although continuous culture systems (e.g., chemostats) are technically challenging to maintain, they offer more precise control. Chemostats allow microbial populations to be maintained in a constant environment over extended periods, while enabling deliberate manipulation of specific selective forces such as nutrient levels or antibiotic exposure[51].

Molecular drivers of nutrient induced antibiotic sensitivity changes

An important aspect highlighted throughout this thesis is that changes in antibiotic effect are often unique to specific nutrient-antibiotic combinations. While significant progress has been made through the rapid development of genomic databases cataloging resistance-conferring mutations ('resistomes')[52], these approaches primarily correlate known mutations with antimicrobial resistance profiles. Less conventional mechanisms, such as metabolism-driven resistance, are often underrepresented in these databases, as they are embedded within more complex networks[25]. Resistance-associated mutations in such

networks typically arise after prolonged periods of selection pressure (**Chapter 6**), whereas phenotypic adaptive responses can cause a transient reduction in antibiotic efficacy during earlier phases of exposure[48], exemplified in **Chapter 3**. Although this thesis did not explicitly investigate molecular mechanisms, the observed nutrient-antibiotic-specific effects emphasize the need for future studies to dissect the specific biochemical responses involved.

Transcriptomics, proteomics, and metabolomics probe molecular layers that lie much closer to the phenotype than genomic data, and thus provide the biochemical resolution needed to dissect antibiotic-response mechanisms. For example, whole-genome sequencing of two *P. aeruginosa* isolates accounted for only part of their divergent β-lactam phenotypes, whereas transcriptomics uncovered additional resistance-linked expression changes invisible at the DNA level[53]. Likewise, quantitative proteomics detected the early induction of heat-shock chaperones, proteases, and metabolic enzymes when *P. aeruginosa* was exposed to sub-inhibitory tobramycin, highlighting rapid adaptive pathways[54]. In **Chapter 5**, it was shown how metabolomics can track nutrient utilization, antibiotic-induced metabolite signatures[21], and how those metabolic read-outs can be exploited by supplementing targeted nutrients to enhance antibiotic sensitivity[9]. Each technique carries its own analytical limitations and, most critically, captures only a snapshot of an inherently dynamic system.

Fluxomics adds this missing temporal dimension by tracing how carbon and energy flow through metabolic networks under changing conditions. Such data reveal compensatory pathways that support survival during nutrient stress and antibiotic pressure[55]. Yet, building isolate-specific flux models for CF infections is data-intensive, and the necessary parameters for making robust extrapolations are seldom available.

Recent genome-scale metabolic reconstructions, which integrate hundreds of reactions and multi-omics inputs, improve confidence in flux predictions. For example, a model combining transcriptomics, proteomics, and metabolomics successfully captured lipopolysaccharide remodeling during polymyxin resistance in *P. aeruginosa*[56]. These integrative models better capture time-dependent processes such as fluctuating drug concentrations or nutrient availability.

Most -omics platforms are advancing toward single-cell resolution because that level of detail can reveal subtle phenotypic adaptations during

antibiotic exposure. Yet, despite their analytical power, single-cell studies have limited biological impact if they are not anchored to a broader mechanistic framework. The heterogeneity of bacterial populations is well established, as repeatedly illustrated by phenomena like antibiotic persistence[57]. Instead, research efforts should begin with established analytical techniques, integrate their datasets, and first map *P. aeruginosa* responses to each antibiotic. Once the antibiotic mode of action and the population-level biochemical responses have been well defined, single-cell approaches can then illuminate the fine-scale heterogeneity that underlies treatment failure.

7.3. Overall conclusion

In conclusion, this thesis demonstrates that shifts in nutrient and oxygen availability can rewire *P. aeruginosa* physiology and alter antibiotic pharmacodynamics. These findings confirm that effective drug evaluation must consider antibiotic mechanism, pathogen physiology, and microenvironment as interdependent factors, especially for heterogeneous infection sites such as the CF lung. The work presented here lays the groundwork for explicitly incorporating nutrient and oxygen levels into antimicrobial sensitivity assay development and systems-level analyses of bacterial responses. Looking ahead, combining detailed infection-site mapping with environment-aware experimental assays represents a necessary first step toward patient-specific antibiotic therapy.

7.5. References

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