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Model-informed design of antibiotic therapy against antimicrobial resistance

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Chapter 1

Introduction

Antibiotics represent a fundamental component of modern healthcare. While primarily employed in the treatment of bacterial infections, their use also extends to prophylactic applications during invasive medical procedures, such as surgical interventions, organ transplantation, and chemotherapy-induced immunosuppression, to prevent opportunistic infections[1]. As such, the modern healthcare system is heavily reliant on the availability of effective antimicrobial agents, and a system without functional antibiotics is virtually inconceivable[2]. However, the rise of antibiotic resistance is making many of these antibiotic treatments ineffective, thereby posing a serious threat to the foundation of modern healthcare[2,3].

Antibiotic resistance refers to the ability of bacteria to survive and proliferate despite the presence of antimicrobial agents that would normally inhibit or kill them[3]. At the cellular level, resistance can arise through various mechanisms, including the production of drug-degrading enzymes, activation of efflux pumps that expel antibiotics from the cell, reduced membrane permeability that limits drug uptake, or the utilization of alternative cellular pathways that bypass the drug's molecular target[4,5]. Resistance to a single antibiotic may sometimes involve multiple overlapping mechanisms[6], contributing to the complexity of effectively counteracting or reversing resistance once established.

Historically, the primary strategy to combat antibiotic resistance centered on the continuous development of new antibiotics[7]. Particular emphasis was placed on discovering novel classes with distinct mechanisms of action, as these were more likely to bypass existing resistance mechanisms. However, resistance to newly introduced antibiotics often emerges rapidly after their clinical deployment, diminishing their long-term utility[3,8]. This problem was compounded by the view within pharmaceutical companies that new antibiotics are economically unappealing: they are short-course therapies with relatively low prices, limited sales due to stewardship restrictions, and small market sizes[9]. As a result, a significant decline in antibiotic discovery has persisted since the late 20$^{\text{th}}$ century[8] Taken together, the stagnation in antibiotic innovation and the relentless evolution of resistance underscore the urgent need for alternative strategies to manage antibiotic resistance beyond traditional drug discovery efforts.

Antibiotic treatment strategies to combat antimicrobial resistance

As the continuous development of new antibiotics has proven unsustainable, alternative strategies are urgently needed to preserve the effectiveness of the drugs currently available. This requires not only evaluating how existing antibiotic therapies are used but also ensuring that the treatment strategy can reliably clear the infection and minimize

the risk of resistance development. By placing increased emphasis on maximizing the utility of existing antibiotics, rather than relying solely on the discovery of new compounds, we can extend the lifespan of current treatments and strengthen our ability to manage drug-resistant infections.

One of the main strategies to prevent the emergence of antibiotic resistance is to optimize dosing regimens. The goal of such efforts is to ensure that drug exposure is high enough to clear the infection and to minimize the chance that concentrations fall into a sub-optimal range where bacteria can survive and adapt to the antibiotics. This strategy relies on defining a drug exposure threshold above which bacterial eradication is expected[10]. The threshold is derived from the drug's pharmacokinetics (PK), which describes its absorption, distribution, and elimination in a patient's body, as well as its pharmacodynamics (PD), which describes how the drug affects the growth of the infecting pathogen. The resulting exposure metrics, known as pharmacokinetics-pharmacodynamics (PK-PD) indices, are commonly used to capture the relationship between drug levels and bacterial response[11]. Common examples include the ratio of the area under the concentration-time curve (AUC) to the pathogen's minimum inhibitory concentration (MIC), percentage of the dosing interval that the drug concentration remains above the minimum inhibitory concentration (%T>MIC), or ratio of maximum concentration to minimum inhibitory concentration (C_{max}/MIC)[10–12]. For each antibiotic–pathogen combination, specific threshold values of these indices, referred to as PK-PD targets, have been linked to antibacterial activity and clinical response. Dosing recommendations are then designed to maximize the probability of achieving these PK-PD targets in a given patient population. This framework has become a cornerstone of antibiotic regimen design and also guides therapeutic drug monitoring where maintaining adequate exposure is critical[12]. Although the use of PK-PD targets to guide antibiotic dosing has been successful in the clinic, it relies largely on the static sensitivity metric MIC and is therefore limited in its ability to account for resistance development.

Another key strategy against antibiotic resistance is the coordinated use of multiple antibiotics to prevent the development or establishment of resistance[13]. Combination antibiotic therapy is widely used in clinical practice for several key reasons. Currently, the main reason to combine antibiotics is to broaden the antibacterial spectrum[14,15], which is particularly important in empirical therapies where the infecting organism has not yet been identified[16]. Using multiple antibiotics increases the likelihood that at least one will be effective against the pathogen, which can become critical in severe or life-threatening infections[16,17]. This expanded spectrum is also beneficial in the treatment of polymicrobial infections, which involve multiple microbial species. Another major reason to combine antibiotics is to reduce the likelihood of resistance emergence during treatment, as the probability for a pathogen to simultaneously acquire resistance to multiple drugs is significantly lower than to a single agent[15,18–20]. This strategy has already proven effective in the treatment of chronic infections that require prolonged antibiotic therapy, such as tuberculosis. During tuberculosis treatment, combination therapy is used as standard practice to prevent the emergence of antibiotic-resistant bacteria and to improve treatment outcomes[21]. The application of combination therapies have also been proposed for their potential synergistic effects[13]. Synergy refers to a pharmacodynamic interaction in which the combined effect of two antibiotics exceeds the sum of their individual activities, resulting in enhanced bacterial killing or growth inhibition[13,22]. Such combinations can improve treatment outcome, particularly by accelerating bacterial clearance early in the course of therapy[22].

While combination therapy holds promise, its broader application presents several

challenges. Multiple factors can limit the effectiveness of this strategy. In some cases, resistance mechanisms such as the upregulation of broad-spectrum efflux pumps may significantly reduce the efficacy of multiple antibiotics simultaneously[23]. Additionally, while synergistic antibiotic combinations can enhance antibacterial potency, they may also intensify selective pressure on bacterial populations, potentially promote the emergence of resistant mutants[13]. Careful selection of antibiotic pairs is therefore critical in designing combination treatments[24]. Moreover, patient-specific differences in drug absorption, distribution, metabolism, and excretion can complicate combination dosing strategies. Thus, maintaining effective drug concentrations for both agents without exceeding toxicity thresholds requires careful optimization of the dosing regimen[25]. Altogether, these challenges underscore the need for rigorous studies that integrate insights from laboratory and clinical observations with statistical analyses to support the development of effective antibiotic therapies in a translational study.

Beyond the core rationales of suppressing resistance emergence, expanding antibacterial spectrum, and achieving synergy, combination therapy can also be used to manipulate the bacterial evolutionary landscape[26]. Resistance mutations to one antibiotic often have pleiotropic effects, which influence other bacterial traits such as fitness[27] or susceptibility to additional antibiotics[13,28]. When these effects alter susceptibility to another antibiotic, they are referred to as collateral effects[28]. Collateral resistance (CR), or cross-resistance, occurs when resistance to one drug also confers resistance to another, which is generally undesirable in clinical settings. In contrast, collateral sensitivity (CS) arises when resistance to one antibiotic increases susceptibility to another, offering a potential therapeutic advantage[13,28]. By strategically selecting antibiotics and designing dosing regimens that exploit CS, it may be possible to steer bacterial evolution into a situation where adaptation to one drug increases vulnerability to the other, thereby constraining the pathogen's evolutionary escape routes towards developing cross-resistance[28–31].

Despite its potential as a strategy to counter antibiotic resistance, translating CS into clinical practice remains challenging. Firstly, the clinical applicability of CS depends on the consistency and stability of CS interactions[32]. It is therefore essential to identify antibiotic pairs that exhibit robust and reproducible CS effects, particularly under clinically relevant conditions. Throughout this thesis, we demonstrate how targeted antibiotic susceptibility assays can be used to identify pathogen-specific CS patterns that are consistent across experimental settings. In addition, we show that large-scale antibiotic surveillance data can be leveraged to detect apparent CS relationships in clinical populations, providing further support for their potential translational relevance. Secondly, it is still unclear how a combination antibiotic therapy can be optimally designed to effectively exploit CS[30,33]. To address this gap, this thesis illustrates how model-based approaches can inform the rational design of CS-based antibiotic treatment strategies.

The patient, the drug, and the pathogen

The outcome of antibiotic therapy depends on several interconnected factors. Developing an effective treatment strategy therefore requires integrating knowledge about three critical aspects, namely the patient, the drug, and the pathogen.

The first key factor is understanding how antibiotic concentrations change over time in the patient's body after a dose is administered, which defines the PK of the drug[11]. To characterize these concentration time profiles, PK models provide essential quantitative tools for describing drug behavior and predicting exposure under various dosing conditions. Population PK models extend this by capturing the expected variability in PK across patients. These models are especially important for evaluating how well a

therapy will be able to achieve a pre-defined antibiotic exposure target in patient groups with special conditions, allowing researchers and clinicians to quantify how the patients' specific physiology or clinical procedures influence antibiotic exposure. For example, patients with reduced renal function may experience higher or prolonged exposure to renally cleared antibiotics, whereas dialysis can temporarily and intermittently accelerate antibiotic clearance in this patient population. In such cases, a population PK model tailored to patients with renal impairment can provide more accurate predictions of drug exposure than a model developed for individuals with normal kidney function.

The second key factor is information on the physicochemical characteristics of the drug. One example is plasma protein binding, which is strongly influenced by drug lipophilicity[34] and affects the pharmacologically active antibiotic concentration in the plasma. In relation to the first factor on how antibiotics are distributed within the patient's body, it is also important to consider the drugs' distribution to the site of infection, such as the blood, lungs, gastrointestinal tract, or central nervous system, which is governed by interactions between drug-specific properties and human physiological factors. A quantitative understanding of antibiotic tissue distribution is essential, as antimicrobial efficacy ultimately depends on the drug concentration at the target site. Physiologically-based pharmacokinetics (PBPK) models provide a useful framework for predicting antibiotic distribution by leveraging knowledge on human physiological parameters. In one of the chapters in this thesis, we combined PBPK modeling with quantitative structure-property relationship (QSPR) models, which link chemical and physicochemical drug properties to physiological outcomes to predict antibiotic concentrations in the lung.

The final key factor is understanding how an antibiotic interacts with the infecting bacterial population, which is collectively captured as an antibiotics' PD characteristics. This includes characterizing how bacterial growth changes when exposed to different antibiotic concentrations[11]. These concentration-response relationships are typically quantified through preclinical *in vitro* experiments, which provide foundational insight into antibiotic effects on bacterial growth. The MIC assay is the most common approach to evaluate how antibiotics affect bacterial growth, in which the lowest antibiotic concentration that inhibits visible growth is determined[11,35]. Although MIC assays are rapid and practical for clinical use, such assays provide only a time-collapsed measure and cannot describe the dynamic nature of bacterial responses. Time-kill studies address this limitation by tracking bacterial counts over time and defining the concentration effect relationship, thereby revealing both concentration and time dependent antibiotic effects[11]. Such experiments can be performed under static drug concentrations to characterize bacterial response at a fixed exposure level, or under dynamic concentrations to mimic the changing drug levels encountered *in vivo*. *in vitro* studies are essential for evaluating drug interactions in combination regimens and for estimating the probability and rate of resistance emergence under different conditions. In particular, understanding resistance evolution is critical for designing therapies that counter antibiotic resistance. This is especially important for CS–based therapies, which relies heavily on a detailed comprehension of how resistance develops and shifts in response to drug exposure. Together, these PD insights shape expectations of how an antibiotic will perform against a given pathogen and form a critical component of treatment design.

Information on the PK and PD characteristics of an antibiotic can also be quantified simultaneously in a combined PK-PD study. One common approach is the *in vivo* dose fractionation study, which empirically quantifies how different levels of drug exposure influence bacterial burden during an infection. Such approaches are frequently

performed in neutropenic murine models in a preclinical study, and results from such studies are often used as a basis to identify the relevant PK-PD index[36]. While informative, such experiments typically allow only limited sampling and usually span no more than the first 24 hours. The 24-hour limitation of *in vivo* dose fractionation studies often restricts the ability to observe the effects of slowly killing antibiotics[37]. These limitations also restrict the use of murine infection models in characterizing the impact of resistance development towards an antibiotic therapy. In addition, antibiotic PK in a murine model may not accurately reproduce human exposure[37]. An alternative to *in vivo* PK-PD studies is the hollow fiber infection model (HFIM), which uses an *in vitro* compartmental system to simulate infection dynamics under controlled drug exposure conditions. Unlike animal models, HFIM systems allow precise control of concentration time profiles that can closely mimic human PK[37]. Ease of sample collection from HFIM systems also supports a more frequent and extended measurement of bacterial density, enabling detailed assessment of resistance emergence and its impact on exposure targets. Data generated from PK-PD studies are highly valuable for efforts to optimize antibiotic dosing[36,37].

Pharmacometrics for the development of antibiotic treatment strategies

The combined information from *in vitro* experiments, preclinical studies, and clinical observations are essential for the development of effective antibiotic treatments. Nevertheless, these data are often spread across multiple studies, each offering distinct but partial insights. Given the complexity and heterogeneity of laboratory and clinical data, a robust analytical approach is essential to extract, integrate, and interpret information from these diverse sources. Throughout this thesis, we demonstrate how pharmacometric approaches can be used to integrate knowledge and extract actionable insights from multiple sources of information.

Pharmacometrics is an analytical discipline that applies mathematical models to approximate the dynamics of phenomena in biological, pharmacological, and physiological systems[38]. In the context of antibiotic therapy, pharmacometric models serve as powerful tools to summarize observations (descriptive), test hypotheses (analytical), and predict treatment outcomes (predictive). These capabilities make pharmacometrics particularly well suited for integrating laboratory and clinical observations on the PK and PD of antibiotics, ultimately informing the rational design of combination therapies. In this thesis, pharmacometric models are used extensively to characterize both laboratory and clinical data, to explore potential pathways of resistance development, and to simulate treatment scenarios for dose selection and optimization across different therapeutic contexts.

In recent years, pharmacometrics approaches have been increasingly used to support translational studies in the development of antibiotic therapies. In particular, semi-mechanistic PK-PD models have proven valuable in preserving the time-course dynamics of antibiotic exposure, its effects on bacterial populations, and the associated development of resistance. Some studies have even demonstrated that well-developed semi-mechanistic models can be used to derive PK-PD indices, yielding results comparable to those obtained from preclinical *in vivo* studies. Beyond characterizing the PD of single agents, PK-PD models also have the capacity to describe complex interactions in combination therapies and to capture the dynamics of resistance emergence. This makes them especially useful for guiding dose selection in scenarios involving multiple antibiotics or a high risk of resistance. Nevertheless, dose selection using PK-PD models is often limited to a predefined set of dosing schedules, which may overlook more

effective or optimized regimens. To address this limitation, in this thesis, we present the development of a framework for the systematic optimization of antibiotic dosing regimens, enabling model-based dose selection to be both more efficient and better tailored to therapeutic goals.

Scope

The main objective of this study is to leverage the use of pharmacometric and statistical modeling methods to support the design of effective antibiotic therapies against antimicrobial resistance. The work presented in this thesis integrates *in vitro* and clinical observations with statistical analyses to extract insights into the evolution of antibiotic resistance and to evaluate how such information can guide the development of an effective antibiotic treatment strategy. Finally, this study demonstrates how PK-PD insights can guide the rational design of dosing strategies that leverage CS to obtain an effective antibiotic treatment that also minimizes the establishment of resistance during the treatment.

In **Section I – The Pharmacodynamics of Antibiotics and Resistance Evolution**, we applied pharmacometric approaches alongside microbiological assays to better characterize antibiotic PD and the processes underlying resistance development. The outcomes of these analyses were used to derive clinical drug exposure targets for a specific antibiotic, identify potential mechanisms of specific resistance evolution, and evaluate the potential impact of bacterial community composition on antibiotic therapy.

In **Chapter 2**, we developed and applied PD models to characterize bacterial population dynamics and resistance emergence under antibiotic pressure. Using teicoplanin as a model drug, we conducted experiments with dynamic drug concentrations mimicking patient PK profiles to capture resistance dynamics under clinically relevant conditions. The study included several highly virulent pathogens, including *Enterococcus faecium*, methicillin-sensitive *Staphylococcus aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), and *Staphylococcus epidermidis*. In **Chapter 3**, we showed how the integration of mechanistic knowledge, such as drug influx, efflux, and degradation kinetics, into PD models can be beneficial for evaluating potential mechanism of antibiotic resistance. The resulting model was then used to explore potential resistance mechanisms to the β-lactam and β-lactamase inhibitor combination piperacillin-tazobactam in *Klebsiella pneumoniae*. In **Chapter 4**, we investigated how the antibiotic sensitivity of a bacterial strain can be influenced not only by its intrinsic properties but also by interactions with other bacterial species present in polymicrobial infections. This study highlights the importance of accounting for microbial community context when evaluating treatment strategies.

In **Section II – From PK-PD to Clinical Dose Optimization**, we underlined the importance of PK knowledge to support the development of an effective antibiotic therapy and demonstrated how the integration of PK knowledge with PD insights is essential for translating experimental findings into a safe and effective therapy.

In **Chapter 5**, we developed a teicoplanin population PK model for renally impaired patients undergoing maintenance hemodialysis. We then demonstrate the utility of this model in optimizing teicoplanin dosing for this patient group. In **Chapter 6**, we explored the use of a PBPK model to predict antibiotic concentrations at pulmonary infection sites, particularly within the lung epithelial lining fluid and alveolar macrophages. This work highlights how drug properties and physiological variability can influence pulmonary drug distribution and demonstrates the potential of QSPR

approaches to support PBPK modeling practices to predict target-site exposure for antibiotics with known structure but limited distribution data. Finally, in **Chapter 7**, we developed and evaluated an analytical framework that integrates PK and PD information to support dose and schedule selection, with combination antibiotic therapy as the primary case study. The resulting dosing regimen optimization framework offers a flexible and clinically informed approach to designing rational combination antibiotic therapies grounded in both quantitative modeling and real-world clinical insights.

In **Section III – Collateral Sensitivity**, we characterized and evaluated the use of CS as a potential strategy for combination antibiotic therapy to combat antimicrobial resistance. Chapters in this section focused on two major challenges related to the application of CS: the consistency of CS emergence and its practical implementation in clinical settings.

In **Chapter 8**, we conducted a large-scale investigation of CS occurrence among clinical isolates. This study also examined both intra- and inter-species consistency in CS emergence among clinically relevant pathogens. By identifying conserved CS relationships, the study aimed to uncover broad-spectrum CS pairs that could potentially be useful against multiple pathogenic species. In **Chapter 9**, we investigated the emergence of consistent CS among antibiotic-resistant *Streptococcus pneumoniae* mutants from a well-characterized laboratory strain. This study revealed the heterogeneity of the resulting mutants' antibiotic sensitivity profiles, highlighting the complexity and stochastic nature of resistance evolution. Experimental characterization of these strains was then used to inform the development of (PK)PD models, which were in turn used to evaluate the therapeutic potential of CS-based combination antibiotic strategies. Our findings suggest that a certain CS-based dosing regimen could be strategically leveraged to suppress the establishment of resistance during systemic *S. pneumoniae* infection. While Chapter 9 evaluated CS emergence using a single laboratory strain as the parental background, **Chapter 10** expanded this investigation by exploring CS occurrence in four clinical *Pseudomonas aeruginosa* isolates. This study also accounted for potential PD interactions between antibiotic pairs, adding an additional layer of complexity to the assessment of combination therapies. As in Chapter 9, experimental data were used to develop (PK)PD models, which then served as platforms to evaluate the efficacy of CS-informed antibiotic combinations. In addition, an *in vitro* treatment simulation was conducted to demonstrate that the proposed CS-based therapy identified through model-based analysis can be used to suppress or delay the establishment 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.

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