Model-informed design of antibiotic therapy against antimicrobial resistance

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Chapter 11

General Summary and Discussion

Antibiotic resistance poses a major threat to global healthcare. The continued emergence of antimicrobial resistance, coupled with the slowdown in the development of new antibiotics, underscores the urgent need to design antibiotic therapies that extend the utility of existing drugs and slow further development of resistance[1,2]. Efforts to design antibiotic therapies to combat antimicrobial resistance therefore require an understanding of how antibiotics influence bacterial growth (pharmacodynamics (PD)), how resistance evolves, and how drugs are absorbed, distributed, metabolized, and eliminated in the human body (pharmacokinetics (PK)). Moreover, the integration of this knowledge is essential to translate complex biological and pharmacological information into actionable strategies. Studies outlined in this thesis employed statistical and pharmacometric approaches to integrate knowledge from experimental and clinical observations to support the development of antibiotic treatment strategies against resistance development. In the first section of this thesis, we used antibiotic sensitivity assays and *in vitro* infection models to characterize the PD of antibiotics against specific pathogens and to investigate mechanisms by which pathogens may acquire resistance or lose sensitivity to an antibiotic. In the second section, we focused on the PK of antibiotics and how information derived from population PK models can be used to inform the design of antibiotic treatment. In the final section of this thesis, we investigated and evaluated the potential implementation of collateral sensitivity (CS) – a phenomenon in which resistance to one antibiotic leads to increased sensitivity to another – as a key rationale for designing antibiotic therapies to limit resistance development.

Section I - The Pharmacodynamics of Antibiotics and Resistance Evolution

Antibiotics exert their effects by inhibiting bacterial growth or killing bacterial cells. Therefore, understanding the impact of antibiotic exposure on the growth behavior of infecting pathogens is essential for evaluating the potential impact of an antibiotic treatment. Moreover, understanding how these exposure-response characteristics may change during resistance development can help inform strategies to limit or counter resistance. In the studies outlined in **Section I**, we used antibiotic assays and *in vitro* infection models to characterize the PD of antibiotics against specific pathogens and

to evaluate how resistance and ecological interactions may affect PD characteristics. Throughout these studies, we demonstrated the utility of pharmacometric models to help characterize antibiotic PD, predict potential treatment outcomes, and support the investigation of resistance mechanisms by serving as a hypothesis-testing platform.

HFIM for the characterization of antibiotic PK-PD targets

In **Chapter 2**, we demonstrated how dynamic time-kill (DTK) experiments in hollow fiber infection model (HFIM) systems can be combined with model-based analyses to inform selection of antibiotic therapeutic exposure targets[3]. This study focused specifically on teicoplanin, a glycopeptide antibiotic widely used to treat Gram-positive infections. Despite its long-standing clinical use, the drug exposures required to reliably achieve successful outcomes remain poorly defined, particularly across different pathogen species, as previous investigations have primarily focused on exposure-response relationships in methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA)[4–7]. Accordingly, in this chapter, we aimed to identify a teicoplanin exposure target applicable across a broader range of clinically relevant Gram-positive pathogens. Hollow fiber DTK experiments were used to characterize bacterial growth, antibiotic PD, and resistance development in *Staphylococcus epidermidis*, MSSA, MRSA, and *Streptococcus pneumoniae* under dynamic teicoplanin concentrations designed to reflect human PK profiles. The design and dose selection of this DTK experiment were informed by a preliminary PD model developed using less labor-intensive static time-kill (STK) assay data. This approach allowed us to perform a more targeted HFIM assay to characterize antibiotic PD in different pathogens that have distinct growth and antibiotic response characteristics. From these experiments, exposure levels required to achieve bacteriostasis, 1-log10 kill, and 2-log10 kill were derived for each pathogen. Substantial variability in unbound unbound fraction of the area under the concentration-time curve ($fAUC$)-based pharmacokinetics-pharmacodynamics (PK-PD) targets was observed across strains, which ranged between 10.5 to 75.4 mg.h/L for achieving 1-log10 kill target. Notably, this variability persisted even after normalization of PK-PD targets to the corresponding teicoplanin minimum inhibitory concentration (MIC). We hypothesized that this variability may be partly associated with differences in the development of transient resistance during the experiment, as reflected by the reduced impact of the second antibiotic dose on the bacterial population. Although the current analysis identified an $fAUC$ target of 75.4 mg.h/L as sufficient to achieve at least a 1-log10 kill across the four tested pathogens, further studies are required to confirm whether pathogen-specific exposure targets are necessary for optimal suppression of infection with teicoplanin.

Regarding the methodology for the study in this chapter, we employed an *in vitro* HFIM system to support the determination of PK-PD targets. This approach contrasts with the more commonly used murine infection models for determining PK-PD target[8,9]. While *in vivo* animal models provide physiologically relevant conditions, including physiological nutrient, pH, and temperature conditions[8,9], they are typically limited by sparse sampling, short observation periods, and low experimental throughput. These limitations restrict the ability of *in vivo* models to assess resistance development over time and to evaluate antibiotic activity across multiple pathogen species. Furthermore, differences between animal and human PK[8] may complicate the extrapolation of resistance-related findings to clinical settings. In **Chapter 2**, HFIM experiments were used as a complementary approach to *in vivo* models to characterize antibiotic exposure-response relationships under PK profiles that more closely reflect human exposure[3]. The HFIM enabled dense sampling and extended observation periods of up to 48 hours, which,

when combined with model-based analyses, allowed a more detailed characterization of bactericidal effect and reduction of teicoplanin sensitivity under clinically relevant PK conditions. In addition, the higher throughput of the HFIM facilitated the evaluation of teicoplanin PD across multiple pathogens. Collectively, these findings highlight the value of HFIM-based experiment and model-based analysis as a complement to animal models for deriving PK-PD targets[10], allowing the assessment of cross-species generalizability of PK-PD targets, and evaluating the potential impact of observed decrease in teicoplanin sensitivity during the treatment period.

Semi-mechanistic model identified porin loss and increased efflux as key resistance mechanisms to β-lactam/β-lactamase inhibitor combination

In **Chapter 3**, we investigated potential resistance mechanisms to the β-lactam/β-lactamase inhibitor combination piperacillin-tazobactam in *Klebsiella pneumoniae*. In contrast to the empirical dose-response PD model developed in **Chapter 2**, this study developed a mathematical semi-mechanistic PD model that accounts for the distribution of piperacillin-tazobactam to its target site in the bacterial periplasm, including the influx, efflux, and degradation of piperacillin and tazobactam in the periplasm by leveraging theoretical knowledge about these cellular processes. Using this model, we identified porin loss and efflux pump upregulation as two potential mechanisms that may independently confer resistance to piperacillin-tazobactam in the tested pathogen strain. The identification of reduced porin expression and increased efflux pump activity as contributors to piperacillin-tazobactam resistance was found to be consistent with prior experimental studies demonstrating the role of porin loss and enhanced efflux in resistance to β-lactam/β-lactamase inhibitor combinations[11,12]. Nevertheless, unlike alterations in β-lactamase activities, modifications in porin and efflux pump function are commonly regarded as secondary or accessory resistance mechanisms[13]. Our findings indicate that each of these mechanisms alone can fully account for the level of piperacillin-tazobactam resistance observed in this study. Importantly, the expression of both porins[14] and efflux pumps[15] was linked to the regulation of bacterial cellular osmolarity and the resulting turgor pressure exerted on the bacterial cells, a critical cellular event that is highly affected by β-lactam exposure[16]. This finding suggested that regulatory systems governing porin and efflux pump expression to be conserved across most, if not all, Gram-negative organisms[17]. Given the critical role of osmotic pressure in β-lactam–induced cell lysis[16], it is plausible that exposure to piperacillin-tazobactam activates this shared regulatory circuitry. Consequently, modulation of porin and efflux pump expression, together with associated genetic changes, may represent a common resistance pathway among Gram-negative bacteria.

The current study demonstrates how empirical knowledge from experimental observations can be integrated with theoretical knowledge to develop semi-mechanistic PD models for evaluating antibiotic resistance mechanisms. These models were used as hypothesis-testing tools by assessing the behavior of hypothetical bacteria harboring cellular characteristics that differ from those described by the theoretical knowledge applied during model development. While such semi-mechanistic models are particularly useful for addressing mechanistic questions, they depend strongly on the accuracy and completeness of the underlying biological knowledge. When such knowledge is limited, simplification of the biological system is needed during the model development process to provide a generalized representation of cellular events. In this study, for example, the binding of piperacillin to multiple variants of its target protein, the penicillin-binding protein (PBP), and its associated bactericidal effects were represented using an empirical

concentration-effect relationship even though previous studies reported the expression of multiple PBP variants in *K. pneumoniae*. This simplification precluded exploration of PBP-specific resistance mechanisms with our model. Nevertheless, this simplification was required given the limited information available on the differences in expression and piperacillin-tazobactam binding characteristics of these PBP variants. This highlights the trade-off between the maintenance of biological complexity in the model and feasibility, where empirical simplifications allow meaningful inference in the absence of sufficient mechanistic data. Overall, the approach described in this chapter is considered of relevance to help identify key knowledge gaps and guide future experimental studies aimed at resolving mechanistic uncertainties.

The effect of polymicrobial interactions on antibiotic PD

In **Chapter 4**, we investigated how the antibiotic sensitivity of a bacterial strain can be shaped not only by its intrinsic properties but also by its surrounding environment. This was examined in the context of polymicrobial infections (PMIs), i.e., infections caused by multiple bacterial species[18–20], where ecological interactions between co-infecting organisms can reshape bacterial growth conditions and, in turn, modulate the PD characteristics of antibiotics acting on individual species. Using antibiotic sensitivity assays in conditioned growth media[21], we investigated how the presence of a secondary pathogen or bacterial population alters the growth dynamics and antibiotic sensitivity of *Pseudomonas aeruginosa*. In these assays, we identified the prevalence of reduction in antibiotic sensitivity in *P. aeruginosa* when cultured in medium conditioned by a secondary bacterial species. This observation indicated the presence of an extracellular factor produced by the secondary species that alters antibiotic sensitivity of *P. aeruginosa*. Further identification of these extracellular factors may therefore help inform antibiotic treatment strategies that account for PMIs. In this study, the characterization of antibiotic sensitivity and PMI were performed using PD models based on antibiotic assays. This enabled us to further integrate information from published PK studies to develop PK-PD models that can be used to evaluate how PMI-associated changes in bacterial growth and antibiotic sensitivity may influence the outcomes of specific antibiotic treatments. Overall, this study underscores the importance of accounting for microbial community context when evaluating treatment strategies[21]

Section II - From Pharmacokinetics to Clinical Dose Optimization

Delivering effective antibiotic therapy requires more than an understanding of PD interactions between drugs and bacteria. Equally important is knowledge of how the patients' body shapes drug concentrations over time, *i.e.*, the PK of the antibiotic. In particular, the studies in this chapter evaluated the importance of antibiotic distribution to tissues where infections occur, as this determines the actual drug exposure experienced by the infecting bacterial population. In addition, inter-individual variability in antibiotic PK within patient populations was considered, demonstrating how information from population PK models can be leveraged to inform the optimization of antibiotic treatment strategies for a patient population.

The variability of teicoplanin clearance in hemodialysis patients

The study in **Chapter 5** again focused on teicoplanin – a predominantly renally cleared glycopeptide whose clearance is closely associated with renal function. It is generally associated with a lower risk of nephrotoxicity compared to the glycopeptide vancomycin[22].

In this study, we evaluated the effectiveness of teicoplanin treatment in achieving established PK-PD targets in patients with renal impairment undergoing maintenance hemodialysis. Specifically, we assessed a thrice-weekly teicoplanin dosing regimen, which was commonly selected in this patient population to align with the often required thrice-weekly hemodialysis schedule. A population PK model for teicoplanin was developed to characterize its PK and the associated inter-individual variability (IIV), enabling the evaluation and design of dosing regimens that maximize population-level attainment of teicoplanin exposure targets. The study revealed substantial variability in teicoplanin clearance among dialysis patients, which is consistent with the heterogeneity in renal function expected across this population. Simulations using the population PK model identified a risk of suboptimal target attainment following the first dose. The population PK model was used to propose an optimized dosing strategy incorporating a loading dose and a framework for individualized dosing that accounts for variability in inter-dose intervals inherent to the thrice-weekly regimen[23,24]. While this optimized regimen can improve the attainment of exposure target, the high variability in drug clearance expected from this patient population limits the ability of a fixed dosing strategies to consistently achieve exposure targets throughout the patient population. Consequently, these findings highlight the potential value of implementing observation-based dose adjustment such as therapeutic drug monitoring (TDM)[25] to ensure the attainment of effective and safe teicoplanin exposure in renally impaired patients undergoing thrice weekly teicoplanin therapy.

Predicting lung antibiotic exposure using PBPK and QSPR approaches

Accurate prediction of antibiotic concentrations at the site of infection is critical for assessing treatment effectiveness. Accordingly, in **Chapter 6**, we evaluated several modeling approaches to support the prediction of antibiotics lung concentrations of different antibiotics based on their known properties. Specifically, we employed a physiologically-based pharmacokinetics (PBPK) model to predict antibiotic concentrations at pulmonary infection sites, including the lung epithelial lining fluid (ELF) and alveolar macrophage (AM). The study evaluated the application of several lung PBPK models, including a general passive permeability-limited model, a drug-specific permeability-limited model, and a quantitative structure-property relationship (QSPR)-informed perfusion limited model. In the latter model, a QSPR modeling approach was used to estimate antibiotic distribution between plasma and lung compartments (ELF or AM) using drug physicochemical properties[26,27]. While drug-specific permeability data proved essential for accurate predictions, the QSPR-informed model offers a more broadly applicable alternative when such data are unavailable. This work highlighted how drug properties and physiological variability can influence pulmonary drug distribution and demonstrated the potential of PBPK-QSPR approaches to support target site exposure predictions for antibiotics with a known structure but limited distribution data.

PBPK modeling approaches are valuable tools for early-stage antibiotic development. Firstly, PBPK modeling approaches can be used to evaluate potential differences and trade-offs between plasma PK and target-site exposure, providing valuable insight for the drug design process. For example, simulations using a passive diffusion PBPK model suggested that more lipophilic compounds may exhibit enhanced passive diffusion into the lung. While increased lipophilicity may be advantageous for lung-targeted antibiotics, previous studies have associated higher lipophilicity with increased systemic drug clearance[28,29], thus indicating a potential trade-off between these physiological characteristics. Consequently, optimizing compounds solely for lung diffusion may result

in rapid plasma clearance, ultimately limiting drug distribution to the lung. These findings highlight the need to simultaneously consider both plasma and lung PK during antibiotic development. Second, integrating *in vitro* PD knowledge with PBPK models describing target-site distribution provides a means to predict the potential effectiveness of candidate antibiotics at specific sites of infection early in development. Together, this PBPK-PD framework offers a rational basis for evaluating candidate compounds, supporting informed compound selection and lead optimization before extensive clinical data become available.

Multi-objective framework for population dosing regimen optimization

In **Chapter 7**, we presented a novel analytical framework to support dosing regimen optimization. The selection and optimization of antibiotic dosing regimens require balancing multiple treatment objectives[30], including efficacy targets such as infection clearance and resistance suppression, as well as safety constraints related to drug toxicity. This task is further complicated by IIV in PK and PD characteristics that are expected within a patient population[31,32]. To address this complexity, model-based analyses are increasingly used to support dose selection and optimization[30,33]. In recent years, several studies showed the utility of numerical optimization methods alongside PK-PD modeling to improve dose optimization[34–37]. Nevertheless, the development of a framework that can simultaneously account for IIV and the multi-objective nature of dosing optimization remained challenging. As a result, most applications focus on a single objective and/or individual. One study proposed the dose optimization for a patient population by using the typical individual as a surrogate for the whole population[8]. While such approaches can be informative, they do not ensure population-level optimality. Other studies have incorporated IIV into dosing optimization[34], but the simultaneous handling of multiple heterogeneous objectives typically requires extensive hyperparameter tuning, introducing additional layers of complexity. In this study, we developed an analytical framework that leverages information from population PK and PD to support dose and schedule selection. This study was built based on the idea presented by Colin *et al.*, which demonstrated the use of probability of target attainment (PTA) to guide antibiotic dosing[38]. Here, we further generalize this concept by considering population-level attainment of any treatment objective (*e.g.* efficacy, safety, and efficiency objectives) as the primary performance metric. The framework applies general optimization algorithms to identify dosing regimens that maximize population-level attainment of all treatment objectives.

During the development of this framework, we recognized the importance of clinical judgment in the treatment design process[39]. To address this need, our framework allows the incorporation of expert input through two complementary approaches. The first approach integrates clinical judgment by assigning objective weights, which requires translating qualitative clinical priorities into quantitative metrics. In this study, we demonstrated how this can be achieved using multi-criteria decision analysis (MCDA), a method previously employed for clinical decision-making[40,41]. A key advantage of MCDA is its ability to summarize input from a group of stakeholders. Nevertheless, MCDA-based weighting provides limited insight into how assigned weights translate into the final optimal solution. For example, in the initial optimization of combination antibiotic dosing regimens, safety targets were attained to a substantially greater extent than efficacy targets despite equal objective weights. This mismatch suggested a potential divergence between expert expectations and actual optimization outcomes when objective weights are used as a reflection of clinical priorities. The second approach incorporates expert input through gate optimization after experts are presented with an

overview of the potential solution space. By explicitly revealing trade-offs among competing objectives during a preliminary grid-based exploration, this approach may enable more informed clinical decision-making. However, integrating input from multiple experts is less straightforward than with MCDA-based methods, and the approach is more computationally intensive due to the need for preliminary grid exploration. By supporting multiple mechanisms for expert input, we aim to develop a dosing optimization framework that enables continued refinement of methods for quantifying and integrating clinical expertise into the optimization process.

Section III - Collateral Sensitivity

The final three chapters of this study focused on CS, a phenomenon in which the evolution of resistance to one antibiotic increases susceptibility to another. For several years, the potential benefit of exploiting CS in antibiotic combination therapy has been theorized following its repeated observation in laboratory studies. Nevertheless, translation of CS into practical treatment strategies has remained limited by several challenges. Firstly, evaluation on its occurrence in the clinical settings needed. Secondly, there has been a lack of quantitative evaluation to determine whether the magnitude of increased sensitivity caused by CS can influence treatment outcomes. Lastly, while theoretical evaluations of the design of CS-based therapy have been performed[42], the development of an actual CS-based regimens from experimentally determined antibiotic sensitivity profiles, together with subsequent *in vitro* validation, remains an open area of investigation.

Occurrence and development patterns of CS in clinical settings

The study presented in **Chapter 8** aimed to investigate the occurrence of CS among clinical isolates. We conducted a large-scale analysis of CS patterns using data from three antibiotic surveillance datasets. This study also examined both intra- and inter-species consistency in CS emergence across clinically relevant pathogens, with a particular focus on members of the ESKAPEE group (*Enterococcus faecium*, *S. aureus*, *K. pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa*, Enterobacter *spp.*, and *Escherichia coli*)[43–45]. Through this retrospective analysis, we provided an evaluation on the consistency of CS within individual pathogen species in clinical settings. In addition, the study helped identify CS relationships conserved across multiple pathogen species, providing a potential foundation for developing broad-spectrum CS-informed combination therapies.

Our study revealed distinct patterns in CS development across antibiotic classes. CS is commonly thought to arise mainly between antibiotics belonging to different drug classes[46]. In line with this assumption, our analysis showed that most CS interactions occurred between antibiotics from distinct classes, with β-lactams representing a notable exception. Among β-lactam antibiotics, intraclass CS was observed more frequently, particularly within the cephalosporin subgroup. This enrichment of intraclass CS among β-lactams may reflect evolutionary constraints associated with the development and diversification of β-lactamase enzymes[47]. Additional studies are required to better understand the mechanisms driving the apparent restriction of intraclass CS interactions to β-lactam antibiotics.

Stochastic modeling of CS-based combination therapy in S. pneumoniae

In **Chapter 9**, we investigated the consistency of CS in *S. pneumoniae*. The heterogeneity in antibiotic sensitivity profiles among resistant mutants observed in this study

highlighted the stochastic nature of resistance and CS evolution. Experimental data collected in this study were subsequently used to inform the development of PK-PD models that incorporated stochastic mutation dynamics by implementing a simplified τ-leap Gillespie simulation framework[48]. To explore the translational relevance of these findings, we used the developed PK-PD models to evaluate the impact of CS in combination antibiotic therapies. Specifically, we simulated treatment outcomes for a unidirectional CS pair (rifampicin-fusidic acid) and a bidirectional CS pair (linezolid-fusidic acid) under simultaneous administration and 12-hour cycling regimens, with monotherapy as a comparator. Across the simulations, CS-based combination therapies were found to be more effective than fusidic acid, linezolid, or rifampicin monotherapies in limiting resistance emergence and clearing infection. The bidirectional CS between fusidic acid and linezolid consistently outperformed the unidirectional fusidic acid-rifampicin combination.

Our simulation-based analysis revealed the importance of the order of administration towards treatment outcomes, with regimens initiated using the antibiotic whose resistance induced the strongest CS effect (linezolid or rifampicin) achieving greater suppression of resistance. Additionally, we identified simultaneous administration as the most effective dosing regimen to prevent mutant establishment. However, prior studies have raised concerns about the selection of double-resistant mutants under combined antibiotic pressure[42,49]. Evaluating this risk would require the isolation and characterization of double-resistant mutants, which were not observed in the present study. Consequently, this risk was not directly assessed. Therefore, future studies are encouraged to investigate the emergence of dual resistance and its implications for the long-term effectiveness of CS-based therapies.

Model-informed design and in vitro evaluation of a CS-based antibiotic combination therapy in P. aeruginosa

In **Chapter 10**, we explored the consistent emergence of CS across four clinical *P. aeruginosa* isolates. The PD models developed in this study also accounted for potential PD interactions between the selected antibiotic pair ciprofloxacin (fluoroquinolone) and tobramycin (aminoglycoside), adding a critical layer of complexity to the evaluation of combination therapies. As in **Chapter 9**, experimental data were used to develop PK-PD models, which then served as platforms to assess the efficacy of CS-informed antibiotic combinations. We then implement the dosing regimen optimization framework previously developed in **Chapter 7** to design dosing regimens aimed at maximizing the effectiveness of the combination while suppressing resistance development and maintaining safety. An additional *in vitro* treatment simulation demonstrated the efficacy of the proposed CS-based therapy (alternating doses of 2.42 mg/kg ciprofloxacin and 1.28 mg/kg tobramycin every 12 hours) in suppressing or delaying the emergence of resistant bacterial populations during the treatment of pulmonary P. aeruginosa infections. These findings further support the translational potential of CS as a resistance management strategy. Notably, this study is the first to propose an optimized CS-based dosing regimen and evaluate its efficacy in an *in vitro* infection model.

Despite the consistency and reproducibility of tobramycin CS in ciprofloxacin-resistant *P. aeruginosa* observed in **Chapter 10** and in other *in vitro* studies[50,51], this CS interaction was not identifiable from the clinical dataset analyzed in **Chapter 8**. This absence of tobramycin CS in clinical ciprofloxacin-resistant isolates reflected the constraints of the analytical approach used in **Chapter 8**, which can only detect CS interactions that reproducibly occur across multiple clinical isolates, thereby capturing phenotypes driven by a dominant and widely distributed resistance mechanisms, or by distinct mechanisms

that converge on similar phenotypic outcomes. Consequently, CS relationships that are readily selected under controlled laboratory evolution but are less prevalent in clinical populations may not be detected, likely due to the greater heterogeneity and less predictable evolutionary pressures present in real-world environments[52,53].

Previous laboratory evolution studies linked aminoglycoside CS in ciprofloxacin-resistant *P. aeruginosa* to mutations in *gyrA-B*, *mexS* (*mexEF-oprN*), and *nfxB* (*mexCD-oprJ*) genes[54]. The absence, or limited prevalence, of these genotypes in the clinical dataset may reflect either the presence of alternative ciprofloxacin resistance mechanisms that do not confer aminoglycoside CS, the emergence of compensatory mutations that negate this phenotype, or epistatic interactions between resistance determinants and the broader genetic background that give rise to complex pleiotropic effects – all of which may prevent the identification of aminoglycoside CS in ciprofloxacin-resistant pathogens performed in Chapter 8. Consistent with this interpretation, other studies have highlighted the complexity and variability of aminoglycoside CS evolution in clinical ciprofloxacin-resistant isolates[55]. It is also important to note that aminoglycoside CS reported in previous *in vitro* studies[50,54] and in **Chapter 10** were observed in ciprofloxacin-resistant strains that had been pre-exposed to ciprofloxacin, suggesting a role for prior antibiotic exposure in shaping the evolutionary pathways leading to aminoglycoside CS. Future studies are therefore encouraged to further evaluate the relevance of antibiotics pre-exposure effects to the development of CS phenotype in clinical or *in vivo* environments.

Pharmacometrics as a knowledge integration platform

Efforts to understand antibiotic resistance and to develop strategies that can prevent or slow its emergence requires the integration of knowledge from a wide range of sources[56,57]. Experimental systems and clinical data each capture different facets of antibiotic effects and bacterial adaptation. A framework that brings these pieces together in a quantitative and structured way is therefore essential for translating biological observations into actionable treatment strategies. Pharmacometrics models provide such framework by allowing information from diverse experiments and patient studies to be combined and interpreted through mechanistic or semi-mechanistic models[58,59].

The type of model chosen in a pharmacometrics analysis depends on the purpose of the study and the nature of the available information. In this thesis, population PK and PK-PD models, along with treatment targets such as PK-PD indices or other treatment objectives, form the foundation of most analyses due to their ability to link drug exposure to expected bacterial response. This makes population PK and PK-PD models suited for evaluating and designing antibiotic treatments. In the past, population models have been used to evaluate and design antibiotic dosing regimen. PK-PD models are particularly useful for evaluating resistance development, as they provide a representation of infecting bacterial populations together with their antibiotic susceptibility and resistance dynamics. For example, in **Chapter 4**, we demonstrated how a PK-PD model can be adapted to predict treatment outcomes when the susceptibility of the focal pathogen is altered by the presence of another bacterial species within the infection. Extensions of the bacterial population structure further enable the representation of resistance heterogeneity, as demonstrated in **Chapter 9** through the inclusion of multiple bacterial subpopulations. Although multi-population PD models have previously been used to capture resistance development[60,61], these earlier studies typically employed one to three subpopulations to empirically represent differences in antibiotic sensitivity. Our framework expands this approach by incorporating up to ten exper-

imentally characterized resistant bacterial subpopulations per-antibiotic. While this remains a simplification of the greater heterogeneity likely present in clinical settings[62], our model enables systematic evaluation of heterogeneous resistance dynamics and their implications, especially CS.

In contrast to PK and PK-PD models, PBPK models have a more focused purpose: to describe drug distribution across tissues based on physiological structure and transport processes[63]. For this purpose, PBPK models are particularly valuable when direct measurement of drug concentration at the infection site is difficult or requires invasive clinical procedures. In **Chapter 6**, we showed how a PBPK modelling framework can be applied to predict lung concentrations of antibiotics, using either an active transport model, a passive diffusion model, or a QSPR-assisted PBPK model. Our analysis demonstrated that a PBPK-QSPR approach can serve as a viable alternative to active transport models when *in vitro* or *in vivo* permeability data are unavailable.

The incorporation of known biological processes into empirical PD models can also help reveal potential mechanisms underlying observed bacterial responses. In **Chapter 3**, we showed this utility of model-based analysis by expanding of an empirical PD model with mechanistic knowledge related to drug transport into and out of the bacterial periplasm. This resulted in a semi-mechanistic PD model that included the mathematical representation of cellular processes previously associated with resistance. By varying parameters corresponding to these resistance-associated processes in the model, we evaluated the potential impact of specific cellular changes and identified candidate resistance mechanisms. This work highlights how semi-mechanistic PD modeling can support hypothesis generation and guide further investigations aimed at identifying effective strategies to counter resistance development.

Altogether, these studies demonstrate how pharmacometric modeling provides a flexible platform for integrating knowledge from laboratory experiments, preclinical systems, and clinical observations. The type of information used to build a model ultimately determines the predictions it can generate, which underscores the importance of selecting the right modeling framework for the research question at hand. Choosing an appropriate model not only ensures scientific relevance but also enables the resulting insights to contribute meaningfully to the design of effective antibiotic therapies.

Conclusions

Building on the overarching objective of this thesis to leverage pharmacometrics and statistical modeling to support effective antibiotic therapy design, the work presented here synthesizes how experimental and clinical evidence can be integrated to address antimicrobial resistance. Efforts to develop strategies against antibiotic resistance span the full spectrum of research, from laboratory experiments to clinical studies. Initial investigations into changes in antibiotic sensitivity often begin in controlled laboratory settings, are subsequently examined in more complex *in vitro* treatment simulations, and are ultimately evaluated in clinical contexts. This progression reflects an ongoing process rather than a confined focus to any single study type. As a result, the development of antibiotic dosing strategies to counter resistance often requires the consideration of information from multiple experimental and clinical sources. Each source provides complementary yet incomplete insight into drug exposure, bacterial response, and resistance evolution, making the ability to extract, integrate, and synthesize actionable information from these sources essential for the effort.

This thesis demonstrated how model-based and statistical analyses can complement

experimental and clinical studies to support the rational design of antibiotic therapies that effectively treat infection while suppressing the development of resistance. It demonstrates how PD and PK-PD models can serve as powerful platforms for integrating data from both experimental and clinical sources to inform effective treatment design. Our work shows how model-based analyses can complement experimental studies by providing deeper insight into the mechanisms, dynamics, and pleiotropic effects of resistance development. Several chapters also emphasized the essential role of PK knowledge in translating PD findings into clinically relevant insights, and how integrated PK-PD models can guide the development of optimal dosing strategies for antibiotic therapies. In particular, this thesis investigated CS as a promising foundation for designing resistance-limiting combination antibiotic therapies. We addressed two critical factors for translating CS-based strategies into clinical application: the reproducibility of CS patterns and the design of treatment regimens that can leverage CS to achieve effective therapeutic outcomes. Notably, this study is the first to propose a potential CS-based dosing schedule and to evaluate its effectiveness in suppressing the establishment of resistance *in vitro*. Together, the findings, strategies, and modeling frameworks presented in this thesis offer valuable tools and perspectives to support the rational design of antibiotic therapies and inform future efforts to curb the spread of resistance.

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