Developing predictive PK/PD models to optimise dosing regimens for antibiotics for gram-negative infections

A thesis submitted in partial fulfilment of the requirements of Liverpool John Moores University for the degree of Doctor of Philosophy

November 2025

Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Acknowledgements

I came from Vietnam, a developing country where opportunities for higher education are not always available. To be able to attend Liverpool Johnn Moores University is not only an excellent chance that I have been given but it also brings a huge sense of pride to my family. This opportunity means a lot to me. It is like a door that has opened for me to a better future with knowledge and possibilities that I have been looking for. I would firstly like to express my deep gratitude to my supervisors who guided me during my PhD journey.

To Neil, your unwavering support welcomed me from the moment I set foot in Liverpool. I do appreciate you taking the time to answer all of my silly questions. Your great support helped me achieve my first significant milestones, allowing me to understand how a project should be planned and run to produce qualified results. I cannot thank you enough for believing in me and pushing me to do better.

To Suzanne, your extensive experience guided me through challenges I once thought were impossible. I now truly appreciate that pursuing a PhD is not merely about finishing a project myself; instead, I should learn how to actively collaborate with others to achieve the goals. The way you patiently supported me in contacting hospitals for data collection and staying on track with deadlines helped me to feel confident and productive with my work.

To Judy, your profound knowledge of complex modelling has transformed my perspective. Throughout this 4-year journey, you have been very kind and understanding since the first steps of my studies. Your valuable advice helped me to look at the details that often go unnoticed but might lead to biased results. Without your supervision, I would not have finished this challenging journey.

To Peter, your insightful advice helped me understand the miracle of R, using it to visualise the findings of a systematic review and modelling. I have applied it to all aspects beyond my research findings. Whenever I encountered difficulties, your thought-provoking questions helped me reframe my perspective and tackle the issues more effectively.

To my dear family, I want to express my deep love for joining me on this challenging journey. To my wonderful husband, Thuan, thank you for your boundless love that made the hard times here more bearable. To my beloved son, I could not believe

that you did not cry even when you could not say an English word when you first came to England. Your strength during those moments has touched my heart more than words can express. To my dearest Mom, thank you for your constant care while I navigated this new chapter of my life. You always encourage me to face challenges with confidence because the family is all beside me.

Even though you are far away, Dad, your presence is always in my heart. Your spirit lights my way, reminding me that I am never alone.

Thank you all for making this journey an unforgettable and rewarding experience.

Abstract

Pharmacokinetic/pharmacodynamic (PK/PD) modelling and simulation are increasingly recognised as powerful tools for optimising antibiotic dosing. This approach plays a critical role in enhancing the effectiveness of the older generation of antibiotics, preserving the efficacy of new generation agents and overcoming the increasing problem of antibiotic resistance. Yet, despite this potential, their use in clinical practice is still limited, especially in low- and middle-income countries. Here, a lack of patient data, the complexity of models, and the absence of supporting frameworks often result in suboptimal dosing and unfavourable outcomes. These issues become even more challenging when treating severe infections caused by multidrug-resistant Gram-negative bacteria (MDR GNB).

This thesis aims to provide practical solutions by developing population PK models from existing published data and integrating them with simplified PD models. Together, these models offer a framework to test dosing regimens that can be adapted to real-world clinical settings.

The work is presented in three phases. Phase I was a systematic review that identified PK/PD targets for antibiotics against MDR GNB, with the aim of determining which indices, whether EUCAST-recommended or more stringent thresholds, were most relevant for guiding dosing in clinical practice. Phase II examined current antibiotic use at two study sites: Cho Ray Hospital (Vietnam) and the Countess of Chester Hospital NHS Foundation Trust (UK), using retrospective data collected from both. Eligible data were analysed and simulations applied to assess the likelihood of achieving PK/PD targets across different MIC values. Phase III focused on meropenem and ceftazidime/avibactam, developing PK/PD model that incorporated assumptions of synergistic activity against KPC-producing *Klebsiella pneumoniae*. Simulations showed that no single regimen consistently achieved targets, underlining the limitations of empirical dosing.

Overall, this thesis provides a practical framework for dual antibiotic modelling and highlights the importance of precision, model-informed strategies to improve outcomes in MDR GNB infections.

Abbreviations

Abbreviation	Full term
AAG	Alpha-1-acid glycoprotein
ADME	Absorption, Distribution, Metabolism, and Excretion
AIC	Akaike Information Criterion
ARC	Augmented renal clearance
AUC	Area Under the Curve
BMI	Body Mass Index
BSIs	Bloodstream infections
BSV	Between-subject variability
CI	Continuous infusion
Cl/CL	Clearance
C_{max}	Maximum concentration of a drug in blood plasma
C_{min}	Minimum concentration of a drug in blood plasma
CoCH	The Countess of Chester Hospital
C_{peak}	Peak (Maximum) Plasma Concentration
CRAB	Carbapenem-resistant *Acinetobacter baumannii*
CrCl	Creatinine Clearance
CRE	Carbapenem-resistant *Enterobacterales*
CRH	Cho Ray Hospital
CRPA	Carbapenem-resistant *Pseudomonas aeruginosa*
CRRT	Continuous Renal Replacement Therapy
C_{trough}	Trough (Minimum) Plasma Concentration
CVVHDF	Continuous Veno-Venous Hemodiafiltration
DPA	Data Protection Act
DTR	Difficult-to-treat
EMA	The European Medicines Agency
ESBLs	Extended-Spectrum Beta-Lactamases
E(t)	Effect at time t (drug effect at a given time point)
*f*T>MIC	The time that the unbound antibiotic concentration remains above the Minimum Inhibitory Concentration
*f*AUC/MIC	Ratio of the area under the unbound drug concentration–time curve to the Minimum Inhibitory Concentration

Abbreviation	Full term
*f*Cmax/MIC	Ratio of the maximum unbound drug concentration to the Minimum Inhibitory Concentration
GNB	Gram-negative bacteria
GOF	Goodness-of-Fit
HRA	Health Research Authority
IC_{50}	Half maximal inhibitory concentration
ICU	Intensive care unit
IDSA	Infectious Diseases Society of America
IIV	Interindividual variability
IV	Intravenous
LJMU	Liverpool John Moores University
KPC	*Klebsiella pneumoniae* carbapenemase
MD	Maintenance doses
MDR GNB	Multidrug-resistant gram-negative bacteria
MIC	Minimum Inhibitory Concentration
MIPD	Model-informed precision dosing
NaN	Not a Number
NCA	Noncompartmental analysis
NDM	New Delhi metallo-beta-lactamase
NHLBI	The National Heart, Lung, and Blood Institute
Non-RCTs	Non-randomised clinical trials
ODEs	Ordinary differential equations
PAE	Post-antibiotic effects
PBPK	Physiologically based pharmacokinetic
PK/PD	Pharmacokinetic/Pharmacodynamic
PopPK	Population PK
PTA	Probability of target attainment
RCT	Randomised clinical trial
RoB 2	The Cochrane risk-of-bias tool for randomised trials
ROBINS-I	The Cochrane Risk of Bias In Non-randomised Studies of Interventions
R.S.E.	Relative Standard Errors
SAEM	Stochastic approximation expectation-maximisation

Abbreviation	Full term
SSIs	Surgical site infections
$t_{1/2}$	Elimination Half-life (Time required for the drug concentration to reduce by half)
TDM	Therapeutic drug monitoring
US FDA	The United States Food and Drug Administration
UTIs	Urinary tract infections
VAP	Ventilator-associated pneumonia
Vd	Volume of distribution
VPC	Visual predictive checks
XDR	Extensively drug-resistant

List of Tables

List of Figures

Table of Contents

Chapter 1 Introduction

1.1 Hospital-acquired gram-negative infections

Hospital-acquired infections (HAIs) remain a major global health challenge, particularly for low- and middle-income countries. Research conducted by Balasubramanian et al. (2023) indicates that middle-income countries experienced approximately 119 million annual cases of HAIs attributable to drug-resistant bacteria, with about 86.98 million cases (approximately 73%) of these cases caused by drug-resistant gram-negative bacteria (Balasubramanian et al., 2023). The economic impact of HAIs places a significant burden on both societies and healthcare organisations. According to the World Health Organisation (WHO), the average incidence of hospital-acquired infections globally in 2023 was 1 in every 10 patients (WHO, 2023). This issue is even more critical among critically ill patients. Notably, the EUROBACT-2 international cohort study, which assessed 333 intensive care units (ICUs) across 52 countries, revealed that roughly 78% of hospital-acquired bloodstream infections occurred in ICU patients (Tabah, 2023).

Gram-negative bacteria (GNB) were identified as the predominant pathogens, accounting for 59% of these cases in this study. *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacteriaceae* (including *Klebsiella pneumoniae* and *Escherichia coli*) are of particular concern. These pathogens are associated with severe infections such as ventilator-associated pneumonia (VAP), bloodstream infections (BSIs), urinary tract infections (UTIs), and surgical site infections (SSIs). In its 2017 report, WHO highlighted these bacteria as critical priority strains, raising an urgent demand for action (Tacconelli et al., 2018). Their resistance to treatment contributes to millions of deaths each year (Ferri et al., 2017; Mudenda et al., 2023). GNB have developed several complex mechanisms to resist antibiotics, including the following:

i. Enzyme production: enzymes such as extended-spectrum beta-lactamases (ESBLs) and carbapenemases inactivate antibiotics by hydrolysing beta-lactam rings or through the O-phosphorylation of aminoglycosides (Drawz et al., 2010; Ramirez et al., 2010).
ii. Efflux pumps: help bacteria to survive in high concentrations of antibiotics by actively pumping the antibiotics out of the cell (Li et al., 2009).

iii. Permeability reduction: commonly found in gram-negative strains due to differences in the structure of their outer membrane, which allows them to limit the diffusion of antibiotics (Nikaido, 2003).

iv. Target alteration: prevent antibiotics from binding effectively by modifying the specific bacterial molecule or site that an antibiotic normally binds to, thereby reducing or preventing the drug's ability to exert its effect (Wright, 2007).

v. Biofilm formation: several bacteria, including *K. pneumoniae* and *P. aeruginosa*, can form biofilms that impede antibiotic penetration (Bjarnsholt et al., 2008; Hoiby et al., 2010).

A systematic review by Murray et al. (2022) established that bacterial resistance caused 4.95 million deaths, and 1.27 million deaths were attributed to it in 2019, making it one of the leading causes of death worldwide (Murray, 2022). If not addressed, it is estimated that this threat may cause approximately 10 million deaths and sustain a global capital loss of $300 billion to $1 trillion by 2050 (O'Neill, 2016; Burki, 2018). Gene mutations in the inner or outer membranes of microorganisms have primarily resulted in the mechanisms outlined above, which lead to reduced antibiotic effectiveness and the rapid development of antimicrobial resistance across multiple anti-infective agents. This has consequently reduced the number of treatment options available to healthcare providers. Consequently, antibiotic resistance has discouraged pharmaceutical investment in new drug development (Chen, 2020). This is because, unlike other medications, once an antibiotic becomes resistant, it is no longer viable or profitable for these companies. The appropriate use of both existing and novel antibiotics is essential to ensure their effectiveness and preserve them for future use.

1.2 The challenge of treating hospital-acquired infections

Nosocomial infections caused by MDR GNB represent a challenge in ICUs, where critically ill patients face heightened risks. These patients often have compromised immune systems that make them more susceptible to infections, alongside the frequent use of invasive devices such as catheters and ventilators, which can serve as entry points for pathogens (Blot et al., 2022). Furthermore, the complexity of treating gram-negative healthcare-associated infections is amplified by multiple contributing factors. The inherent biological characteristics of these resistant

bacteria, as mentioned, play a crucial role, as they have mechanisms that allow them to reduce the amount of antibiotics going through the cell wall, thereby limiting available therapeutic options (Chacko et al., 2023).

Moreover, the presence of underlying health conditions or comorbidities, such as renal or hepatic impairment, critical illness, obesity, or altered perfusion, can significantly alter a patient's pharmacokinetic parameters, including drug absorption, distribution, metabolism, and elimination (Lea-Henry et al., 2018; Morales Castro et al., 2023). These physiological changes not only complicate the management of infections but also increase the risk of subtherapeutic or toxic drug concentrations. As a result, a more individualised and adaptive approach to antibiotic dosing is required to optimise therapeutic efficacy and suppress the resistance in these vulnerable patient populations.

In clinical practice, antibiotic therapy is often prescribed empirically in practice, meaning that prescribers may start treatment based on their prediction of the most likely pathogens and best available evidence. Delaying the start of antibiotics until microbiological culture results are confirmed might contribute to the high rate of 30-day all-cause mortality (De la Rosa-Riestra et al., 2024). However, after the isolated bacteria have been identified, the continued use of antibiotics empirically without a systematic method for optimising key treatment components, such as the appropriate dosage, administration routes, and infusion duration, was not recommended. This is especially the case for older antibiotics, which may not have been updated in line with emerging resistance patterns, placing additional difficulties in the pathway to effective treatment.

In particular, the use of antibiotics in developing countries has been reported to be sub-optimal due to the lack of resources, overcrowding and infrastructure (Gandra et al., 2020; Sulis et al., 2023). Additionally, co-infections caused by multiple pathogens are commonly reported in hospitals, particularly involving drug-resistant strains. Research by Ny et al in 2015, reported that approximately 27% of infections caused by *K. pneumoniae* might be infected together with carbapenem-resistant *P. aeruginosa* or *A. baumanni* (Ny et al., 2015). Such co-infections may exacerbate the clinical symptoms, resulting in higher mortality rates and prolonged hospital stays (Tamma et al., 2012; Adediran et al., 2020; Sophonsri et al., 2023).

This further complicates the antibiotic treatments, often necessitating the simultaneous use of multiple antibiotic classes. Co-administration of two or more antibiotics may result in pharmacokinetic interactions, leading to either reduced or elevated drug levels, thereby altering the risk profile for adverse effects (Meyer et al., 2019; Greve et al., 2022; Simoni et al., 2024). Combination therapy is increasingly employed in clinical practice to address complex infectious diseases (Torella et al., 2010; Tyers et al., 2019; Bognár et al., 2024). Such an approach has been considered to extend bacterial coverage while limiting resistance, particularly in polymicrobial, septic, or multidrug-resistant infections.

1.3 Combination of antibiotics in clinical practice

Originating from the research on the treatment of tuberculosis, the combination of antibiotics might reduce the rate of development of bacterial resistance (Fox et al., 1999; Kerantzas et al., 2017). Whilst for a single antibiotic, the bacteria may develop resistance in a short period of time, the ability to develop resistance to several antibiotics when prescribed simultaneously is low. This has implications for the treatment of MDR tuberculosis. Using two, three or four different antibiotics reduces the chance of the emergence of this most deadly bacterial resistance (Larkins-Ford et al., 2023). The United States Food and Drug Administration (US FDA) supports the use of antibiotic combinations for life-threatening cases of tuberculosis (FDA, 2013). Considerable research has examined antibiotic combinations, yet systematic reviews and meta-analyses have reported conflicting results. For example, the study by Paul et al. (2003) found no superiority of beta–lactam–aminoglycoside therapy over beta-lactam combinations for MDR-GNB, whereas Guillamet et al. (2023) later supported aminoglycoside use in sepsis (Paul et al., 2003; Paul et al., 2014; Guillamet et al., 2023). Cheng et al. (2018) found that colistin monotherapy and colistin-based combinations showed comparable efficacy against carbapenem-resistant GNB (Cheng et al., 2018). Similarly, the study conducted by Samal et al. (2021) indicated no significant differences between colistin monotherapy and combination therapy, although they noted that the latter was more beneficial for managing severe infections. Conversely, in 2019, the guidelines from six international organisations, including the American College of Clinical Pharmacy (ACCP), the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), the Infectious Diseases Society of America (IDSA), the International

Society for Anti-infective Pharmacology (ISAP), the Society of Critical Care Medicine (SCCM), and the Society of Infectious Diseases Pharmacists (SIDP), suggest using colistin in combination with one or two additional agents, typically a beta-lactam or tigecyclines or aminoglycosides, against Carbapenem-resistant *Enterobacterales* (CRE) where meropenem MIC below 8 mg/L or against *Carbapenem-Resistant P. aeruginosa (CRPA)* with susceptible MIC (Tsuji et al., 2019). Combination therapy demonstrated superior cure rates relative to colistin–beta-lactam alone (Tsuji et al., 2019).

Since the publication of the 2019 international consensus guidelines on polymyxin use, which cautiously endorsed colistin-based combination therapy for MDR GNB infections due to its synergistic potential and role in preventing resistance, the 2024 IDSA guidelines have reflected a more selective and evidence-driven approach (Haddad, Mamta Jain, et al, 2024). While the 2019 guidelines supported combination regimens, particularly with meropenem, tigecycline, rifampin, or aminoglycosides, for pathogens such as CRE, Carbapenem-resistant *A. baumannii* (CRAB), and CRPA, the 2024 update limits the use of colistin primarily to situations where newer, less toxic agents such as newer beta-lactams or cefiderocol are unavailable. Infections caused by CRE or CRPA should preferentially be treated with active beta-lactams (i.e., ceftazidime-avibactam, cefiderocol) as monotherapy, reserving combination therapy with polymyxins only for refractory cases or CRAB infections lacking alternative options. This shift reflects growing clinical trial evidence, heightened awareness of polymyxin-associated nephrotoxicity, and improved access to novel antimicrobials. Nonetheless, the 2024 IDSA guidelines continue to recommend various antibiotic combinations (refer to Table 1-1) for treating multidrug-resistant gram-negative infections, such as NDM-producing strains, or for individuals with severe infections (Tamma et al., 2024). Although some studies have shown that the mortality rate or treatment effectiveness between the mono-antibiotic therapy group and the combination group is not statistically significant, antibiotic combinations, if applied appropriately, may be favoured compared to using a single antibiotic, such as the use of ceftazidime-avibactam and aztreonam in treating NDM and other MBL-producing *P. aeruginosa* as recommended by the 2024 ISDA guidelines. In low-resource hospital settings, where the new antibiotics might not always be available, the antibiotic combination therapy has still been common in clinical practice (Tyers et al., 2019). Additionally,

the use of antibiotic combinations under PK/PD model-guided approach to optimisation of dosing regimens has not been well established.

Table 1-1. The combination of antibiotics recommended by ISDA 2024

Antibiotic combination therapy	Bacteria and infections
Meropenem + polymyxins/aminoglycosides	Carbapenemase-producing *K. pneumoniae* bloodstream infections with meropenem MIC is 8-16 μg/ml
Imipenem-cilastatin-relebactam/ imipenem-cilastatin + colistin	Gram-negative organisms not susceptible to imipenem (i.e. *Enterobacterales*)
Ceftazidime-avibactam + aztreonam	NDM and other Metallo-beta-lactamases (MBLs) -producing *Enterobacterales* infections MBL-producing *P. aeruginosa*
Aztreonam + meropenem-vaborbactam/imipenem-cilastatin-relebactam	NDM and other MBL-producing *Enterobacterales* infections
Imipenem-cilastatin-relebactam + tobramycin/polymyxin B	Difficult-to-treat (DTR) *P. aeruginosa*
Sulbactam-durlobactam + a carbapenem (i.e. imipenem-cilastatin or meropenem) *Or* **high-dose ampicillin-sulbactam (total daily dose of 9 grams of sulbactam) + at least 1 other agent (i.e., polymyxin B, minocycline > tigecycline, or cefiderocol)**	CRAB

1.4 Traditional therapeutic drug monitoring and model-informed precision dosing

Therapeutic drug monitoring (TDM) is essential for optimising antibiotic dosing. Traditional TDM informs prescribers of the pharmacokinetic profile by using antibiotic concentrations measured at designated time points to ensure they remain within the therapeutic range (Del Valle-Moreno et al., 2023). However, traditional TDM has predominantly focused on antibiotics with narrow therapeutic indices, such as aminoglycosides, teicoplanin, colistin, vancomycin, and daptomycin. These

antibiotics require specific TDM as even minor variations in concentrations can result in inadequate therapeutic outcomes or, conversely, adverse toxic effects. Depending on the specific pharmacokinetic properties of each antibiotic, either the trough (C_{trough} or C_{min}) or peak concentrations (C_{peak} or C_{max}) will be monitored to ensure that these concentrations stay within the established therapeutic range, thereby minimising the risk of toxicity and ensuring the effectiveness of the treatment. Figure 1-1 shows the example of amikacin concentrations measured in five patients from the study by D'Arcy et al. (2012), using the C_{min} and C_{max} to predict the volume of distribution and clearance of amikacin and by doing so optimising the dosing regimens for patients with continuous venovenous haemodiafiltration (CVVHDF) (D'Arcy et al., 2012).

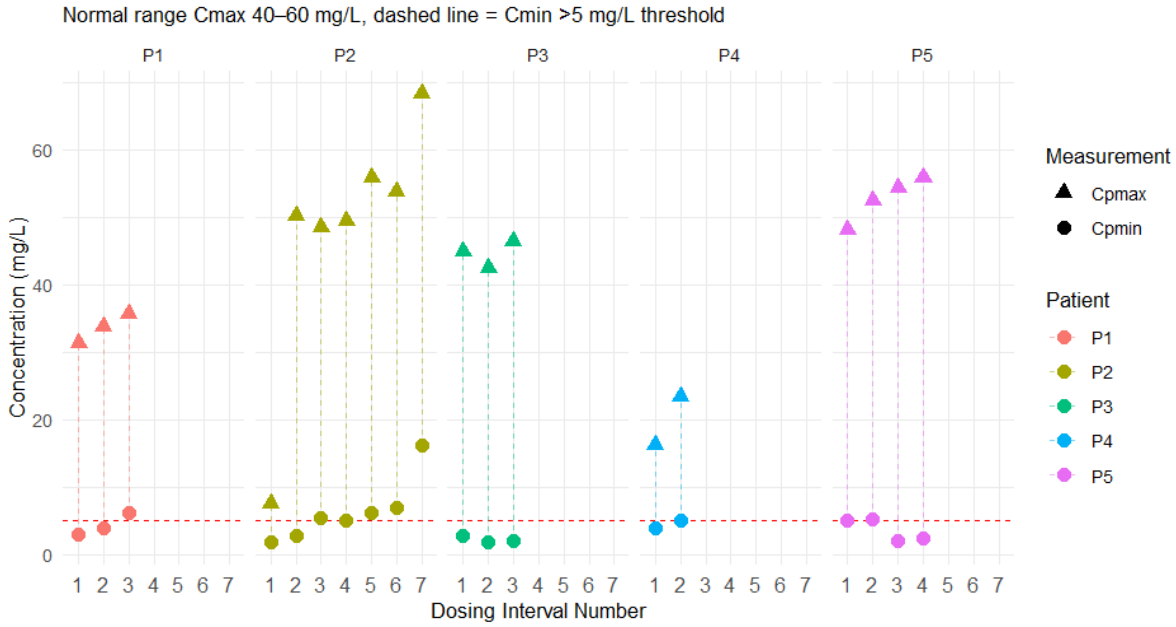


Figure 1-1. Example of amikacin TDM using findings from the study of D'Arcy et al, 2012.

The Figure 1-1 shows the TDM results for five patients receiving amikacin, with a C_{max} range of 40–60 mg/L and a C_{min} toxicity threshold of 5 mg/L. Two patients demonstrated optimal levels within the therapeutic range (P3 and P5), indicating effective and safe dosing. One patient showed subtherapeutic peaks with borderline toxic troughs (P1), suggesting a need for dose adjustment. Another patient had therapeutic peaks but elevated trough levels, raising concerns about potential toxicity (P2). The remaining patient exhibited low peak concentrations despite safe troughs, indicating underdosing (P4). These findings underscore the importance of individualised dosing based on TDM to optimise efficacy while minimising toxicity.

Nevertheless, the adjustment related to traditional TDM is often delayed until the concentrations stabilise, which takes approximately four to five half-lives to achieve, resulting in a timeframe of at least 12 hours and possibly up to 10 days after administration (Rybak, 2006; Estes et al., 2010). In contrast to traditional TDM, model-informed precision dosing (MIPD) allows proactive dose selection based on predicted concentration-time profiles and individual patient factors before reaching subtherapeutic or toxic levels. It has also been widely utilised for various categories of antibiotics, extending beyond those with a narrow therapeutic index and applicable to a broader range of patients, not just those who are critically ill. This approach enhances the effectiveness and safety of antibiotic use. With the advancement of modelling and simulation, PK/PD modelling is an upgraded discipline of traditional TDM to inform the dosing regimens for each individual, by integrating the patient demographics and individual plasma concentrations using a population PK model or PK/PD model, to administer the most appropriate antibiotic therapies (Nielsen et al., 2013). MIPD facilitates the practice of using antibiotics by optimising dosing regimens based on individual patient characteristics and pharmacokinetic/pharmacodynamic principles. Figure 1-2 highlights several characteristics of each strategy; on balance, MIPD is the more favourable approach.

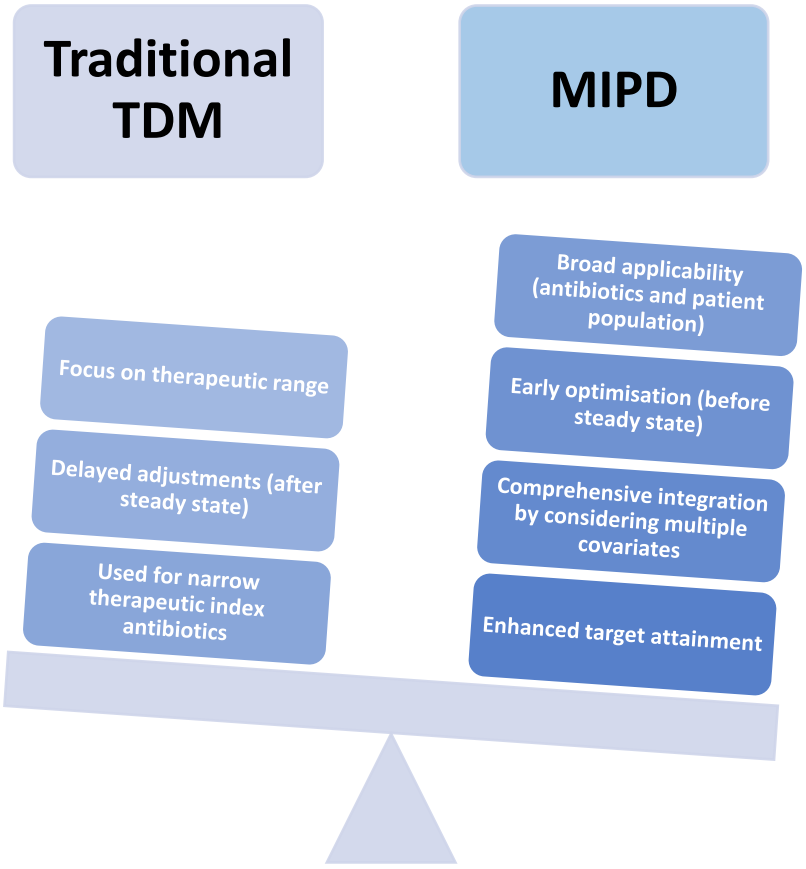


Figure 1-2. TDM and MIPD in the optimisation of antibiotic dosing regimens.

However, there are barriers hindering the practical application of MIPD, such as model complexity, a lack of awareness and training, and inadequate legal frameworks for model adoption in practice (Kantasiripitak et al., 2020; Dibbets et al., 2024). To implement a PK/PD model, serial blood sampling is required to determine a patient's pharmacokinetic parameters, such as peak or trough concentration of free drug or the time the concentrations remain above the MIC (*f*T>MIC). This prevents the majority of prescribers from using this approach to modify the dosage for individuals. In order to measure the concentrations to calculate Area under the curve (AUC), C_{max}, C_{min}, specific antibiotic kits for quantitation and on-site machines are required to determine the concentration-time profiles of the antibiotics (Parthasarathy et al., 2018). These resources are unlikely to be found in clinical settings, particularly in low-resource areas, making their implementation challenging. Additionally, there is still no consensus regarding the optimal PK/PD targets that can achieve the best clinical efficacy, which makes it difficult to use PK/PD to optimise dosing regimens. Furthermore, the value of PK/PD parameters derived from *in vitro* studies or by using animal models may not be desirable targets for achieving favourable outcomes in real-world situations. When translating such exact preclinical data to patients, it is crucial to urge caution, especially for those suffering from multidrug-resistant bacterial infections, as the anticipated improvements may not be achieved with these PK/PD values. Hence, there is a need to identify and validate impactful PK/PD targets of antibiotics in treating gram-negative microorganisms that can effectively address the complexities posed by real-world clinical scenarios.

1.5 Understanding PK/PD relationships in antibiotic therapy

1.5.1 Pharmacokinetic parameters

Pharmacokinetics (PK) describes how the antibiotic is absorbed, distributed, metabolised and eliminated (ADME) in the body. The concentration of an antibiotic (in plasma and tissues) will change over time due to the ADME process, which determines the drug's PK profile. Several metric factors are used to examine the association between the dosage and the efficacy of antibiotics, including the bioavailability (F), volume of distribution (Vd), protein binding, clearance (Cl), and half-life ($t_{1/2}$). These factors affect the robustness of PK and PD modelling.

1.5.1.1 Bioavailability

Bioavailability (F) significantly impacts PK model predictions by influencing key parameters like AUC, C_{max}, and time above MIC (T>MIC) (Ullah et al., 2020). Accurate estimation and incorporation of F are necessary to ensure the reliability of PK models for antibiotics, particularly for oral or non-intravenous routes (Van den Broek et al., 2021). The bioavailability of parenteral antibiotics is generally considered 100% when administered via intravenous (IV) injection or infusion because the drug is directly introduced into the systemic circulation, bypassing absorption processes in the gastrointestinal tract and first-pass metabolism.

1.5.1.2 Volume of distribution (Vd)

The Volume of distribution (Vd) represents a proportionality constant relating the total amount of an antibiotic (dose) to the plasma concentration (Cc) at a given time. The influence of Vd can be explained by this equation:

$$\text{Vd (L)} = \frac{\text{Amount of antibiotic (mg)}}{\text{Plasma concentration of antibiotic (mg/L)}} \qquad \textit{(Equation 1)}$$

When the Vd increases, the plasma concentration of the antibiotics decreases accordingly due to greater dilution. This leads to a lower *f*T>MIC, as well as reduced Cmax and AUC, resulting in decreased *f*AUC/MIC and *f*Cmax/MIC (See Section 1.5.3 for details).

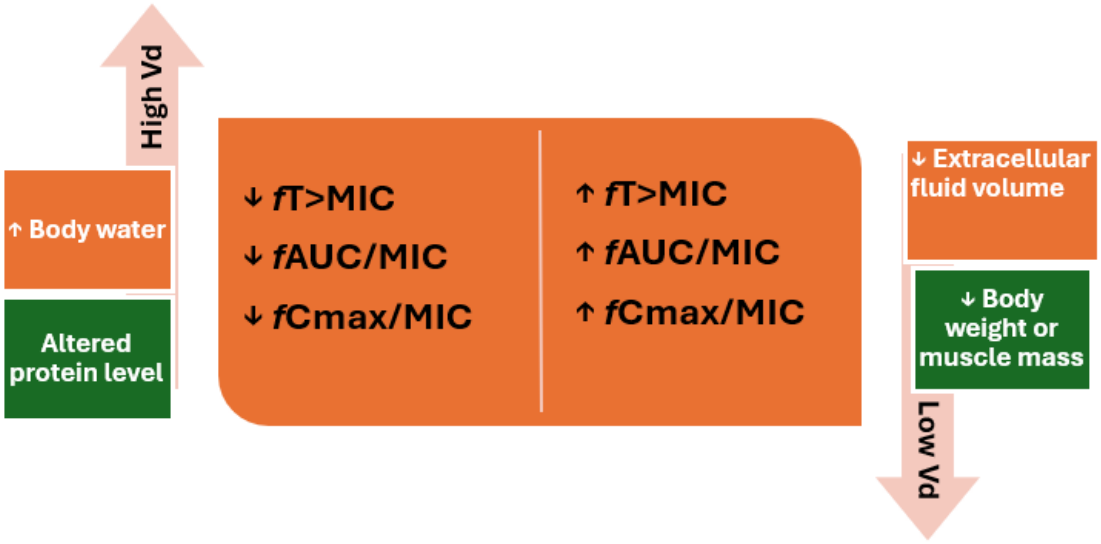


Figure 1-3. Effects of patient pharmacokinetic alteration on PK/PD targets

1.5.1.3 Clearance

Clearance (Cl) is a PK parameter that indicates the volume of plasma completely cleared by the antibiotic per unit of time. Like Vd, Cl is a key parameter in PK and PD that influences drug exposure, elimination, and therapeutic efficacy. In PK/PD modelling, accurate estimation of Cl is essential for predicting drug concentrations, optimising dosing regimens, and ensuring effective treatment. This is because Cl is a primary determinant of drug exposure and directly affects the accuracy and robustness of PK and PD models. The relationship between Vd and Cl is as follows:

$$Cl = \frac{Vd \times ln2}{t_{1/2}} \qquad \textit{(Equation 2)}$$

- Higher Cl → Faster drug elimination → Shorter half-life → More frequent dosing required.
- Lower Cl → Slower drug elimination → Longer half-life → Potential for drug accumulation.

In a one-compartment PK model, Cl determines the elimination phase by this equation:

$$C_t = C_0\, e^{-kt} \text{ , where } k = \frac{Cl}{Vd} \qquad \textit{(Equation 3)}$$

While, in two or more compartment models, Cl affects both distribution and elimination phases, making the models more complex.

- Low Cl → Higher drug accumulation → Increased toxicity risk (i.e., vancomycin nephrotoxicity).
- High Cl → Lower drug exposure → Risk of subtherapeutic levels (i.e., ceftazidime underdosing in ICU patients).

1.5.1.4 Protein binding

Drugs in the bloodstream exist in two forms:

- Bound drug → Attached to plasma proteins (i.e., albumin, α1-acid glycoprotein).
- Free drug (unbound) → Pharmacologically active and able to distribute to tissues.

Protein binding significantly influences key pharmacokinetic parameters such as Vd, Cl and ultimately, antibiotic efficacy. This is particularly relevant for time-dependent antibiotics like beta-lactams, whose pharmacodynamic activity relies on maintaining free drug concentrations above the minimum inhibitory concentration (*f*T>MIC) throughout the dosing interval. Since only the unbound fraction of a drug is microbiologically active, alterations in protein binding can substantially affect therapeutic outcomes. Conditions such as hypoalbuminemia, often caused by malnutrition or inflammation, reduce protein binding and increase the unbound fraction of drugs (Sharif, 2025). While this may enhance efficacy, it also raises the risk of toxicity.

1.5.1.5 Half-life

The half-life ($t_{1/2}$) of an antibiotic is the time required for its plasma concentration to decrease by 50%. It is a fundamental PK parameter influencing drug dosing regimens, frequency, and therapeutic effectiveness. In PK models, half-life directly affects drug accumulation, steady-state levels, and clearance predictions.

$$t_{1/2} = \frac{ln2 \; x \; Vd}{Cl} \qquad \textit{(Equation 4)}$$

- An increase in Vd → Increases half-life (drug remains in the body longer).
- An increase in Cl → Decreases half-life (drug is eliminated faster).
- Drugs with short half-lives (<2 hours, i.e., meropenem) require frequent dosing to maintain effective concentrations.
- Drugs with long half-lives (>12 hours, i.e., azithromycin) require less frequent dosing (once-daily or less).
- Steady-state is reached after 4-5 half-lives, meaning longer half-life drugs take longer to stabilise.

1.5.2 PK modelling

Antibiotic PK is estimated by fitting mathematical models to measured drug concentrations in patients. The simplest is a one-compartment model, which treats the body as a single well-mixed space and uses two parameters, Vd and Cl. If this simple model cannot explain the data, additional compartments can be added to describe distribution phases, each with its own rate constants describing drug

movement between compartments. Population PK modelling applies the same approach to a cohort, providing typical (mean/median) values and variability for parameters like Vd and Cl, thereby quantifying how patients differ from one another. PK parameters act as covariates that describe between-subject variability (BSV) among individuals within a population. By using patient factors such as age, weight, serum creatinine, disease states, and drug interactions, which mainly affect the ADME of the drugs inside the body, the deviation in plasma concentrations of individual patients can be described from the population mean. Covariates may improve the accuracy and reliability of model-based predictions, allowing for better understanding and prediction of drug behaviour and effects in specific patient groups (Sanghavi et al., 2024).

$$\theta_i = \theta_{pop} \times e^{nj} \qquad \textit{(Equation 5)}$$

The equation above represents the BSV in PK and PD modelling derived from nonlinear mixed-effect modelling (NLME), one of the primary frameworks used for population PK and PD models. This approach employs a log-normal distribution to account for variability, assuming that individual parameter values are log-normally distributed around the population mean parameter to ensure that individual parameter values remain positive.

PopPK modelling provides a framework to describe and explain inter-individual variability (IIV) in drug disposition. Unlike classical PK studies that report average parameters, PopPK quantifies both central tendency (typical Cl, Vd) and variability, enabling predictions at both population and individual levels (Bulman et al., 2022; Greppmair et al., 2023).

By incorporating patient-specific covariates, PopPK models identify sources of variability that are clinically meaningful. For example, kidney function, often measured as creatinine clearance (CrCl), is a well-recognised driver of meropenem clearance. Likewise, a patient's body weight and albumin levels can affect how the drug is distributed and bound in the bloodstream. These models therefore help prescribers understand not only that drug exposure differs between patients, but also the reasons behind those differences.

This is particularly important in critically ill patients, where standard pharmacokinetic studies may not capture the full range of physiological extremes seen in the ICU.

Nonlinear mixed-effects modelling, implemented in tools such as Monolix, NONMEM, or nlmixr in R, is flexible enough to handle both rich datasets and sparse sampling. This approach makes it possible to describe variability across diverse patient groups and to translate those findings into dosing recommendations that are more relevant for real-world ICU populations, who often differ significantly from the carefully selected participants in traditional clinical trials (Su et al., 2024).

1.5.3 Pharmacodynamics

While PK ensures that the antibiotic reaches adequate concentrations in the body, pharmacodynamics (PD) ensures that these concentrations are effective against pathogens. PD describes how an antibiotic exerts its effect on bacteria, including killing mechanisms, resistance development, and optimal dosing strategies.

1.5.3.1 PK/PD targets

PK/PD indices

The PK/PD targets of antibiotics are one of the most useful indicators of optimum dosing regimens in both drug development and practice (Asín-Prieto et al., 2015; Rodríguez-Gascón et al., 2021; Tilanus et al., 2023). The EMA and the US FDA have guided the industry to consider PK and PD for antibiotic development since the 2000s (European Agency for the Evaluation of Medicinal Products, 2000; Food Drug Administration, 2003). This has led to the application of PK/PD targets in the selection of optimal dosing regimens in practice (Gallo, 2010; Xiao, 2018; Pereira et al., 2022b).

The ultimate goal of antimicrobial therapy is not simply to achieve certain plasma concentrations, but to ensure that bacterial eradication occurs reliably at the site of infection. PK/PD indices such as the proportion of the dosing interval that free drug concentrations exceed the minimum inhibitory concentration (*f*T>MIC), or the ratio of area under the concentration–time curve to MIC (*f*AUC/MIC), serve as surrogates for clinical efficacy. For beta-lactams such as meropenem, *f*T>MIC is the most relevant index; for ceftazidime/avibactam, both *f*T>MIC of ceftazidime and sustained avibactam concentrations above a threshold are required to protect against beta-lactamase hydrolysis.

By linking PopPK parameters with these PD indices, one can evaluate whether standard regimens are adequate across patient subgroups and microbial susceptibility distributions. For example, while meropenem 1 g every 8 hours infused over 30 minutes may achieve *f*T>MIC against susceptible strains in patients with normal renal function, the same regimen may fail in patients with augmented renal clearance or against isolates with elevated MICs. Without a PK/PD framework, such treatment failures may be attributed merely to “resistance,” when in fact they reflect insufficient drug exposure relative to bacterial susceptibility.

PK/PD parameters are integrated from the pharmacokinetic parameters, which describe the antibiotic concentration-time profile in the body through its ADME and pharmacodynamic aspects, which indicate the association between antibiotic concentrations and their effects on microorganisms (Drusano et al., 2012; Kuriyama et al., 2014). The integration of these two fields, using mathematical models and software tools, assists the prescribers in predicting the effective dosing regimens to support customising individual antibiotic therapies. The pharmacokinetic profile of the antibiotics only describes their time course in different parts of the body. Thus, it cannot elucidate the relationship between the antibiotic concentrations in the body and the response this elicits (i.e., the dose-response relationship). The integration of PK and PD through mathematical models enables the relationship between dosing regimen and response to be determined over a given time period (Derendorf et al., 1999; Pereira et al., 2022b).

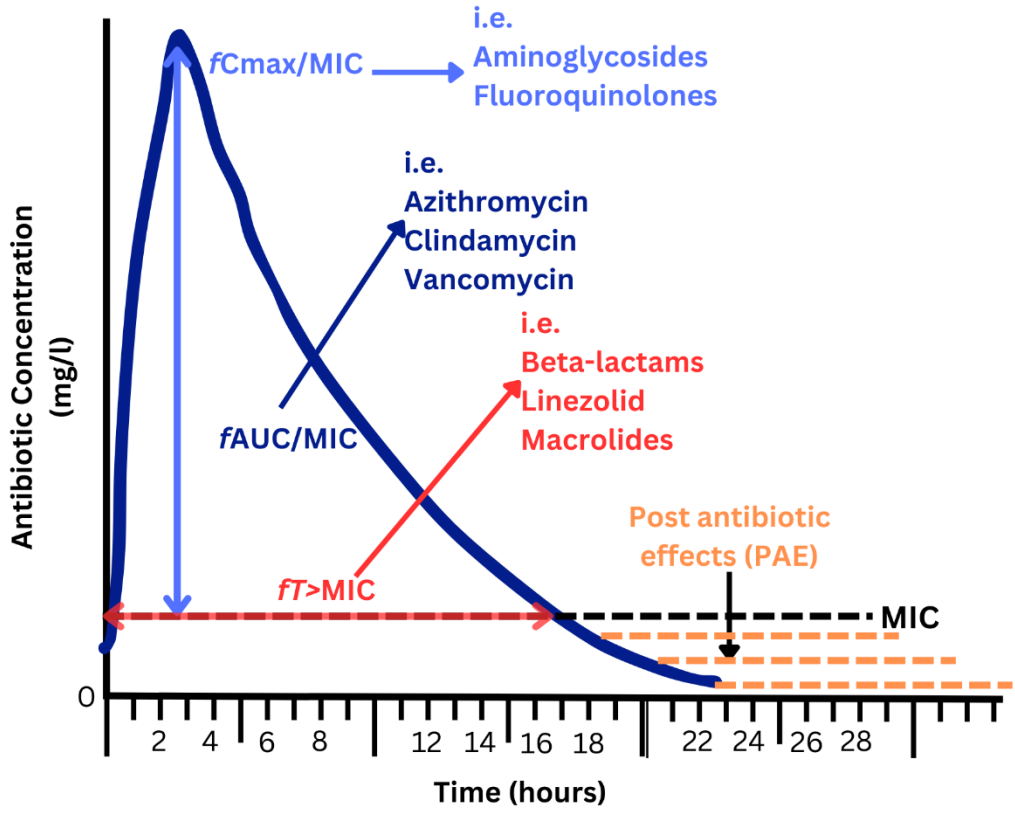


Figure 1-4. The PK/PD index and post-antibiotic effects.

The relationship between exposure, isolated bacteria and antibiotic efficacy is illustrated in Figure 1-4. The free antibiotic concentration (*f*) represents antibiotics in plasma that are unbound to proteins such as albumin, alpha-1-acid glycoprotein (AAG) and by using that, the antibiotic concentrations at the infected sites can be extrapolated (Routledge et al., 2013).

There are three main PK/PD indices, including the time that the unbound antibiotic concentration remains above the MIC (*f*T>MIC), the ratio of the maximal unbound antibiotic concentration to the minimum inhibitory concentration (fC_{max}/MIC), and the ratio of the unbound area under the concentration-time curve to the MIC (*f*AUC/MIC) (William A. Craig, 1998; Mouton, Dudley, Cars, Derendorf, & Drusano, 2005). Based on PK/PD parameters, antibiotics can be classified into different categories. The first category is time-dependent, which describes the duration for which the free antimicrobial concentrations are above the MIC of the infected pathogens (*f*T>MIC) for a portion of the dosing interval, to maximise the efficacy of antibiotics (Reed, 2000; Craig, 2001; Kuti, 2016). There is no requirement for higher concentrations to

achieve positive outcomes, but the duration of antibiotic exposure mainly determines their ability to eradicate the bacteria. The second category is concentration-dependent, which shows the correlation between peak concentration and MIC (*f*Cmax/MIC). The higher the concentrations, the better the ability of bacterial eradication (Jacobs, 2003). Several antibiotics, such as azithromycin, clindamycin, vancomycin, and tetracyclines, exhibit mixed PD properties, with both time-dependent and concentration-dependent characteristics. Their efficacy is best correlated with *f*AUC/MIC rather than purely time (T>MIC) or concentration metrics (C_{max}/MIC) because they obtain the post-antibiotic effects (PAE). Thus, the *f*AUC/MIC can best describe their bacterial killing abilities by maintaining the high concentration and long duration of exposure during dosing intervals (Craig, 1998).

The PK/PD target refers to the minimal value correlated with a high probability of good outcomes for the patient (Linder, 2016). Generally, it takes at least 48 to 72 hours for antibiotics to manifest their effectiveness, causing difficulties in selecting appropriate dosing regimens at the onset (Póvoa, 2008; Veiga et al., 2018). Attainment of antibiotic PK/PD targets is advantageous in predicting more precise dosing regimens, contributing to the success of therapeutics (Asín-Prieto et al., 2015; Tängdén et al., 2020). Indeed, there has been an extensive investigation into the benefits of PK/PD target attainment since the 1990s. The study of Dudley et al. (1991) showed that there was a correlation between the successful microbiological outcomes and the target attainment of fluoroquinolones AUC_{24}/MIC of >33.7 in patients with community-acquired respiratory tract infections (Dudley, 1991). The results of a systematic review conducted by Franzetti et al. (2010 indicated that the achievement of PK/PD targets including Cmax/MIC ≥8 - 10 mg/L for aminoglycosides, AUC_{0-24}/MIC ≥ 87 for fluoroquinolone; AUC_{0-24}/MIC ≥ 400 for vancomycin and ≥50–70% T>MIC for beta-lactam could affect the probability of good outcomes with respect to the patient mortality and pathogen eradication (Franzetti et al., 2010). A multicentre, pharmacokinetic study conducted by Robert et al. (2014) determined that the positive clinical outcomes of critically ill patients were related to the achievement of at least 50% *f*T>MIC for beta-lactam (Roberts, Paul, et al., 2014). Based on the statistical correlation between PK/PD target attainment and clinically successful outcomes, the PK/PD model-informed precise dosing is suggested to be widely applicable in clinical settings to not only maximise the bacterial killing effect but also to suppress resistance (Ferreira, 2021; Haseeb

et al., 2022; Hites, 2021; Roberts, Abdul-Aziz, et al., 2014; Wicha et al., 2021).

Clinical breakpoints

The European Committee for Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) are the two widely used guidelines for identifying which antibiotics are effective for individuals through the Antimicrobial Susceptibility Testing (AST) process (Cusack et al., 2019; Bayot ML, 2024). CLSI and EUCAST have defined Clinical breakpoints as factors that determine bacteria as clinically S – Susceptible, I – Susceptible with increased exposure or R – Resistant (Kahlmeter et al., 2006). Healthcare providers routinely use these criteria, which result from laboratory reports, to determine the appropriate antibiotic therapies for individuals. There has been no internationally harmonised AST. In the United States, CLSI is preferable to EUCAST. On the other hand, in Europe and in low and middle-income countries EUCAST is the favoured standard over CLSI for AST.

PK/PD targets played an important role in defining the clinical breakpoints. The procedure of establishing the clinical breakpoints involved evaluating antibiotic efficacy in *in vitro* studies and clinical trials to determine the pharmacodynamic targets such as *f*Cmax/MIC, *f*AUC/MIC and *f*T>MIC as well as the pharmacokinetic profile of the antibiotics such as Vd and CrCl. Monte Carlo Simulation was then used to predict the exposure of certain antibiotics in the target population and bacteria across various common dosing regimens (Van Ingen, 2012). The clinical breakpoints provide crucial insights into the everyday use of antibiotics in practice.

The EUCAST PK/PD target values were mainly identified by two methods. One of the methods, Classification and Regression Tree analysis, involved an algorithm of iterative splitting by using mathematical procedures and simulations, such as Monte Carlo Simulations, to solve the complexities of the triangular relationship between the exposure, the isolated bacteria and the patients (Mouton et al., 2012). For example, the study conducted by Ambrose et al. in 2003 gathered information on PK/PD index value derived from *in vitro* infected models to apply in human patients with pneumonia (Ambrose et al., 2003). They aimed to evaluate the PK/PD target of fluoroquinolone, at which point a significant statistical difference could be observed between the cured and uncured groups. This study indicated that the

target PK/PD value of fluoroquinolone as *f*AUC/MIC was approximately 34 for the treatment of pneumonia (Ambrose et al., 2003). The second approach derives target values directly from clinical trials, selecting the PK/PD index values achieved in at least 90% of successfully treated patients (Mouton et al., 2012). These methods have furthered the optimisation of antibiotic dosing regimens by informing the clinical points to maximise antibiotic exposure and response for individual patients.

However, it is important to note that the *in vitro* models might involve the wild-type strains of target bacteria, which may not reflect the intensity of acquired microorganisms in real-world settings (Kahlmeter et al., 2019; Maynard et al., 2021). Additionally, dosing regimens in clinical trials were obtained from patients with high antibiotic exposure and low MICs as the population mainly consisted of healthy volunteers or not-critically ill patients. As a result, these suggested breakpoints may not be applicable to real-world patients with severe pharmacokinetic alterations and may not address the issue of bacterial resistance.

1.5.4 PK/PD model-informed precise dosing

Whilst the need for new antibiotics is urgent but still unmet, the personalisation of existing antibiotic treatments is one of the most promising approaches to preserve the efficacy of both old and new antibiotics for future use (Olofsson et al., 2007; Heffernan, 2018). Most study reports agree that a key factor for better outcomes is the early initiation of a suitable antibiotic; however, there is less consensus on ideal doses and timings, especially for the pharmacokinetic/pharmacodynamic (PK/PD) targets of antibiotics in clinical settings which depend on multiple factors, including the choice of antibiotic agents, key patient characteristics, and local resistance patterns. The use of PK/PD model-based dosing has grown considerably in recent years and is now an invaluable predictive tool for optimisation (Asín-Prieto et al., 2015; Tsai et al., 2015). In the context of PK/PD principles, PK/PD modelling and simulations are highly beneficial in drug development (Hochhaus et al., 2000; Pereira et al., 2022a). PK/PD modelling is a mathematically based model integrating PK and PD parameters to elucidate the relationship between antibiotic dosing regimens and their time-course response (Mouton et al., 2002; Nielsen et al., 2011; Nielsen et al., 2013; Pereira et al., 2022b).

1.5.5 The need for the development of a practical PK/PD model in clinical settings

1.5.5.1 Limitations of Empirical Dosing

The traditional approach to antibiotic therapy has been empirical, relying on standardised regimens developed from early-phase pharmacokinetic trials conducted in healthy volunteers or small groups of non-critically ill patients. These regimens, which form the basis of product labelling and clinical guidelines, assume a relatively stable physiological environment. In reality, the critically ill population represents a group with profound heterogeneity in drug disposition. Pathophysiological changes such as augmented renal clearance, impaired hepatic function, increased capillary permeability, and fluid resuscitation contribute to wide variability in antibiotic exposure.

As a result, conventional fixed-dose regimens may fail to achieve PK/PD targets in this population. Underdosing risks therapeutic failure and selection of resistant subpopulations, while overdosing may increase toxicity without enhancing efficacy. This variability makes reliance on empirical dosing increasingly untenable in ICUs, where MDR GNB are common and achieving PK/PD target exposure with first-line or salvage therapy agents such as meropenem or ceftazidime/avibactam can be challenging.

Although PK/PD model-informed precise dosing has been proven to be of significant benefit and there have been extensive studies recommending its application to antibiotic dosing in secondary settings, the broad applicability of PK/PD model to inform individualised dosing at the bedside is still limited (Sinnollareddy et al., 2012; Darwich et al., 2017a; Darwich et al., 2017b; Kluwe et al., 2021; Maier et al., 2022). The development of a PK/PD model requires: a dataset comprising patients' demographics and comorbidities to build up a structural and covariate pharmacokinetic model; patients' clinical use of antibiotics, and the pathogens' profiles, including the MIC of the bacteria, to build up the PD model (Meibohm et al., 1997; Lalonde et al., 2007; Peck et al., 2007). However, it is not feasible to collect these essential components in practice, and thus, a simplified method to generalise the application of the PK/PD model into routine clinical care should be studied. One of the aims of this project is to find a more practical approach to develop the core data set for a predictive PK/PD model of dosing optimisation for gram-negative

infections, thereby saving time and cost for the PK/PD model-informed precise dosing in hospital settings.

The application of PK/PD modelling and simulations to achieve the PK/PD targets to prevent the sub-optimal outcomes in hospital settings would be advantageous but is not practicable on account of the complexity of the methods and the time required. To develop a PK/PD model for the selection of dosing regimens for a particular population, initially, a series of blood samples of at least 2 to 10 per patient is required to determine the pharmacokinetic parameters (Lee, 2001; Sathe et al., 2021). It would be too challenging and costly to monitor the drug concentrations of every antibiotic. In fact, antibiotics that do not have a narrow therapeutic index are not usually considered for monitoring in clinical practice. Recently, there has been a preference for sparse over rich blood sampling, with fewer blood samples required to be drawn, but the ability of dosing prediction of PK/PD models remains stable (Jamsen et al., 2011; Magis-Escurra et al., 2014; Sathe et al., 2021). At the bedside, this approach might be desirable to optimise dosing regimens for a specified individual requiring antibiotic dosing and durations to be evaluated daily (Adembri, Novelli, & Nobili, 2020). However, it is still burdensome for this to be widely applicable on a larger scale. Firstly, the sampling process in routine clinical practice might not be as idealised as those being set up in clinical trials, resulting in inaccurate individualisation of dosing as predicted by PK/PD models (Choi, Crainiceanu, & Caffo, 2013). In hospital settings, blood sampling is commonly drawn from the antecubital fossa area for the measurement of drug concentrations. The antibiotic concentration collected from peripheral veins may not accurately represent measurements from arterial or central veins (Kuepfer et al., 2016). Secondly, sampling time points, according to the prescribers' orders, might not be strictly adhered to (Choi et al., 2013). The PK profiles that mainly rely on the time of antibiotic measurements will be constructed with inappropriate data, resulting in less optimal dosing regimens. Thirdly, it might not be possible to implement ultrafiltration in every setting, especially in developing countries where the healthcare infrastructure is still less developed; this is required for accurately measuring the antibiotic concentrations to determine the pharmacokinetic parameter value. These challenges can prevent the implementation of PK/PD model-informed precise dosing in practice.

To resolve these issues, simplified methods to achieve the PK/PD targets should be investigated. Based on the guidance of the EMA that encouraged studies applying in silico simulations to aid dosing regimens (rather than performing extensive sampling when developing PK/PD models for paediatric dosing), applying prior knowledge derived from simulations is a practical approach (EMA, 2006). A dataset that combines readily available demographic information for patients, their use of antibiotics and simulations based on these relevant real-life data can offer an effective and widely applicable approach in practice (Kibbelaar et al., 2020; Timsit, 2020). In this project, the antibiotic concentrations used for the development of the pharmacokinetic model will be simulated using relevant software instead of drawing blood samples. This approach is to simplify the process of dosing individualisation that follows PK/PD principles in practice.

Software

Table 1-2 presents widely used software tools in the development of PK/PD models for antibiotics. For this study, Monolix and R were selected for PK/PD modelling and simulation due to their combined efficiency, flexibility, and accessibility. Monolix was chosen for its robust implementation of the stochastic approximation expectation maximisation (SAEM) algorithm, which is particularly effective for population PK modelling and well-suited for sparse clinical data. It also provides an intuitive graphical interface and strong model diagnostics. Importantly, Monolix offers a free academic licence, making it a cost-effective option for student researchers. R, an open-source statistical programming environment, was used in parallel for PK/PD simulations, data processing, and visualisation, utilising packages such as mrgsolve and deSolve (Soetaert, 2010; Barrett, 2018). Its flexibility and full integration with statistical workflows further supported model evaluation and dosing optimisation. Together, these tools offered a powerful, accessible platform for conducting comprehensive PK/PD analyses.

Table 1-2. Comparison of the software widely used in modelling and simulations for antibiotics.

Software	NONMEM	Monolix	Phoenix NLME	Simcyp Simulator	R
Website	https://www.iconplc.com	https://lixoft.com	https://www.certara.com	https://www.certara.com	https://cran.r-project.org
Estimation method	Nonlinear mixed-effects modelling	Stochastic approximation expectation maximisation (SAEM)	Nonlinear mixed-effects modelling	Mechanistic modelling, Monte Carlo simulations, and IVIVE techniques	Monte Carlo Simulation (mrgsove, deSolve)
Application	Population	Population	Population	Individual	Population, Individual
Cost	Paid licences	Paid licences/ freely available for students	Paid licences	Paid licences	Free
Features	Handles complex models and large datasets, widely used in PK/PD modelling for antibiotic dosing optimisation	SAEM is efficient for handling antibiotic dosing optimisation	Early-phase drug development and antibiotic regimen optimisation	Physiologically based pharmacokinetic (PBPK) modelling and simulation of drug-drug interactions	Strong integration with data visualisation and analysis tools for predicting drug behaviour, PTA simulations.

1.6 Aims and Objectives

Aim: The aim of this thesis was to develop and apply a simplified PK/PD model to optimise antibiotic dosing regimens for GNB infections, using evidence from published literature and real-world clinical data.

Research Questions: This thesis was guided by the following key research questions:

1. What PK/PD targets are associated with improved clinical outcomes in the treatment of GNB infections?
2. How are antibiotics for GNB infections currently used in routine clinical practice across different healthcare settings?
3. Can simplified PK/PD models, based on real-world data, be developed to evaluate the adequacy of current antibiotic dosing regimens and inform optimisation strategies?

Objectives: To achieve this aim, the following objectives were defined:

1. To identify and summarise clinically relevant PK/PD targets associated with treatment success for GNB through a systematic review and meta-analysis (Phase I).
2. To collect and characterise real-world antibiotic usage data from two hospital settings (Vietnam, namely Cho Ray Hospital and the UK, namely The Countess of Chester Hospital), including information on dosing practices, patient characteristics, and infection profiles (Phase II).
3. To develop and validate population PK models for antibiotic combination therapy using real-world patient data, the selection of antibiotics was determined based on the availability of data resulting from Phase I and Phase II (Phase III).
4. To integrate the PK models with an E_{max} pharmacodynamic model and simulate the Probability of Target Attainment (PTA) for different dosing regimens (Phase III).
5. To evaluate the adequacy of current dosing regimens and explore alternative strategies that may improve clinical outcomes (Phase III).

The diagram (Figure 1-5) visualised the flow of work.

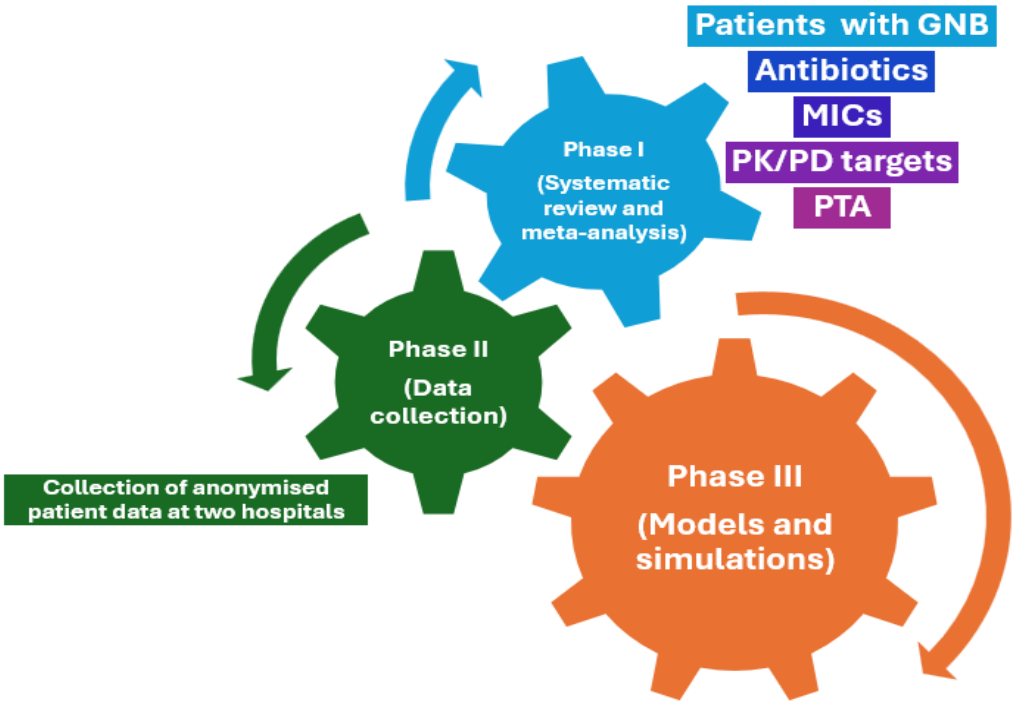


Figure 1-5. The connection between the three phases of this project.

GNB: Gram-negative bacteria; MICs: Minimum inhibitory concentrations;
PK/PD: Pharmacokinetics/Pharmacodynamics; PTA: Probability of target attainment.

1.6.1 Phase I systematic review and meta-analysis

The systematic review, undertaken in Phase I aimed to identify and summarise the PK/PD targets of antibiotics treating GNB infections, as reported in the literature. The aim was to study the impact of PK/PD targets observed in practice and to explore whether these PK/PD target values have the potential to be applied when making clinical decisions to optimise treatments for patients with MDR GNB infections. This phase of the project aimed to address the following questions:

- To what extent do PK/PD targets derived from *in vitro* studies align with those observed in real-world clinical settings for patients with GNB infections?
- What are the most impactful PK/PD parameters that best predict the clinical efficacy of antibiotics treating gram-negative infectious episodes in clinical settings?
- Will these clinically derived PK/PD target indices likely establish closer to optimum dosing regimens for patients with drug-resistant GNB infections?

1.6.2 Phase II antibiotic usage data collection

This retrospective observational study was conducted at two hospitals to collect real-world data on antibiotic use among patients with confirmed GNB infections. Anonymised information on patient demographics, infection characteristics, and antibiotic regimens was systematically gathered. To ensure consistency and reliability, data were recorded using standardised forms and procedures. This phase of the project aimed to address the following questions:

How can real-world antibiotic use data from patients with gram-negative infections support the development and evaluation of optimised dosing strategies based on PK/PD targets?

1.6.3 Phase III PK/PD modelling and simulations

Based on the findings from Phase I, a systematic review of impactful PK/PD targets and Phase II, a retrospective observational study of antibiotic use in clinical practice, meropenem and ceftazidime/avibactam were identified as commonly used antibiotics for treating gram-negative bacterial infections. Consequently, population pharmacokinetic (PopPK) models were developed for these agents using patient-level data. These models were later integrated with a PD E_{max} model using the findings of synergy effectiveness between meropenem and ceftazidime/avibactam to simulate drug exposure and evaluate the PTA against relevant gram-negative pathogens. By integrating the PK models with an E_{max}-based PD model, the study assessed the PTA for each patient, providing insight into the effectiveness of current dosing strategies in routine practice. This phase aimed to answer the following questions:

- Can separate PopPK models for meropenem and ceftazidime/avibactam be developed using patient-level data from Phase I?
- How well do the developed PK models predict drug concentrations under real-world dosing regimens when evaluated through internal validation and Monte Carlo simulations?
- Can integration of PK models with an E_{max}-based PD model provide an effective framework to assess the PTA in individual patients receiving meropenem and ceftazidime/avibactam in clinical practice?

Chapter 2 Methods

This PhD research followed a stepwise, mixed-methods design structured in three interrelated phases to inform and optimise antibiotic dosing strategies against GNB infections. Phase I involved a systematic review to identify impactful PK/PD targets for antibiotics used in the treatment of GNB infections. Findings from this review guided the selection of antibiotics of interest for the subsequent phases. In Phase II, an observational study was conducted at two hospitals to collect retrospective real-world data on antibiotic use in patients with culture-confirmed GNB infections. This included anonymised patient demographics, infection characteristics, and detailed treatment regimens. Finally, in Phase III, patient-level data from Phase II were used to develop and validate population PK models for the selected antibiotics. These models were then integrated with an E_{max}-based PD model to simulate drug exposure and evaluate the PTA under real-world dosing scenarios. The sequential design allowed for data-driven model development, grounded in both current evidence and clinical practice.

2.1 Phase I systematic review and meta-analysis of PK/PD targets study design

This systematic review was carried out and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines (Page et al., 2021). The protocol was registered on PROSPERO (CRD42022376130).

The terms ("pharmacokinetic" OR "pharmacodynamic" OR “PK PD”) AND (“antibiotics” OR “antibiotic”) AND ("resistant" OR "resistance") were searched from 1st January 2000 to 24th March 2023 to identify the PK/PD targets of antibiotics treating GNB. PK/PD targets for antibiotic dosing optimisation have only been researched since the 2000s; therefore, the search period commencing from 2000 was selected.

The following databases were searched: Cochrane Central, Web of Science, PubMed, Embase, & Scopus. Only papers or articles published in the English language were included. Reference lists of eligible systematic reviews were

manually screened to capture further studies.

i. Initial searches: this was conducted using PubMed to identify relevant keywords. This included MeSH terms derived from the Cochrane Library.
ii. Intensive search: relevant keywords and synonyms established in step one were used in the respective databases.
iii. Extra search: references and bibliographies of these papers collected from step two were searched.

2.1.1 Inclusion and exclusion criteria

2.1.1.1 Inclusion criteria

Participants/population: patients with GNB infections treated in part or whole with parenterally delivered antibiotic therapy.
Intervention(s), exposure(s): PK/PD indices obtained from antibacterial therapies to treat gram-negative infectious episodes in Clinical settings.
Comparator(s)/control: Compared to the EUCAST PK/PD indices of the same antibiotics as the intervention arm.

- Studies reporting PK/PD data for antibiotics used to treat GNB infections.
- Studies that assessed PK/PD targets (i.e., %*f*T>MIC, AUC/MIC) or PTA.
- Studies that reported relevant clinical or microbiological outcomes (i.e., treatment success, bacterial eradication, mortality).
- Eligible study designs included population PK studies, clinical PK/PD modelling studies, randomised controlled trials, observational cohort studies, and case studies.
- Publications in English.

2.1.1.2 Exclusion criteria

Studies were excluded from the current systematic review based on the following conditions: qualitative studies, meta-analysis studies, *in vitro* studies, in silico studies, protocol, review articles, commentaries, meeting abstracts, editorials, correspondences, letters to the editor, guidelines, book chapter(s), and veterinary studies.
Studies were excluded if:

- Studies were not published in English
- Studies had not been completed

- Studies reported only gram-positive infections
- Studies investigated healthy participants
- Studies did not investigate parenteral antibiotics (i.e., oral antibiotics, inhaled antibiotics, antibiotics for surgery prophylaxis, antibiotics not administered)
- Studies reported PK/PD of antibiotics that were unapproved at the time of investigation

2.1.2 Selection and data extraction

All search results were numbered after removing duplicates and then reviewed by the primary investigator. A second investigator randomly checked 5% of the search results at each screening stage. The random list was yielded by using a Microsoft Excel random formula from the list of numbers. A member of the research team was consulted if necessary to resolve selection discrepancies. In the first screening stage, titles and abstracts were consulted to eliminate studies that were clearly irrelevant. In the second stage, the full text of the articles was evaluated to determine whether each study met the inclusion criteria. Any disagreement was resolved through discussion (involving a third member of the team if necessary). Missing data or information was evaluated and solved by applying Cochrane's missing data guidelines where possible.

The process of selecting relevant papers was fully documented following PRISMA guidelines. A reference manager, namely EndNote X20, and Microsoft Excel 365, provided by Liverpool John Moores University, were used for data extraction and storage/indexing during this project. The search results can be found in Appendix 1, and details of the included articles are in Appendix 3.

2.1.3 Data collection

The primary investigator extracted data from all journal articles using a standardised data collection form. Study characteristics were extracted, including (where available) title, author(s), year of publication, study design, aspects of the study population (sample size, age group, gender, Body Mass Index (BMI) or weight if applicable), bacteria, infections, study antibiotics, study antibiotic dose, duration, PK/PD parameters and values, PTA and clinical outcomes. If the studies did not report the data of each individual patient, the mean or median value was collected.

Patient outcomes consisted of the PTA, bactericidal result, and patient status, including recovery, death or loss of follow-up. If there were no follow-up activities following the antibiotic treatment, it was assumed that follow-up processes were missing or patients were missing from follow-up.

2.1.4 Quality assessment

The Cochrane risk of bias tool[1] was utilised to assess the quality of the studies and the risk of bias. Version 2 of the Cochrane risk-of-bias tool for randomised trials (RoB 2) was used to assess the risk of bias in randomised trials; The Risk of Bias In Non-randomised Studies of Interventions (ROBINS-I) tool was used for the assessment in non-randomised studies. The NHLBI (National Heart, Lung, and Blood Institute) tool was used to assess the risk of bias in case series and case studies (NHLBI, 2013; Sterne et al., 2016; Sterne et al., 2019).

A risk of bias assessment was undertaken by the primary investigator and agreed upon by the research team.

2.1.5 Data analysis

Eligible studies were initially classified into three categories, including randomised clinical trials (RCTs), non-randomised studies and case studies for systematic review. Studies were classified according to study design for quality assessment. The characteristics of data were grouped by antibiotics for narrative synthesis. PK/PD target values of antibiotics resulting from dosing regimens in a group of patients or individuals from eligible studies were extracted to compare with the PK/PD breakpoints recommended by the EUCAST. These breakpoints represent MIC thresholds below which a microorganism is considered susceptible to a particular antibiotic, taking into account not only *in vitro* activity but also human PK, PD, and where available, clinical outcome data. For instance, for meropenem, the susceptible breakpoint against Enterobacterales is ≤2 mg/L, based on a PK/PD target of approximately 40% *f*T>MIC, with simulations indicating robust target attainment at standard dosing regimens (EUCAST Rationale Document, version 3.0, 2020) and for ceftazidime-avibactam, a breakpoint of ≤8 mg/L is used for *Enterobacterales* and *P. aeruginosa*, based on data showing that about 45% to 55%

[1] https://methods.cochrane.org/bias/resources

*f*T>MIC is predictive of microbiological success at recommended dosing (EUCAST Rationale Document, version 1.0, 2017).

This data is aimed at helping researchers select the appropriate dosage for clinical trials and prescribers to choose the right dosing regimens. Comparison of PK/PD parameters from eligible articles in terms of index selection and clinical relevance was made to evaluate the impact of the PK/PD targets in daily clinical situations.

2.1.6 Data for meta-analysis

2.1.6.1 Pooled data

This meta-analysis examined the main aspect of achieving the PK/PD targets in clinical settings. Data for the meta-analysis were extracted from the studies into two groups: those that monitored the achievement of PK/PD target with labelled dosing regimens, and those with modification of labelled dosing regimens. The data extraction included the number of patients who achieved the PK/PD targets by modifying the dosing regimens or remaining on the same instructions of use as guided by the label in each trial.

2.1.6.2 Statistical Methodology

The number of PK/PD target achievements from included studies was combined using a meta-analysis method with a random effects model with weighted estimation (Curtin, 2017). Data were analysed using the R system (version 4.3.2, The R Foundation for Statistical Computing, Vienna, Austria) with the function “metaprop” in the “metafor” package (R Core Team, 2023). The “metaprop” was selected to pool proportions from the interventions that were made with the dosing regimens to achieve the PK/PD target. Proportional meta-analysis was used in this study to synthesise single-group patients that experienced the modification of duration or dosage, to establish the effect of modifying labelled dosing regimens accordingly to the PK/PD targets. The individual weights that each study, which contributed to the pooled estimate, with associated 95% confidence intervals, and the statistical tests for heterogeneity using the random effects model were calculated.

2.2 Phase II collection of antibiotic usage data in two hospitals

2.2.1 Study design

Building upon the findings of Phase I, Phase II focused on evaluating the use of included antibiotics in real-world clinical settings. To achieve this, anonymised data from patients who had been prescribed any of the key antibiotics for which robust PK/PD targets had been established in literature at two participating hospitals were collected. This approach enabled an in-depth examination of current antibiotic prescribing practices and their alignment with established PK/PD principles identified in the systematic review. Phase II was a retrospective study to develop a dataset to use in a predictive PK/PD model of dosing regimens. The data collection period involved collecting anonymised patient data, relating to the use of included antibiotics to treat four investigated pathogens from the hospital database between January 2023 and December 2023; the patient database was anonymised; no identifiers were required for the PK/PD model development. The sample size was estimated at 308 unidentified patients (See Section 2.2.5 for details). Patient data were collected from two hospitals, one in the United Kingdom and one in Vietnam.

The data intended to be collected was detailed in
Appendix 3. Patients prescribed one of nine selected antibiotics, including amikacin, cefepime, ceftazidime, ceftazidime-avibactam, ciprofloxacin, gentamicin, imipenem, meropenem, piperacillin-tazobactam, which were selected from the findings of Phase I, and had microbiological culture results confirming infection with one or more of the following pathogens, including *E. coli*, *K. pneumoniae* or *A. baumannii*, and *P. aeruginosa,* were included for analysis. These two criteria were initially applied to ensure that the included antibiotic regimens were likely prescribed in response to a confirmed infection with a susceptible organism, thereby strengthening the relevance of the data for PK/PD analysis.

2.2.2 Ethics

Ethical approval and data governance procedures were obtained from relevant institutions prior to the commencement of data collection. The study was approved by the Research Ethics Committee at Liverpool John Moores University (LJMU) on 6th April 2023 under the University Research Ethics Committee (UREC) minimal risk registration number Hope - 23/PBS/003. In the United Kingdom, further approval was granted by the Health Research Authority (HRA) on 27 April 2023, with project

number 322658 and REC reference 23/HRA/1641. For the Vietnam site, authorisation was provided by the Director of Cho Ray Hospital under Decision No. 3399/QĐ-BVCR, dated 1 August 2024. Data sharing agreements were established between LJMU and the Countess of Chester Hospital (CoCH) on 9 January 2024, and between LJMU and Cho Ray Hospital (CRH) on 1 August 2023, ensuring compliance with institutional and regulatory requirements for the handling of anonymised patient data.

2.2.3 Eligibility criteria

2.2.3.1 Inclusion criteria

Inclusion criteria are as follows: (1) patients with a recorded positive culture for at least one of the following bacteria: *E. coli, K. pneumoniae, A. baumannii, P. aeruginosa*; (2) patients who were prescribed parenteral antibiotics to treat gram-negative infections, including amikacin, cefepime, ceftazidime, ceftazidime-avibactam, ciprofloxacin, gentamicin, imipenem, meropenem, piperacillin-tazobactam; (3) recorded patient outcomes available; and (4) availability of the MIC data; (5) available of cultures.

Outcome assessments:

- Success: The elimination of infecting pathogens confirmed by repeat negative cultures or a positive patient clinical response (no additional therapies
- required)
- Failure: Death (all-cause mortality with an absence of follow-up cultures) or no improvement (additional therapies required including admission to ICU; need for vasopressor drugs such as dopamine, dobutamine, epinephrine, norepinephrine, vasopressin, and phenylephrine; need of mechanical ventilation).

2.2.3.2 Exclusion criteria

Exclusion criteria are as follows: (1) patients with combined gram-positive and gram-negative infections; (3) no dosing regimen information; (4) use of antibiotics delivered only via non-parenteral routes; (5) use of antibiotics for prophylaxis only.

2.2.4 Data collection

Anonymised patient data were collected retrospectively from CoCH and CRH. The

data collection process followed local ethical approvals and data governance protocols at each site.

At CoCH, the hospital information technology (IT) initially generated a list of anonymised patients who had been prescribed at least one of nine selected antibiotics commonly used to treat GNB infections. The selection of these antibiotics was based on the findings of Phase I. Following anonymisation, the researcher manually screened the records using a read-only account to access the CoCH Cerner service via VMware Horizon, thereby confirming the presence of a positive microbiological culture for GNB. This service allowed external researchers to collect the data remotely. Only anonymised patients with confirmed GNB infections were included to extract relevant data for final analysis. The anonymised dataset comprised demographic information (i.e., age, sex), infection site, microbiological findings, renal function, and detailed antibiotic administration records.

At CRH, the IT staff used the two conditions as mentioned to identify patients with confirmed microbiological culture results positive for GNB who received at least one systemic antibiotic during their hospital stay. Subsequently, the data were anonymised before being shared with the researcher.

All data were recorded in a standardised electronic form to ensure consistency across both sites. No identifiable patient information was accessed at any stage by the research team.

Patient demographics, including age, ethnicity (if applicable), gender, weight, renal function (eGFR (or CrCl)), and serum albumin level (if applicable), were collected. These data were collected because they might influence the models' prediction ability.

2.2.5 Statistics and data analysis

2.2.5.1 Sample size

The aim of this phase of the project was to collect data on anonymised patients' clinical use of antibiotics to treat gram-negative infectious episodes and to enable the development of a PK/PD model. The data were collected from CoCH and CRH over a period of one year, from 01/01/2023 to 31/12/2023.

According to the annual counts and rates of *E. coli* bacteraemia, *Klebsiella spp*. and *P. aeruginosa*, published by the UK Health Security Agency (updated in 2022), approximately 184 cases of GNB were reported at CoCH during 2022 (UK Health

Security Agency, 2022a, 2022b, 2022c). According to the report provided by CRH, there were about 6980 cases of GNB infections in 2022.

A sample size met the inclusion and exclusion criteria with a 95% confidence level, 30% of the likely proportion and a 5% margin of error is about 308 (Adapted from Daniel WW's formula (Daniel, 1999)), as below:

$$n = \frac{N \times X}{X + N - 1} = \frac{7164 \times 322.69}{322.69 + 7164 - 1} = 308.8 \qquad \textit{(Equation 6)}$$

where,

$$X = \frac{{Z_{\alpha/2}}^2 \times p \times (1 - p)}{\mathrm{MOE}^2} = \frac{1.96^2 \times 0.3\,(1 - 0.3)}{0.05^2} = 322.69 \qquad \textit{(Equation 7)}$$

- $Z_{\alpha/2}$: the critical value of the Normal distribution at α/2 (for a confidence level of 95%, α is 0.05 and the critical value is 1.96),
- MOE: the margin of error (5%)
- P: the likelihood of proportion (hereby given that 30%, applying the rate of positive culture of GNB at the hospital from the study of Peleg et al. in 2010 (Peleg et al., 2010))
- N: the population size (hereby referring to 7164 cases at two investigated sites)

In the scenario that the cases collected from the two hospital databases met the criteria larger than the calculated sample size, the latest cases, defined by the date of admission, will be chosen only. If the cases collected from the hospitals were not sufficient, a justification based on the number of identified cases would be sought.

2.2.5.2 Data analysis

This approach combined hypothesis testing (t-test) with a measure of practical relevance (Cohen's d) to provide a comprehensive understanding of the results. By doing so, the general information on how antibiotics were used in practice was analysed.

Data Preparation: Anonymised data from patients included in the study were tabulated and analysed to understand the population demographics, the clinical use of antibiotics, and patient outcomes. Patients were categorised according to their age, categories included those aged below 18; those between 18 and 65; and those above 65.

Grouping: Patients were categorised into several groups, such as by the number of pathogens they were infected with, and the number of antibiotics they were prescribed. The outcome metrics included clinical outcomes such as mortality or recovery rates.

Statistical Analysis:

Independent Samples t-Test: A two-sample t-test was conducted to compare the mean outcomes between different groups.

Effect Size (Cohen's d): Cohen's d quantified the practical significance of these differences, helping to determine the Clinical relevance of observed effects.

Cohen's d was calculated to measure the magnitude of the difference between the two groups.

Effect size interpretation followed standard guidelines:

- 0.2–0.49: Small effect
- 0.5–0.79: Medium effect
- ≥0.8: Large effect

A p-value < 0.05 was considered statistically significant for rejecting the null hypothesis.

Software used for statistical analysis: R (version 4.3.2, base R and dplyr, visualised by ggplot2)

2.2.6 Data management

2.2.6.1 Access to data

The anonymised patient databases were provided by COCH and CRH. All data had to be accessible, on request, to responsible members of Liverpool John Moores University [and the relevant hospital(s)] for monitoring and/or audit of the study, to ensure that the study complied with applicable regulations. During data analysis, the data, consisting of the main working files and one backup copy for each, was not left unattended. Files were password-protected and stored using a secure service provided by Liverpool John Moores University (SharePoint). This service was used for archiving research data and was shared only within the research team. Following the completion of the study, all anonymised data will be stored securely on the Liverpool John Moores University Ctera system for a minimum of 10 years, in accordance with the university's data retention policy. Access will remain restricted

to authorised members of the research team. After the retention period, data will be permanently deleted using secure erasure procedures.

2.2.6.2 Quality assurance procedures

The study was conducted in accordance with Data Protection Act (DPA) 2018 with respect to collection, storage, and processing. Two hospitals signed the Data Sharing Agreement (DSA) with LJMU before the study started. All patient data was de-identified by hospital IT staff before being presented to the research team.

2.2.6.3 Participant confidentiality

All investigators and the study site staff complied with the requirements of data protection legislation with regard to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles in future.

2.3 Phase III PK/PD modelling and target attainment analysis by simulations

2.3.1 Study design

This phase of the project involved an in silico simulation study, employing advanced computational tools and mathematical modelling techniques to develop, evaluate, and validate PopPK and PK/PD models for selected antibiotics. The antibiotics and their predefined PK/PD targets were selected based on the findings from Phase I.

The PopPK modelling approach allowed the estimation of typical pharmacokinetic parameters (i.e., Cl, Vd) and the quantification of variability among patients by incorporating individual covariates such as age, renal function, and body weight if applicable. These models were built using non-linear mixed-effects modelling, which enables simulation of drug concentrations across diverse patient populations.

To translate drug concentrations into antibacterial effects, a PD model was developed by integrating the PopPK models with an E_{max}-based model. The E_{max} model is a commonly used sigmoidal (Hill-type) model that describes the maximal drug effect (E_{max}), the concentration required to achieve 50% of this maximal effect (EC_{50}), and the steepness of the concentration-effect curve (Hill coefficient) (Gabrielsson et al., 2001). This model captures the non-linear relationship between

antibiotic exposure and bacterial killing, allowing prediction of PTA across a range of dosing regimens.

Subsequently, patient-level data from Phase II, including demographics and antibiotic prescriptions, were used as inputs to the PopPK models to simulate individual drug concentration-time profiles. These simulations were combined with the E_{max} model to calculate PTA values, reflecting the likelihood that a given dosing regimen would achieve the predefined PK/PD targets associated with optimal clinical efficacy.

Figure 2-1 shows a flowchart detailing the development of a PK/PD model. The outlined steps are designed to establish and validate robust PK/PD models, facilitating the optimisation of antibiotic dosing regimens through in silico simulations while leveraging computational methodologies and patient-specific information.

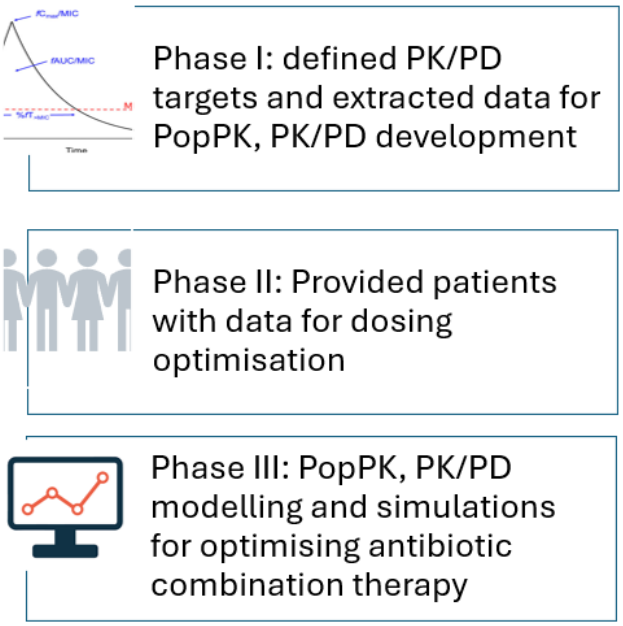


Figure 2-1. The flowchart outlines the step-by-step process of how the dataset was prepared for modelling and simulation

PopPK: Population pharmacokinetic models
PK/PD: pharmacokinetics/pharmacodynamics

2.3.2 Software

The simulation process involved several sequential steps, using different software. First, separate pharmacokinetic models for meropenem and ceftazidime/avibactam were developed by Monolix (version 2024R1); the datasets for the models

depended on the availability of observed concentrations collected from Phase I. The PK parameters derived from these PK models were used for subsequent PK/PD modelling and simulations by R. The deSolve package in R was then used to solve the differential equations and simulate concentration–time profiles for each patient under various dosing regimens. Following this, the predicted concentrations were evaluated against predefined pharmacodynamic targets (i.e. 100% *f*T>MIC or 100% *f*T>4×MIC), which were obtained from Phase I, to determine the PTA. Finally, the PTA values were summarised across patients to assess the likelihood of achieving effective drug exposures with the actual dosing regimens prescribed to patients in Phase II or proposed dosing strategies.

2.3.3 Methodology to develop a population PK model using Monolix

In the initial stage of dataset preparation, data were collated from the articles included in Phase I of this project. The extracted information comprised the selected antibiotics for modelling and simulation, measured antibiotic concentrations, demographic characteristics, and relevant covariates such as age, weight, and renal function. These data were then formatted into a Monolix-compatible structure (i.e., .txt or .csv format), with clearly defined columns for patient ID, time, antibiotic concentration, dosing information, and covariates. Timepoints corresponded to the dosing frequency, beginning at time zero and continuing until the final recorded measurement. Antibiotic doses were recorded in milligrams for consistency across studies. In instances where multiple regimens were reported for a single patient, the most frequently administered regimen or the one closest to the time of measurement was selected. Age was recorded in years and weight in kilograms. Where CrCl values were unavailable, the Cockcroft-Gault equation was used to estimate renal function based on serum creatinine.

The compiled dataset underwent thorough cleaning to address missing values, correct inconsistencies, and ensure accuracy prior to analysis. After preparation, the dataset was imported into the modelling software, and the data structure was defined in accordance with software requirements, such as specification of columns for time, dose, and measured concentration.

Model development began with the selection of an appropriate structural PK model. This choice of a one-compartment, or two-compartment with linear elimination was

guided by existing literature and initial exploratory data analysis. Initial estimates for PK parameters such as Cl and Vd were entered based on published values or fitted from preliminary simulations. Models of interindividual variability (IIV) were applied using exponential, proportional, or additive error structures. Residual error models were similarly specified as additive, proportional, or a combination of both.

Covariate analysis was conducted to explore the influence of patient-specific factors, such as renal function and body weight, on PK parameters. This was facilitated using Monolix's automated covariate search tools. Covariates that significantly improved model fit were retained in the final model to enhance predictive performance. Stepwise regression was used to refine covariate selection. This involved a forward selection process, beginning with no predictors and adding variables sequentially based on statistical significance and model improvement metrics, such as the Akaike Information Criterion (AIC) if applicable. The process continued until no further variables substantially enhanced the model. Covariates in PK models were modelled as a function of the logarithm to reflect the underlying biological and statistical relationships more accurately. For example, renal function measured by CrCl is commonly modelled on a logarithmic scale because the relationship between CrCl and drug clearance is typically non-linear. Using a log-transformed covariate allows for a multiplicative or power-law relationship, which better represents physiological processes such as drug elimination. This approach is consistent with the principles of allometric scaling, where many biological systems follow exponential or power functions rather than linear relationships. Statistically, modelling covariates in logarithmic form can stabilise variance, reduce the influence of extreme values, and improve model fit by linearising skewed relationships. It also enhances interpretability, as the coefficient associated with the log-transformed covariate can be understood as the percentage change in the pharmacokinetic parameter for each per cent change in the covariate. During model development, covariates including body weight, age, and renal function (expressed as either CrCl or log-transformed CrCl) were considered with attention to potential collinearity. To avoid multicollinearity, only one of the two variables CrCl or logCrCl was included in the model at a time. No significant collinearity was observed among the remaining covariates, based on correlation matrix inspection and biological plausibility. The use of log-transformed CrCl was chosen to improve model fit and better reflect the non-linear relationship between renal function and drug clearance.

Model estimation was performed using the SAEM algorithm implemented in Monolix. Throughout the estimation process, convergence diagnostics and parameter estimates were carefully monitored. The resulting model was evaluated through a range of diagnostic methods, including goodness-of-fit plots (i.e., observed vs. predicted concentrations, residuals vs. time), and individual prediction plots. Visual predictive checks (VPC) were carried out to assess the model's ability to reproduce observed data. The precision of parameter estimates was evaluated using standard errors and confidence intervals.

To ensure model reliability, internal validation was performed using bootstrapping or cross-validation techniques. The final model was selected based on comprehensive evaluation of diagnostic plots, statistical goodness-of-fit (GOF), and overall predictive performance. The final output included complete documentation of the model structure, parameter estimates, and validation results.

2.3.4 Development of the PK/PD models

A combined PK/PD modelling and simulation framework was implemented in R (version 4.4.2) using several packages, including deSolve for solving differential equations, ggplot2 for visualisation, and dplyr and tidyr for data manipulation. This approach was applied to determine if the dosing regimen used (data obtained from Phase II) was likely to achieve the target PK/PD value (data obtained from Phase I).

PK models, as mentioned at 2.3.3, were used for the integration with PD models. Drug concentrations were simulated over a 72-hour period using Euler’s method, a mathematical method for approximating the solution for ordinary differential equations (ODEs), with a time step of one hour (Butcher, 2008). For each dosing regimen, 500 replicate simulations were performed to capture variability in concentration-time profiles. All regimens assumed a fixed intermittent infusion for both drugs. A comprehensive grid of regimens was tested, varying doses and dosing intervals for antibiotics. Particularly, meropenem doses ranged from 500 mg to 2000 mg every 6, 8, and 12 hours, while ceftazidime/avibactam (referring to the dose of ceftazidime only) ranged from 750 mg to 2000 mg for every 6, 8, and 12 hours. Both antibiotics were simulated to be infused simultaneously over 3 hours to maximise the duration their concentrations remained above MICs.

First, both meropenem and ceftazidime/avibactam were administered concurrently, assuming that the first infusion of each drug started at time zero and repeated according to their respective dosing intervals.

Second, a staggered-administration scenario was simulated to reflect clinical situations in which the two agents cannot be infused simultaneously. In this simulation, meropenem was initiated at time zero (lag = 0), while ceftazidime/avibactam was introduced after a fixed 3-hour delay (lag = 3).

Euler's method was employed to numerically solve the system of ODEs describing the pharmacokinetics of antibiotics (Butcher, 2008). This approach involves discretising time into small intervals and iteratively estimating drug concentrations based on the rate of change at each time point. Specifically, the concentration at the next time step was approximated using the formula:

$$C_{t+\Delta t} = C_t + \Delta t \times \frac{dC}{dt} \qquad \textit{(Equation 8)}$$

Where $\frac{dC}{dt}$ is the rate of change in drug concentration, determined by the difference between the infusion input and the elimination clearance. For each antibiotic, the rate of change in concentration was calculated as:

$$\frac{dC}{dt} = \frac{infusion\ rate}{V} - \frac{Cl}{V} \times C \qquad \textit{(Equation 9)}$$

The infusion rate was defined conditionally based on the dosing interval and infusion duration (i.e, a dose of 1,000 mg infused over 0.5 hours corresponds to an infusion rate of 2,000 mg/h), allowing simulation of intermittent infusions administered over the time of infusion duration. The time step (Δt) was set to 1 hour to ensure adequate resolution of the concentration–time profiles over a 72-hour period. Euler's method was selected due to its simplicity, computational efficiency, and interpretability, particularly when performing large-scale simulations across multiple dosing regimens. Although more advanced numerical methods such as Runge-Kutta algorithms may offer improved accuracy and stability for stiff systems, Euler's method was sufficient for the purpose of simulating linear PK models with regular dosing inputs in this context (Butcher, 2008).

To characterise the relationship between antibiotic exposure and bactericidal activity, a pharmacodynamic model based on the sigmoid E_{max} equation (Hill equation) was employed (Gabrielsson et al., 2001). This PK/PD model describes the drug effect as a function of concentration and accounts for the saturable nature of antimicrobial activity. The general form of the model is:

$$E(t) = E_{max} \times \frac{C(t)^{\gamma}}{C(t)^{\gamma} + IC_{50}^{\gamma}} \qquad \textit{(Equation 10)}$$

where E(t) is the predicted effect at time at time (t), C(t) represents the drug concentration, E_{max} denotes the maximum possible effect, IC50 is the concentration producing 50% of the maximal effect, and γ is the Hill coefficient indicating the steepness of the concentration-effect curve.

This structure was selected for its well-established ability to describe non-linear exposure–response relationships in antimicrobial pharmacodynamics (Nielsen et al., 2011; Mouton et al., 2012). In this study, the E_{max} model was applied to the combined concentrations of both antibiotics to simulate their joint bactericidal activity over time. Parameter values for E_{max}, IC_{50}, and γ were not fixed a priori but were instead determined during model development based on published literature and empirical fitting to ensure relevance to the bacterial targets and drug combination being studied.

The resulting E(t) profiles were used to quantify the proportion of time the predicted pharmacodynamic effect exceeded predefined thresholds (i.e., E(t) > 1), which served as a basis for calculating the PTA for each dosing regimen. This allowed comparison of different regimens with respect to their ability to maintain effective antimicrobial activity throughout the dosing interval.

PK/PD targets were chosen from the findings of Phase I (i.e, 100% *f*T>MIC and 100% *f*T>4×MIC). The E(t) model offers a more mechanistic view of bacterial kill dynamics and can incorporate the synergistic or additive effects of drug combinations. The key difference between these two approaches lies in their conceptual basis. While PK/PD target (i.e, *f*T>MIC) provides a binary measure of target attainment (i.e., whether the concentration is above MIC or not), E(t) reflects the magnitude of pharmacodynamic effect at each time point, allowing a more continuous and graded evaluation of antibacterial activity. E(t) > 1, for example,

indicates an effect greater than the baseline (or IC_{50}-level) activity and can be used as a threshold to assess meaningful bacterial suppression over the dosing interval.

In this study, both metrics were used complementarily. PK/PD target was applied to evaluate the performance of each antibiotic independently based on established clinical benchmarks, while E(t) provided an integrated assessment of the combined pharmacodynamic effect of antibiotics in combination.

2.3.5 The probability of target attainment (PTA)

PTA using the PopPK parameters was calculated using a Monte Carlo simulation approach, where 500 virtual patient profiles were generated by sampling clearance and volume parameters from log-normal distributions based on Monolix-derived population estimates. Drug concentrations were simulated over time for each profile using Euler's method, and PTA was defined as the proportion of simulated profiles maintaining free drug concentrations above the pharmacodynamic target for the entire dosing interval.

Also, the PTA calculations in the use of PK/PD modelling and simulations were performed using repeated deterministic simulations, in which each dosing regimen was simulated 100 times per patient to capture concentration–time profiles and evaluate pharmacodynamic target attainment. This is because this approach allowed for robust estimation of PTA under fixed PK parameter values and defined patient covariates (i.e., renal function). This simulation did not involve full Monte Carlo simulations. Specifically, the simulations did not include stochastic sampling from population PK distributions, nor were they integrated with a distribution of MIC values typically used in traditional Monte Carlo frameworks. PTA was calculated for all PK/PD targets, allowing assessment of whether dosing regimens achieved either traditional or mechanistic PK/PD goals. Comparing the two provided additional insights into regimen effectiveness, especially when interpreting borderline or suboptimal exposures: a regimen may meet the PK/PD target (i.e, 100% *f*T>MIC) target but still fall short of an adequate E(t) threshold, suggesting insufficient overall bactericidal activity. Data outputs were summarised and visualised using ggplot2, producing heatmap-style plots that illustrated PTA across the full regimen grid, stratified by CrCl and MIC assumptions if applicable. These visualisations supported interpretation of optimal dosing strategies for patients with multidrug-resistant gram-negative infections.

Chapter 3 Results

3.1 Phase I A systematic review to define the target PK/PD parameters Search results

In Phase I of the project, a systematic review was conducted to identify clinically impactful PK/PD targets for antibiotics treating GNB infections. The search strategy followed PRISMA guidelines, as illustrated in Figure 3-1. A total of 25,794 records were identified from electronic databases on 24 March 2023. After removing 8,720 duplicates, 17,075 records remained for title and abstract screening. Articles lacking sufficient information in the abstract were retained for full-text screening.

Extracted data included title, author(s), year of publication, study design, aspects of the study population (sample size, age group, gender, BMI or weight, bacteria, infections, underlying diseases, the severity of underlying conditions by APACHE II or SOFA scores if applicable, renal function), study antibiotics, study antibiotic dose, duration, PK/PD parameters and values, PTA and clinical outcomes. Although serum albumin was known to impact the pharmacokinetics and pharmacodynamics of protein-bound antibiotics like beta-lactams and aminoglycosides (Ulldemolins et al., 2011), none of the included studies examined this covariate.

Of the 44 included studies, 23 were case reports, three were randomised controlled trials (RCTs), and the remaining 18 were non-randomised studies (Non-RCTs). The characteristics of the included studies were summarised in Appendix 3 in accordance with Cochrane Handbook guidelines. A meta-analysis was performed using pooled data from RCTs and eligible non-RCTs. Three non-RCTs were excluded from the meta-analysis because they provided data from individual patients only, not from groups.

PK/PD values for the antibiotics studied were also extracted from the EUCAST Rationale Documents for Clinical Breakpoints and compiled to enable cross-comparison of studies targeting the same bacterial species. In total, the analysis of 185 single records of PK/PD targets and 624 patient data collected from RCTs and Non-RCTs was performed to study the differences between clinical PK/PD and *in vitro* ones.

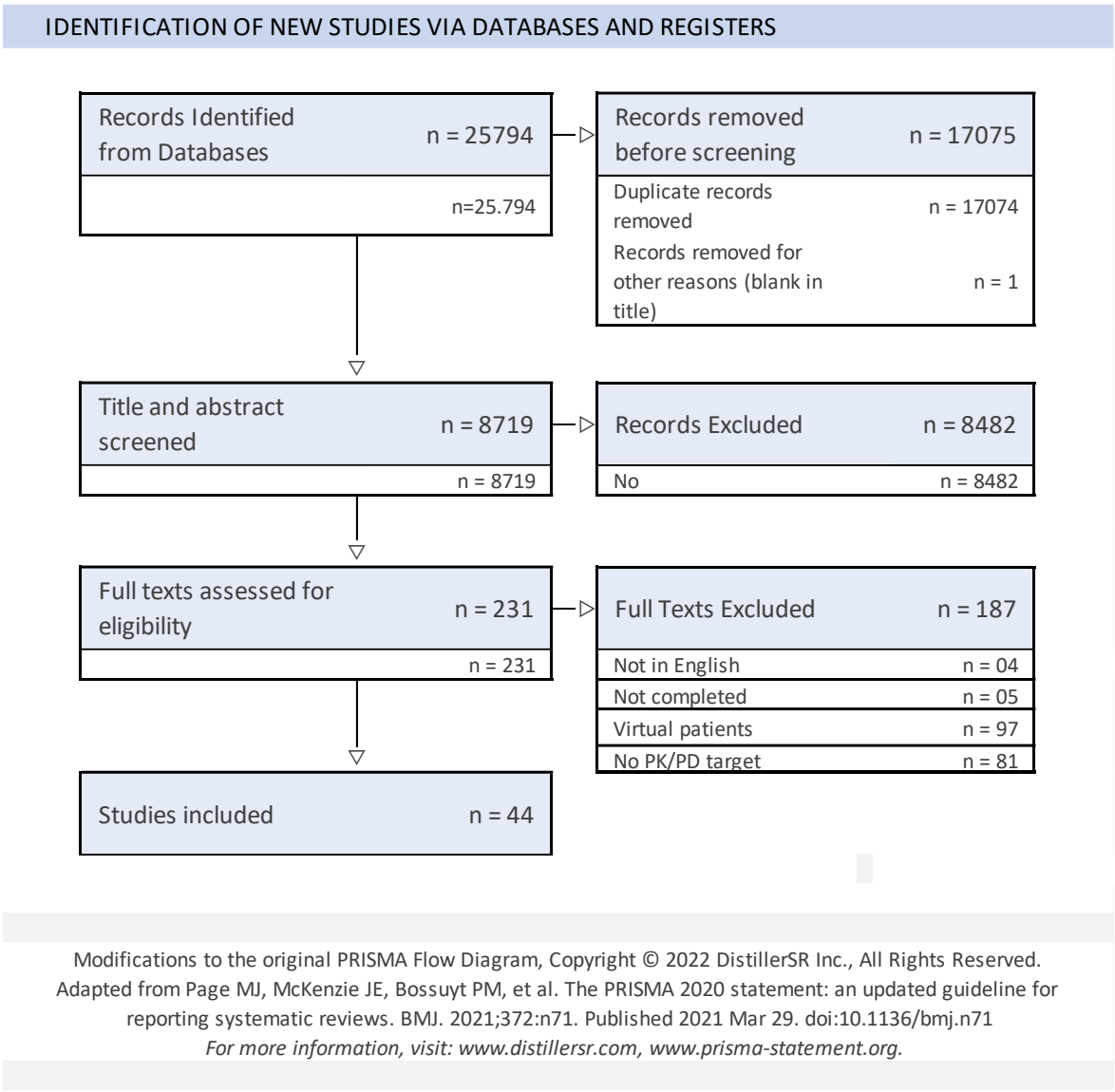


Figure 3-1. The result of the search process in accordance with the PRISMA guidelines.

3.1.1 Included studies

44 out of 231 studies were identified to fulfil the inclusion criteria. Studies were excluded if they assessed PK/PD target attainment using virtual patient simulations or hypothetical dosing regimens rather than real-world data from actual patients, even if they defined relevant PK/PD targets for antibiotics against GNB infections. The majority of eligible studies (51.27%) were case studies.

There were 21 antibiotics, including amikacin, aztreonam, cefepime, cefiderocol, cefoperazone-sulbactam, cefotaxime, cefoxitin, ceftazidime, ertapenem, ceftazidime-avibactam, ceftolozane-tazobactam, ciprofloxacin, colistin, doripenem, fosfomycin, gentamicin, imipenem, meropenem, meropenem-vaborbactam,

piperacillin-tazobactam, tigecycline and three combinations including fosfomycin and cefiderocol, fosfomycin, ceftazidime-avibactam and fosfomycin and meropenem. The study included participants of all age groups: paediatrics (ages 0-17), adults (aged 18-63), and the elderly (aged 64+), representing a diverse range of ages. There was no limitation on underlying diseases, using the APACHE II and SOFA to evaluate the severity of the patient's condition. The participant's renal function was collected using their information on CrCl, serum creatinine or eGFR, and the state of kidney diseases, including acute kidney injury (AKI), continuous renal replacement therapy (CRRT), continuous veno-venous hemofiltration (CVVH), continuous veno-venous hemodialysis (CVVHD), or continuous veno-venous hemodiafiltration (CVVHDF).

3.1.1.1 Randomised controlled trials

Quality assessment

Figure 3-2 displays the results of assessing the risk of bias using version 2 of the Cochrane risk-of-bias tool for randomised trials RoB2 (Sterne et al., 2019). Each study was scored as having either a 'High' or 'Low' level of bias. Out of the three studies, only one studied by Chongcharoenyanon et al. (2021), was deemed to have a low bias (Chongcharoenyanon et al., 2021). The remaining two studies conducted by Zhou et al. (2017) and Jaruratanasirikul et al. (2005) had biased randomisation processes due to the lack of specification of randomisation procedures or allocation (Jaruratanasirikul et al., 2005; Zhou et al., 2017).

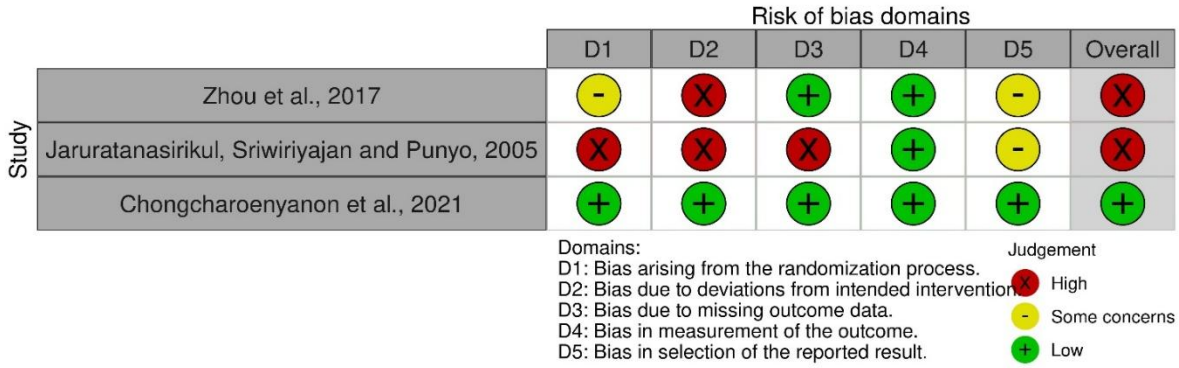


Figure 3-2. Results of quality assessment of 3 RCTs.

Study characteristics and results

Detailed data extracted from the included RCTs, including MIC values, PK/PD targets, infusion regimens, and %T>MIC attainment, are provided in Appendix 3 (see Phase 1_Summary for details) for transparency and reference.

In a paediatric RCT by Chongcharoenyanon et al. (2021) (n=90), piperacillin/tazobactam (100 mg/kg every 8 hours, 4-hour infusion) achieved 50% fT>MIC in 100% of patients at MIC ≤4 mg/L, and in 95.6%, 91.1%, and 75.6% at MICs of 8, 16, and 32 mg/L, respectively. For 50% *f*T>4×MIC, the corresponding attainment was 95.5% (MIC 2 mg/L), 91.1% (MIC 4 mg/L), 75.6% (MIC 8 mg/L), 42.2% (MIC 16 mg/L), and 8.9% (MIC 32 mg/L).

In Jaruratanasirikul et al. (2005) (n=9), patients with ventilator-associated pneumonia received meropenem 2 g every 8 hours as a 3-hour infusion. The mean ± SD %T>MIC was 98.56 ± 3.28% for MIC 1 mg/L, 85.56 ± 16.42% for MIC 4 mg/L, 72.89 ± 22.40% for MIC 8 mg/L, and 57.89 ± 24.26% for MIC 16 mg/L.

Zhou et al. (2017) conducted an RCT in 79 elderly patients with Gram-negative lower respiratory tract infections comparing standard versus model-based individualised meropenem dosing. The target T>MIC was ≥76%, and in the intervention group, the median %T>MIC achieved was 98.9% (IQR: 77.1–100%). MIC values were not reported per patient, but the overall cohort had a median MIC of 1 mg/L (IQR: 0.25–4 mg/L).

Across the three included RCTs, all studies focused on evaluating PK/PD target attainment using the EUCAST-recommended pharmacodynamic index of *f*T>MIC) for beta-lactam antibiotics, but applied more stringent thresholds than standard guidance. For meropenem, while EUCAST suggests a target of approximately 54% *f*T>MIC for maximal bacterial effect, both Zhou et al. 2017 and Jaruratanasirikul et al. 2005 assessed higher thresholds. Zhou et al. targeted 76% *f*T>MIC based on prior model-based optimisation, while Jaruratanasirikul et al. reported %T>MIC outcomes of up to 98% at low MICs. For piperacillin–tazobactam, EUCAST recommends 30–35% fT>MIC; however, the study by Chongcharoenyanon et al. 2021 evaluated target attainment at 50% *f*T>MIC and 50% *f*T>4×MIC across a range of MIC values (2–32 mg/L). These findings highlight a consistent trend toward applying higher PK/PD thresholds, reflecting growing evidence that more

aggressive targets may be necessary to optimise outcomes in critically ill or high-risk patient populations, particularly when treating organisms with elevated MICs.

3.1.1.2 Non-randomised studies

Quality assessment

The ROBINS-I risk of bias assessment was applied to 18 non-RCT studies included in Phase 1. The overall quality of evidence was affected by methodological limitations, with the majority of studies rated as having a moderate to serious overall risk of bias. Seven of the 18 studies were rated as having a serious overall risk of bias (Abhilash et al., 2015; Abhilash et al., 2016; Corcione et al., 2021; Eisert et al., 2021; O'Jeanson, 2021; Pilmis et al., 2021; Chabert et al., 2022). This was largely driven by the absence of adjustments for important confounders such as renal function, severity of illness, or infection site. The remaining 11 studies were rated as "Moderate" in the confounding domain, suggesting that they incorporated at least some level of covariate modelling or attempted to address confounding through statistical or clinical stratification methods (Olbrisch et al., 2019; Philpott et al., 2019; Sorlí et al., 2019; Gómez-Junyent et al., 2020; Yang et al., 2021; Zhou et al., 2021; Wieringa et al., 2022; Zahr et al., 2022; Fresan et al., 2023; Gatti et al., 2023; Zavrelova et al., 2023). These included adjustments such as renal function, albumin levels, or stratification by urine output.

The selection of participants was generally rated at moderate risk of bias. Although most studies clearly defined inclusion criteria, the recruitment process was often not fully described, raising concerns about potential selection bias. Studies relied on convenience sampling or inclusion based on the availability of TDM data.

The classification of interventions was consistently evaluated as low risk. Antibiotic dosing regimens, including intermittent, prolonged, and continuous infusions, were well documented and appropriately categorised in all studies. Similarly, deviations from intended interventions were uncommon, and treatments were typically administered in accordance with established protocols or standard care practices.

Missing data did not present a major concern. Most studies provided complete pharmacokinetic sampling and analytical results, and data gaps, when present, were transparently acknowledged. Measurement of outcomes was also uniformly rated as low risk. The included studies employed validated analytical techniques

such as high-performance liquid chromatography (HPLC) or liquid chromatography-tandem mass spectrometry (LC-MS/MS) to determine antibiotic concentrations.

However, the selection of reported results was rated at moderate risk in many studies. While pharmacokinetic outcomes were generally well presented, several studies offered limited analysis of clinical or microbiological endpoints, and subgroup comparisons were often missing or underdeveloped. Some reports lacked clarity regarding prespecified endpoints or presented only partial PK/PD evaluations without corresponding outcome data.

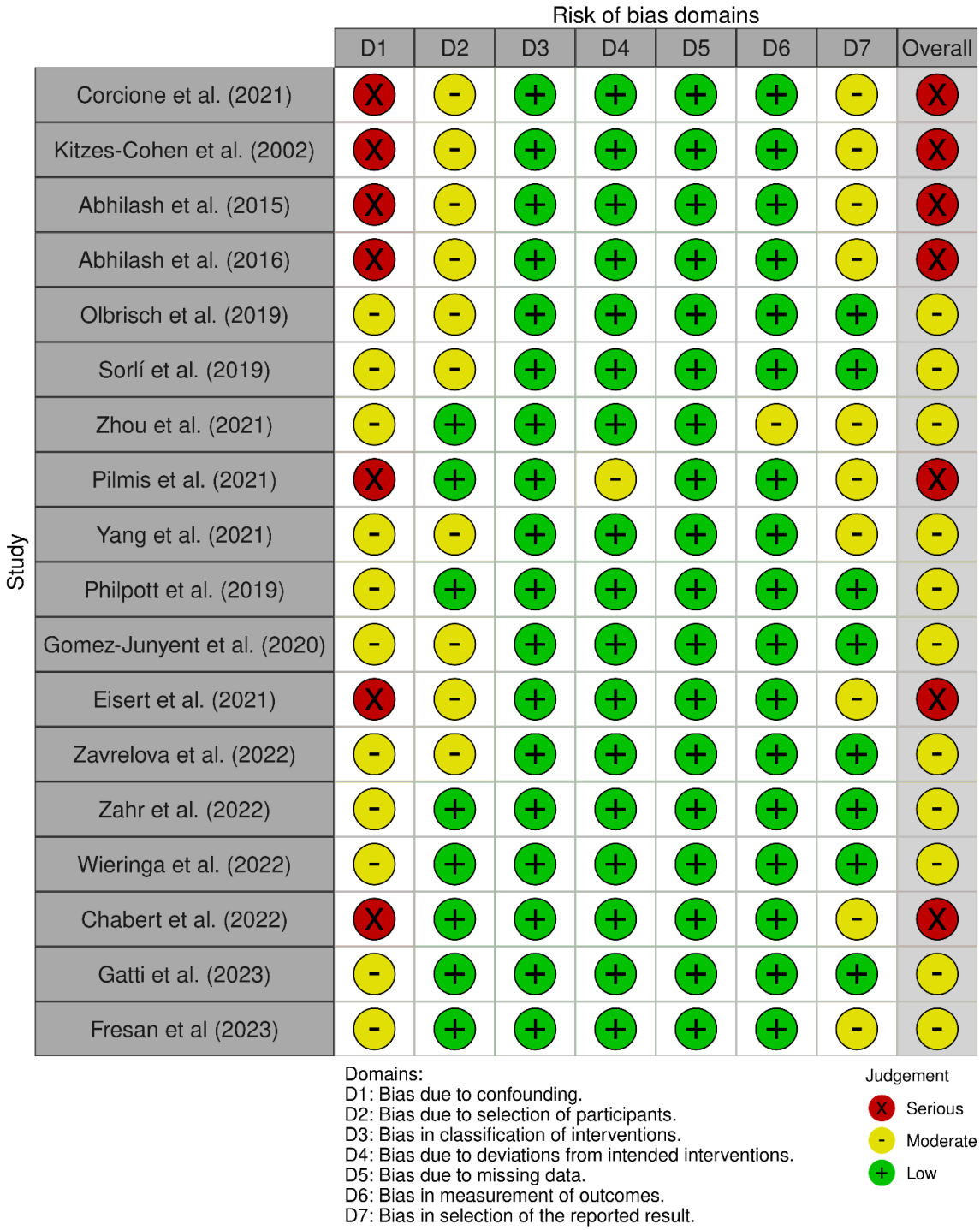


Figure 3-3. The results of evaluating risk of bias for non-RCTs.

Study characteristics and results

Aminoglycosides (amikacin, gentamicin)

Aminoglycosides were studied in two reports focusing on C_{max}/MIC, the relevant PK/PD index. However, these studies were less consistent in how they reported individual patient data, and limited correlation with outcomes was available.

Beta-lactam antibiotics (cephalosporins, carbapenems, penicillin/beta-lactamase inhibitors)

This group includes aztreonam, cefotaxime, cefoxitin, ceftazidime, ceftazidime-avibactam, cefepime, ceftolozane-tazobactam, meropenem, imipenem, and piperacillin-tazobactam. Most studies aimed to achieve *f*T>MIC, the primary PK/PD index for beta-lactams.

13 non-RCTs studied 316 patients and eight of them commonly reported modified infusion strategies, including extended (up to 6 hours) or continuous infusions, to enhance target attainment (Abhilash et al., 2015; Abhilash et al., 2016; Olbrisch et al., 2019; Philpott et al., 2019; Eisert et al., 2021; Pilmis et al., 2021; Yang et al., 2021; Chabert et al., 2022; Fresan et al., 2023; Gatti et al., 2023; Zavrelova et al., 2023). Two studies evaluated higher-than-standard *f*T>MIC thresholds to assess efficacy under EUCAST-compliant dosing (Yang et al., 2021; Wieringa et al., 2022). A subset of studies used fCss/MIC instead of *f*T>MIC, which reflects the magnitude rather than the duration of target attainment (Gómez-Junyent et al., 2020; Gatti et al., 2023). Though conceptually related, this metric allowed the authors to propose much higher thresholds (i.e., *f*Css/MIC ≥10 for ceftazidime) to ensure sustained bacterial killing.

Cefiderocol

Cefiderocol was evaluated against published *f*T>MIC or *f*Css/MIC thresholds. Extended infusions were often used to enhance target attainment despite sparse outcome data.

Glycylcyclines (tigecycline)

Two studies assessed tigecycline using different PK/PD indices (Yang et al., 2021; Zhou et al., 2021). Yang et al. proposed a novel target (AUC_0–12 × V / MIC ≥ 100), while Zhou et al. applied EUCAST thresholds but tailored them to clinical and microbiological endpoints (fAUC/MIC > 0.9 for efficacy; >0.35 for bacterial eradication). This variability underscores the lack of harmonised PK/PD thresholds for tigecycline.

Polymyxins (colistin)

The PK/PD of colistin was evaluated in one study , which adhered to EUCAST's AUC/MIC target of ≥60 mg·h/L (Sorlí et al., 2019). However, the authors increased

the daily dose up to 9 MIU and switched from IV bolus to 30-minute infusions to improve target attainment. This highlights how even well-defined targets may require administration adjustments for critically ill patients.

Overall observations:

Across the 18 non-RCT studies, beta-lactam antibiotics constituted the majority of agents investigated, with *f*T>MIC being the most commonly used PK/PD target. A consistent strategy observed in these studies was the use of extended or continuous infusion to enhance target attainment, particularly in critically ill patients or those with altered renal function. While some studies employed alternative PK/PD indices, such as *f*Css/MIC, these were conceptually aligned with *f*T>MIC but aimed to quantify target magnitude more precisely.

Only half of the studies provided individual-level patient data, which limited the granularity of comparative analysis. Furthermore, although several studies documented whether PK/PD targets were achieved, relatively few linked this explicitly to clinical outcomes or microbiological eradication, highlighting a gap in the integration of PK/PD data with treatment effectiveness. The heterogeneity in study designs, target definitions, and reporting underscores the need for more standardised approaches to PK/PD evaluation in non-randomised studies.

3.1.1.3 Case series and case studies

Quality assessment

Due to the lowest level of case reports in the hierarchy of systematic reviews, all 23 case studies and case series would score ‘Definitely High’ (Sackett, 1989; Burns et al., 2011). However, the NHLBI (National Heart, Lung, and Blood Institute) tool was applied to evaluate the quality of these 23 studies (NHLBI, 2013) (Table 3-1). Yet this tool has not been published, which means that the assessment is not considered as standardised as claimed by NHLBI. Besides, the rating system of “Good, Fair or Poor” implemented by NHLBI was not thoroughly developed. The tool simply provided essential factors that needed to be considered but did not instruct how to rate the overall quality of a study. To determine whether a study is “Good”, “Fair”, or “Poor”, one point is awarded for every “Yes” and zero points for every other answer. The study that met “7-9” criteria was deemed “Good”; the one that met “4-6” was considered “Fair”, and the one that met only “0-3” was evaluated as “Poor”.

The results of these 23 included case studies were tabulated, and more than 78% of them were deemed “Good”.

Table 3-1. Results of bias assessment using the NHLBI tool

Study	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Quality rating
(Aoki et al., 2011)	N	Y	N*	Y	Y	Y	Y	Y	Y	Good
(Heil et al., 2015)	Y	N	N*	Y	Y	Y	Y	CD	Y	Fair
(Kobic et al., 2021)	Y	Y	N*	NR	Y	Y	Y	Y	Y	Fair
(König et al., 2021)	Y	Y	N	Y	Y	Y	Y	Y	Y	Good
(Mornese Pinna et al., 2022)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Good
(Kobic et al., 2022)	Y	Y	N*	N	Y	Y	Y	Y	Y	Good
(Gatti et al., 2023)	Y	N	Y	Y	Y	Y	Y	Y	Y	Good
(Liu et al., 2016)	Y	Y	N*	NR	Y	N	Y	Y	Y	Fair
(Goutelle et al., 2021)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Good
(Wenzler et al., 2017)	Y	Y	N*	NR	Y	Y	Y	Y	Y	Good
(Teng et al., 2022)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Good
(Shah et al., 2021)	Y	Y	N*	Y	Y	Y	Y	Y	Y	Good
(Utrup et al., 2010)	Y	Y	N*	Y	Y	Y	Y	Y	Y	Good
(Menna et al., 2018)	N	Y	Y	Y	Y	Y	Y	Y	Y	Good
(Bulik et al., 2010)	N	Y	Y	Y	Y	Y	Y	Y	Y	Good
(Cojutti et al., 2022)	Y	Y	N*	NR	Y	Y	Y	CD	Y	Fair
(Gatti et al., 2022)	Y	N	Y	N	Y	Y	Y	Y	Y	Good
(Kuti et al., 2004)	N	Y	N*	Y	Y	Y	Y	Y	Y	Good
(Delfino et al., 2018)	Y	N	Y	Y	Y	Y	Y	Y	Y	Good
(Wu et al., 2020)	Y	Y	N*	Y	Y	Y	Y	Y	Y	Good
(Hanretty et al., 2018)	Y	Y	N	NR	Y	Y	Y	CD	Y	Fair
(Zhang et al., 2022)	Y	Y	N	CD	Y	Y	Y	Y	Y	Good
(Soukup et al., 2019)	Y	Y	N*	NR	Y	Y	Y	Y	Y	Good
N: Only one case; NR: not report, CD: can’t determine*										

The nine questions of NHLBI tools are as below:
Question 1. Was the study question or objective clearly stated?
Question 2. Was the study population clearly and fully described, including a case definition?

Question 3. Were the cases consecutive?
Question 4. Were the subjects comparable?
Question 5. Was the intervention clearly described?
Question 6. Were the outcome measures clearly defined, valid, reliable and implemented consistently across all study participants?
Question 7. Was the length of follow-up adequate?
Question 8. Were the statistical methods well described?
Question 9. Were the results well described?

Five (5) studies were evaluated as having “Fair” quality, each scoring 6 out of 9 on the NHLBI case series assessment tool. The study by Kobic et al. (2021) offered a detailed PK and PD evaluation of cefiderocol in a single critically ill patient undergoing CVVHDF, with clear intervention reporting and PK modelling, but lacked statistical analysis and subject comparability. Similarly, the study by Heil et al. (2015) described the use of extended-infusion cefepime and colistin in a hemodialysis patient with MDR *P. aeruginosa*, demonstrating target attainment but reporting no case selection strategy or inferential statistics. The study by Liu et al. (2016) investigated the emergence of cefoperazone/sulbactam resistance in a single patient with hospital-acquired pneumonia due *to A. baumannii*, with a clearly stated objective, detailed intervention reporting, and consistent PK/PD assessments, but lacked case comparability, consecutive enrolment, and statistical analysis. Hanretty et al. (2018) presented the first case of meropenem-vaborbactam monotherapy in a pediatric patient with KPC-producing *K. pneumoniae* bacteremia; the study reported thorough PK monitoring and *f*T > MIC achievement but was limited by its single-case design and absence of statistical modelling. Lastly, Cojutti et al. (2022) documented the use of high-dose meropenem plus fosfomycin guided by real-time therapeutic drug monitoring in a COVID-19 patient with XDR *K. pneumoniae*, providing robust clinical and PK data despite limitations in statistical analysis and generalizability. All five studies contributed meaningful insights but were classified as “Fair” due to methodological constraints inherent to single-case reporting and limited information of statistical methods.

The remaining studies scored between 7 and 9 out of 9. Although these studies involved a limited number of patients (mostly fewer than 10), their clearly stated objectives, well-documented methodologies, and robust PK/PD interpretations supported their classification as high-quality evidence.

Study characteristics and results

A total of 23 case studies involving 58 patients were included in the systematic review. The antibiotics investigated were grouped by class to facilitate analysis, as follows:

Beta-lactams (carbapenems and cephalosporins)

Carbapenems

A total of 15 patients received carbapenem antibiotics, including meropenem (n = 10) and doripenem (n = 5). The primary PK/PD index reported in this group was *f*T>MIC, with defined targets ranging from >40% to >70% of the dosing interval. In two patients, a higher threshold of *f*T>4×MIC was applied to optimise efficacy against resistant organisms. Prolonged infusion strategies were used in 11 of 15 patients, with infusion durations ranging from 3 to 4 hours to enhance time above MIC. PK/PD targets were successfully achieved in 11 of 15 patients (73.3%). In the four patients who did not reach target attainment, the observed causes included high MICs (i.e., 16–32 mg/L for *P. aeruginosa*) and standard infusion durations without dose adjustment.

Cephalosporins

12 patients were treated with third-, fourth-, or fifth-generation cephalosporins. Among these, ceftazidime-avibactam was administered in 8 cases, cefepime in 2 cases, and ceftaroline in 1 case. One additional patient received a cephalosporin–fosfomycin combination.

For ceftazidime-avibactam, the reported PK/PD indices were *f*T>MIC (n = 5), fT>4×MIC (n = 2), and *f*T>1 μg/mL (n = 1, for avibactam). The target thresholds were generally defined as ≥50–100% fT>MIC, and infusion durations ranged from 2 to 3 hours. PK/PD targets were achieved in 6 of 8 patients (75%). The remaining 2 patients failed to achieve the target due to insufficient concentrations relative to MIC, despite standard dosing.

Cefepime, a fourth-generation cephalosporin, was administered in 2 cases with target indices of *f*T>MIC 60–70%. One patient reached the target, while the other did not, likely due to inadequate exposure related to renal function and high bacterial MIC. The single patient treated with ceftaroline, a fifth-generation cephalosporin,

achieved the defined PK/PD target of >60% *f*T>MIC, administered via prolonged infusion.

Among the 12 patients receiving cephalosporins, 7 were treated with prolonged infusions, while 5 received standard bolus dosing. In total, PK/PD targets were achieved in 8 of 12 patients (66.7%).

Cefiderocol

Cefiderocol was used in 23 patients, making it the most frequently investigated antibiotic in the dataset. It was used predominantly for infections caused by carbapenem-resistant GNB, including *P. aeruginosa* (n = 10), *A. baumannii* (n = 8), and *K. pneumoniae* (n = 5). The reported PK/PD indices were *f*T>MIC (n = 18), *f*Css>MIC (n = 4), and Css > predefined thresholds such as 4–10 mg/L (n = 1). Target values ranged from ≥75% to 100% fT>MIC or Css exceeding MIC or fixed thresholds.

All patients received standard cefiderocol regimens (2 g every 8 hours) administered via 3-hour prolonged infusion. PK/PD targets were successfully attained in 20 of 23 patients (87%). In the 3 patients with subtherapeutic exposure, target failures were associated with elevated MICs (i.e., ≥8 mg/L), augmented renal clearance, or the absence of dose escalation strategies.

Fluoroquinolones (Ciprofloxacin)

For ciprofloxacin, only one patient in the review received ciprofloxacin, as examined by the study by Utrup et al. (2010), which used Cmax/MIC >10 and AUC/MIC >125, differing from EUCAST's suggested *f*AUC/MIC ≥140. The therapy did not achieve the target despite standard dosing, which may be explained by a high MIC value in the pathogen or reduced drug exposure due to altered pharmacokinetics. This case highlights the limitations of ciprofloxacin use in the management of highly resistant GNB infections.

Polymyxins (Colistin)

Two patients received colistin, both for infections caused by MDR *A. baumannii*. The studies applied Css > 2 mg/L or AUC/MIC ≥ 60 as the target index. In one patient, the PK/PD target was successfully achieved using high-dose intravenous therapy with concentration-guided adjustments. The second patient did not reach the target,

likely due to dose-limiting toxicity or suboptimal pharmacokinetics in the setting of organ dysfunction.

Combination Regimens

In seven cases, cephalosporins were administered in combination with fosfomycin. Particularly, Cojutti et al. (2022) and Gatti et al. (2022) evaluated regimens combining fosfomycin with meropenem, ceftazidime-avibactam, or cefiderocol. In Gatti et al.'s study, six patients were assessed, and in Cojutti et al.'s study, one patient was assessed using PK/PD indices aligned with EUCAST recommendations. However, both studies applied elevated PK/PD thresholds for fosfomycin, with AUC/MIC targets set at 40.08 and ≥83, respectively. Among the seven patients, only one failed to attain the predefined PK/PD target, with an observed AUC/MIC of 32.4. These combination regimens were primarily used in patients infected with extensively drug-resistant (XDR) or DTR organisms. Dosing strategies frequently involved prolonged infusions and individualised adjustments based on pathogen-specific MIC values or desired %*f*T>MIC targets. Overall, PK/PD target attainment was achieved in six out of seven patients. The overall high rate of attainment supports the efficacy of individualised dosing and combination strategies in treating XDR or DTR infections.

Overall observations

Overall PK/PD Target Attainment

Across all 58 patients, PK/PD targets were achieved in 44 patients (77.2%), while 13 patients (22.8%) failed to meet the predefined thresholds. The most frequently applied PK/PD index was *f*T>MIC, reported in 46 patients (80.7%), followed by *f*T>4×MIC in 4 patients (7%), Css>MIC or fixed thresholds in 6 patients (10.5%), and AUC/MIC in 2 patients (3.5%).

Prolonged infusion was the most common strategy to optimise drug exposure, used in 34 patients (59.6%). A total of 23 patients (40.4%) were treated without dose modification, following standard infusion protocols. Route modification (i.e., switching to continuous infusion) was documented in 2 patients (3.5%).

Bacterial pathogens and resistance profiles

The pathogens treated were predominantly resistant Gram-negative organisms, including *P. aeruginosa*, *K. pneumoniae*, and *A. baumannii*. Resistance

classifications reported were: MDR (n=18), XDR (n=3), DTR (n=3), CRE (n=1), general resistance (n=28), and no resistance (n=6). These findings underscore the critical role of individualised dosing and infusion strategies in maximising PK/PD target attainment, particularly when treating infections caused by resistant GNB.

3.1.2 Meta-analysis

A pooled analysis of 292 patients from three RCTs and three non-RCTs (Details in Table is shown in Figure 3-4.

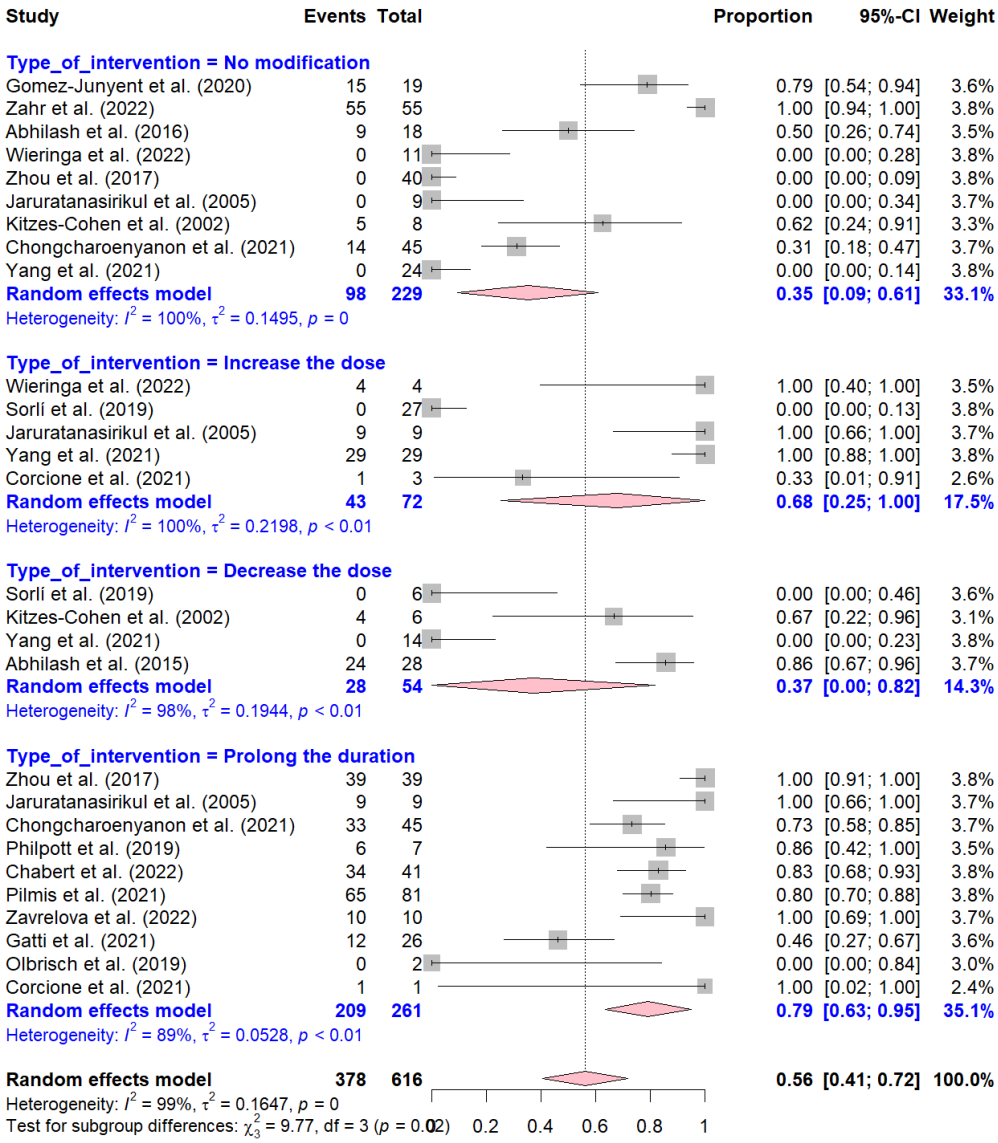


Figure 3-4. Meta-analysis of three RCTs and three non-RCTs to establish the effects of modifying labelled dosing regimens for individuals.

Forest plot showing the proportion of patients who achieved the PK/PD targets across different antibiotic dose modification strategies. Studies are grouped by intervention type: no modification, increase in dose, decrease in dose, or prolonged duration.
The X-axis represents the proportion of events (Events/Total) with corresponding 95% confidence intervals (CI) for each study. A random-effects meta-analysis model was used within each intervention subgroup, and an overall pooled estimate is shown at the bottom.
Heterogeneity was assessed using the I^2 statistic, and subgroup differences were tested using χ^2 tests.

The forest plot summarises the proportion of patients who achieved predefined PK/PD targets under different types of dosing interventions. Each grey square represents the proportion of success in individual studies, stratified by the type of intervention applied: no modification, increased dose, decreased dose, or prolonged infusion duration.

Across all interventions, the overall pooled proportion of patients achieving PK/PD targets was 56% (95% CI: 41%–72%), indicating that dosing adjustments, whether by increasing the dose, prolonging the infusion, or other modifications, which were adjusted according to severity of the infections and clinical status of the patients, were associated with improved target attainment. In contrast, among patients who received standard, unmodified dosing regimens, the pooled proportion achieving PK/PD targets was notably lower, at 35% (95% CI: 9%–61%). This suggests that relying solely on labelled dosing may be insufficient for achieving optimal antibiotic exposure, particularly in the context of resistant or critically ill patient populations.

Among all intervention types, prolonging the infusion duration demonstrated the highest pooled success rate, with 79% (95% CI: 63%–95%) of patients achieving PK/PD targets. Conversely, dose reduction was the least effective strategy, with a pooled success rate of only 37% (95% CI: 0%–82%).

These findings underscore the importance of individualising antibiotic dosing strategies, particularly through prolonged infusion, to improve pharmacodynamic efficacy, rather than relying on standard fixed-dose regimens.

3.1.2.1 Heterogeneity

Heterogeneity in proportional meta-analyses was assessed using the I^2 statistic, which quantifies variability in observed proportions across studies. As noted in

previous research, I^2 values are typically high in proportional meta-analysis due to the statistical nature of proportions and small sample sizes, even when clinical differences are minimal (Borenstein et al., 2021). In this review, the I^2 for the main analysis (Figure 3-4), was 99%. This value reflects the diverse nature of the included studies and should not be interpreted as indicating inconsistency or methodological flaws. Given the heterogeneity expected in this context, statistical approaches assuming large between-study variation (i.e., random-effects models) were appropriately applied throughout the analysis.

3.1.3 The selection of antibiotics for modelling and simulations:

The systematic review highlights key PK/PD targets essential for optimising antibiotic efficacy against Gram-negative infections. For meropenem, the primary targets include maintaining 40%-100% of the dosing interval with *f*T>MIC, with enhanced efficacy observed at 100% *f*T>4×MIC. Similarly, ceftazidime/avibactam achieves optimal activity when 100% *f*T>MIC is maintained, and Css/MIC remain between 3.8 and 6.2. These PK/PD targets provide a robust framework for developing models that simulate drug exposure and bacterial killing dynamics under varying clinical scenarios. Incorporating these parameters into PK/PD modelling and simulation enables the prediction of dosing regimens required to achieve sufficient target attainment, particularly in patients with altered pharmacokinetics or multidrug-resistant infections. This approach ensures the effective use of meropenem and ceftazidime/avibactam in combating Gram-negative pathogens while minimising the risk of resistance development.

Table 3-2. The predefined PK/PD targets that were determined from the systematic review

Antibiotic Classification (WHO)	**Antibiotics**	**PK/PD parameters 1**	**PK/PD parameters 2**	**PK/PD parameters 3**
Aminoglycosides	Amikacin	Cmax/MIC ≥ 8		
Fluoroquinolones	Ciprofloxacin	Cmax/MIC > 10		
Third-generation cephalosporins	Ceftazidime-avibactam	*100% f*T>MIC	Css/MIC ≤ 5	
Aminoglycosides	Gentamicin	Cmax/MIC ≥ 8	100% fT>MIC	
Carbapenems	Imipenem	T>MIC	T>5xMIC	
Fourth-generation	Cefepime	60%-70%	100% *f*T>MIC	*f*Css>3-

Antibiotic Classification (WHO)	Antibiotics	PK/PD parameters 1	PK/PD parameters 2	PK/PD parameters 3
cephalosporins		T>MIC		10xMIC
Third-generation cephalosporins	Ceftazidime	*f*Css>3-10xMIC	*f*T>5xMIC	Css/MIC 3.8-6.2
Carbapenems	Meropenem	Css/MIC 1-4 (100% fT> 4 x MIC)	40%-100% *f*T>MIC	Css/MIC 3.8-6.2
Beta lactam - beta lactamase inhibitor (anti-pseudomonal)	Piperacillin-tazobactam	40%-100% *f*T>MIC	40%-100% *f*T>4xMIC	Css/MIC 3.8-6.2

This systematic review identified the most impactful PK/PD targets used in the treatment of multidrug-resistant MDR GNB infections in clinical practice. Table 3-2 summarises the findings from the systematic review. The included studies covered a range of antibiotic classes, including aminoglycosides, fluoroquinolones, cephalosporins, carbapenems, and beta-lactam/beta-lactamase inhibitor combinations. The PK/PD indices varied by antibiotic class, with aminoglycosides (i.e., amikacin, gentamicin) typically targeting Cmax/MIC ≥ 8, fluoroquinolones such as ciprofloxacin aiming for Cmax/MIC > 10, and time-dependent antibiotics like cephalosporins and carbapenems focusing on *f*T>MIC or Css/MIC ratios. Notably, ceftazidime-avibactam and meropenem were associated with stringent PK/PD targets, including 100% *f*T>MIC and 100% *f*T > 4×MIC, respectively.

Among these agents, meropenem emerged as the most frequently used antibiotic in the management of MDR gram-negative infections across the reviewed studies. Given its prevalence and the established PK/PD targets, meropenem and its relevant PK/PD target were selected for further investigation through model-informed simulations. These simulations aimed to evaluate the PTA for different dosing regimens, thereby optimising its use in clinical scenarios. In addition, the selection of a second antibiotic for PK/PD model development to optimise dosing regimens will be based on the findings from Phase II, which identified the most frequently isolated bacterium in hospital settings.

3.2 Phase II the collection of anonymised patient data on the use of antibiotics in practice

This study retrospectively analysed anonymised patient data on antibiotic use from two hospitals, one in Vietnam and one in the UK, to understand how antibiotics were prescribed and used in routine clinical practice. The primary aim was to identify the most commonly encountered bacterial pathogens and the actual dosing regimens administered to patients. Using this information, a PK/PD model was developed and applied to evaluate the effectiveness of these real-world regimens through simulation. Given that no PK/PD modelling had previously been used to optimise dosing in these settings, this study sought to assess whether the administered antibiotic therapies were likely to have achieved appropriate PK/PD targets, thereby supporting efforts to improve antimicrobial use and guide future dosing strategies.

3.2.1 Patients

Based on Daniel's formula (Daniel, 1999), the required sample size was estimated at 308 patients, using a 95% confidence level, an expected proportion of 30%, and a 5% margin of error. However, after applying the predefined inclusion and exclusion criteria, only 277 eligible patients were available within the study period. Therefore, the final analysis was conducted on 277 patients.

Out of 277 patients with positive microbiological culture results, data were collected for 225 individuals regarding their demographic information, clinical outcomes, and antibiotic usage. The remaining 52 patients were excluded from the analysis due to insufficient data, specifically incomplete records of drug usage and a lack of information on patient outcomes. Additionally, some patients were excluded because they had concurrent infections with gram-positive bacteria, which did not meet the study's selection criteria. Table 3-3 below summarises the population included in the study.

Table 3-3. The summary of the patient population of Phase II

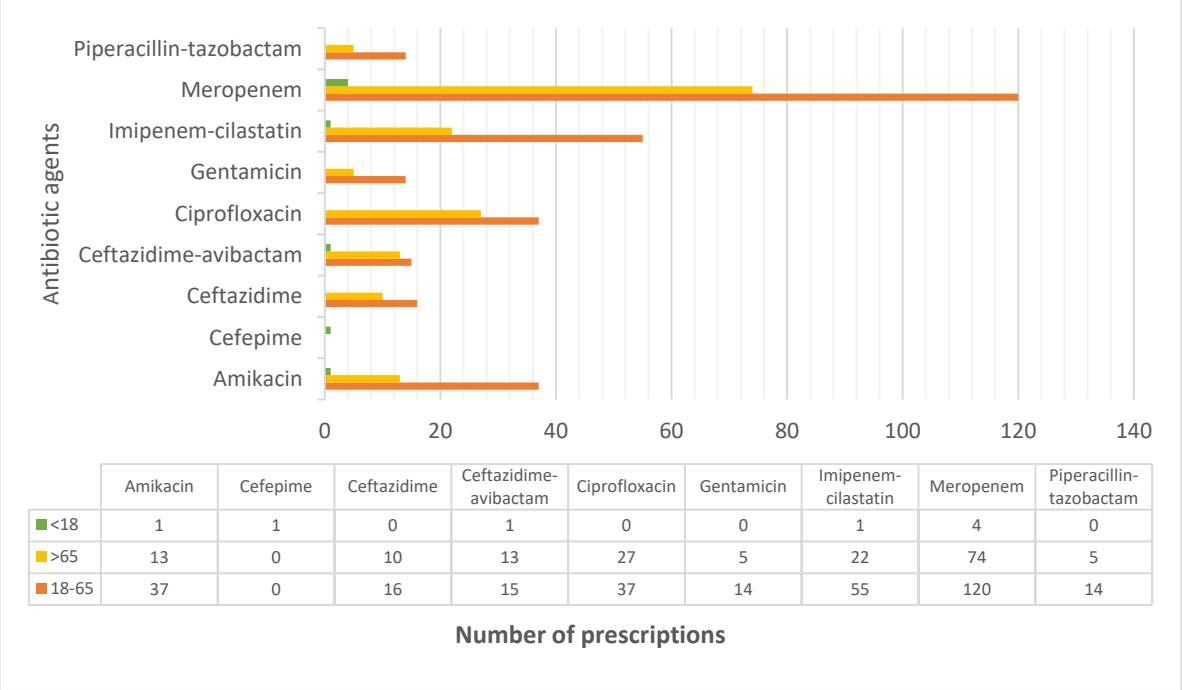
Characteristics	**Population (n=225)**	**Female (n=81)**	**Male (n=144)**
Age (years)	59.0 (1 – 99)	60.0 (15 – 93)	58.5 (1 – 99)
Body weight (kg)	55.0 (9 – 88)	49.0 (41 – 60)	61.0 (9 – 88)

Characteristics	**Population (n=225)**	**Female (n=81)**	**Male (n=144)**
Serum Creatinine (mg/dL)	1.52 (0.30 - 9.83)	1.29 (0.30 - 8.90)	1.65 (0.30 – 9.83)
CRP (mg/L)	94.83 (0.50 - 292.80)	89.93 (0.50 - 292.80)	98.36 (4.80 – 253.00)
Albumin (g/dL)	2.74 (1.27 – 4.00)	2.60 (1.27 - 3.90)	2.81 (1.50 – 4.00)
Procalcitonin (ng/mL)	11.79 (0.02 – 198.0)	6.46 (0.00 - 56.47)	14.65 (0.00 – 198)
Length of hospital stay (days)	28.71 (5 – 177)	29.40 (5 – 107)	28.32 (5 – 177)
Antibiotics	Amikacin, ceftazidime, ceftazidime/avibactam, cefepime, gentamicin, imipenem, meropenem, piperacillin/tazobactam. ciprofloxacin was not included in the population.		
	Amikacin: n=50, mean daily dose: 890 ± 262mg Cefepime: n=1, mean daily dose: 1.000 ± 0mg Ceftazidime: n=17, mean daily dose: 5.227 ± 1536mg Ceftazidime-avibactam: n=24, mean daily dose: 5.053 ± 2578mg Ciprofloxacin: n=46, mean daily dose: 850 ± 374mg Gentamicin: n=16, mean daily dose: 197 ± 90mg Imipenem-cilastatin: n=69, mean daily dose: 1.656 ± 686mg Meropenem: n=192, mean daily dose: 2.735 ± 1476mg Piperacillin-tazobactam: n=18, mean daily dose: 15.773 ± 3712mg		
Bacteria	*A. baumannii, E. coli, K. pneumoniae, P. aeruginosa* Percentage of patients infected with a single bacterium: 67.56% (n=152, with A. baumannii n=15, E. coli n=11, K. pneumoniae n=115, P. aeruginosa n=11). Percentage of patients co-infected with two or more bacteria: 32.44% (n=73).		
Patient outcomes	Deaths: 5.78% (n=13) Failure: 34.67% (n=78)		

Characteristics	Population (n=225)	Female (n=81)	Male (n=144)
	Recovery: 59.56% (n=134)		
Rate of ICU admission	17.33% (n=39/186 cases)		
Patient demographic data was fixed to the first study day. The median was applied for age and body weight, and the other patient factors were used as mean and range or standard deviation. *C-reactive protein (CRP) is a liver-derived acute-phase reactant that rises rapidly during inflammation and is widely used to aid the detection and monitoring of bacterial infection.*			

3.2.2 The use of antibiotics

Figure 3-5 shows the distribution of investigated antibiotics across the three age groups (below 18, 18-65, and above 65). Meropenem was the most commonly used antibiotic among the eight investigated.



	Amikacin	Cefepime	Ceftazidime	Ceftazidime-avibactam	Ciprofloxacin	Gentamicin	Imipenem-cilastatin	Meropenem	Piperacillin-tazobactam
<18	1	1	0	1	0	0	1	4	0
>65	13	0	10	13	27	5	22	74	5
18-65	37	0	16	15	37	14	55	120	14

Figure 3-5. Distribution of antibiotic usage across age groups.

Figure 3-6 shows the percentage of single or combination antibiotic use across three age groups. The preference for single antibiotics was predominant among the elderly compared to the other two age groups. The percentage of multiple antibiotic use was reported to be higher in the group aged below 18 (50%) compared to 35%

in the group aged 18-65 and 30% in the group aged above 65. However, the difference was not statistically significant between these three age groups (p-value 0.560); the data indicate that the patients' age did not influence the choice of multiple antibiotics for treating MDR GNB.

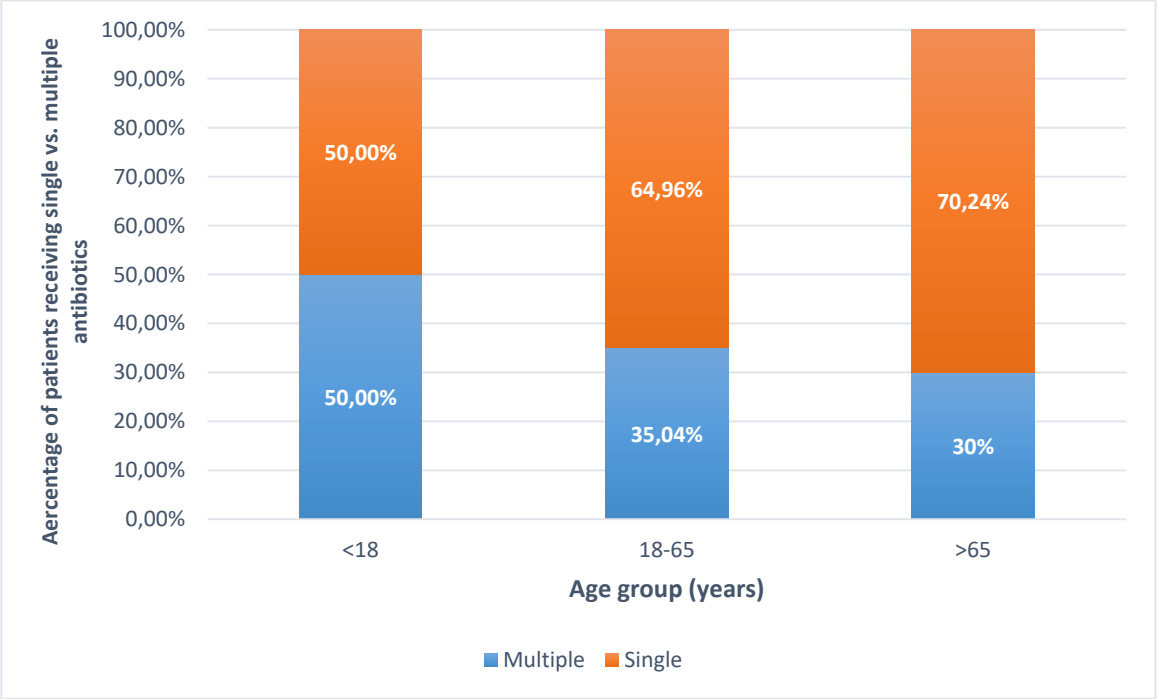


Figure 3-6. The distribution of therapy types (single vs. multiple antibiotics) across the three age groups.

Among 225 patients at two hospitals, 75 received two or more antibiotics, representing 33.33% of the population, while 150 were prescribed a single antibiotic to treat multi-resistant gram-negative strains. However, there was no statistically significant difference in patient outcomes between the single and the combined antibiotic therapy groups (p-value 0.570, as can be seen in Figure 3-7). Of 225 patients, 153 were infected with a single bacterium, while the remaining 72 were infected with two or more bacteria. The mortality and treatment failure rate for those with a single bacterium infection was 38.56%, whereas the rate for those with multiple bacterial infections was 44.44%, but there was no statistically significant difference between the group of single bacterium infection and multiple bacterial infections in patient outcomes (p-value 0.658).

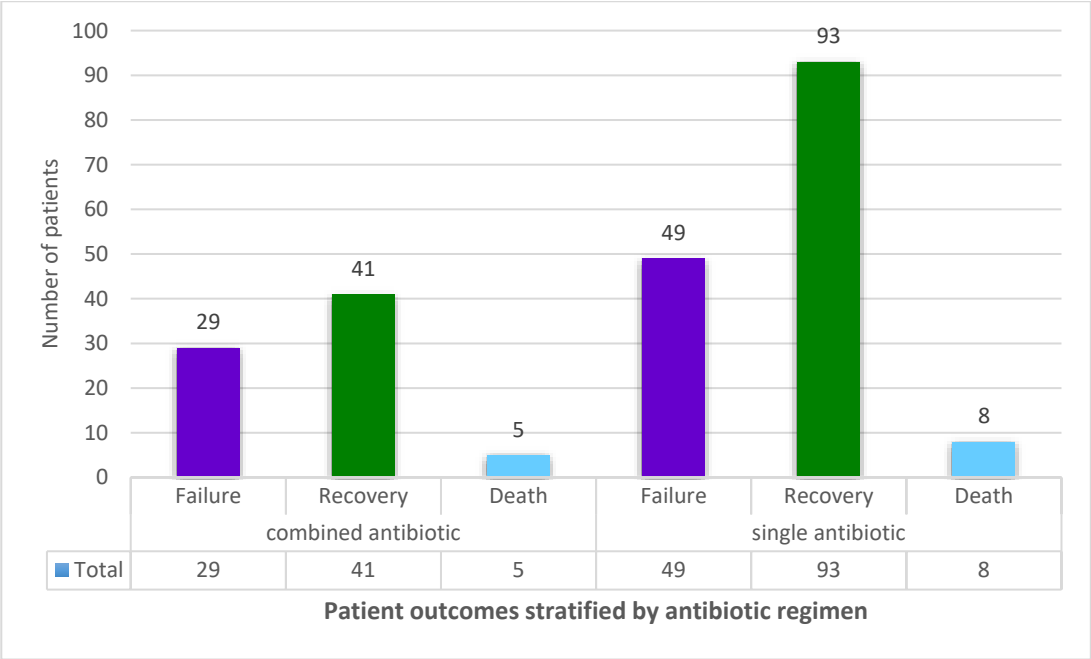


Figure 3-7. Patient outcomes by antibiotic groups (X2: 1.12, p-value: 0.570).

The average duration of antibiotic use typically approached 10 days for most antibiotics; however, only one case of cefepime use was documented for about one month. There have been no restrictions on antibiotic usage in hospital settings because the duration of antibiotic therapies is determined by several factors, including the type of infections, the patient's clinical response, the isolated bacteria, and the severity of the patient's clinical status. While the duration varies among patients, the standard duration of use typically ranges from 10 to 14 days. The case that was prescribed with cefepime for 27 days was a child diagnosed with pneumonia, sepsis shock and microbiological culture showing the presence of *K. pneumoniae.* The dosing regimen of each antibiotic followed the instructions of use, but there was no customisation of the use of antibiotics corresponding to each patient's factors.

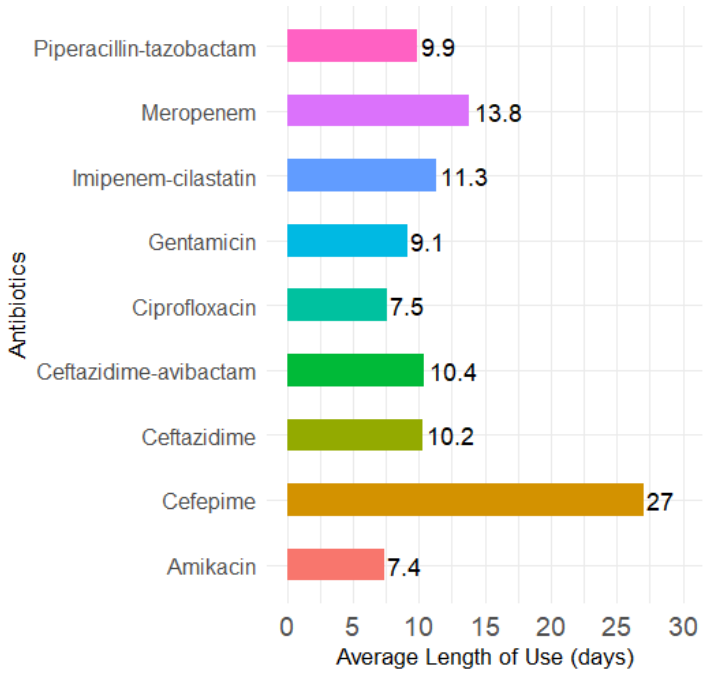


Figure 3-8. Average length of antibiotic use

3.2.3 Microbiological findings

Of the 225 included patients, a single pathogen was identified in 153 patients (68%). The other cases were infected with at least two bacteria (72 cases, 32%). The most commonly isolated microorganisms were *K. pneumoniae* (116 cases, 75.8%), A. baumannii (15 cases, 9.8%) and *E. coli* (11 cases, 7.1%) and *P. aeruginosa* (11 cases, 7.1%). The risk of co-infection with two gram-negative bacteria in both hospitals is around 30%. The treatment failure rate is approximately 40% higher than in cases involving only one strain of bacteria but there was no statistically significant difference in clinical outcomes between the two groups (p 0.658).

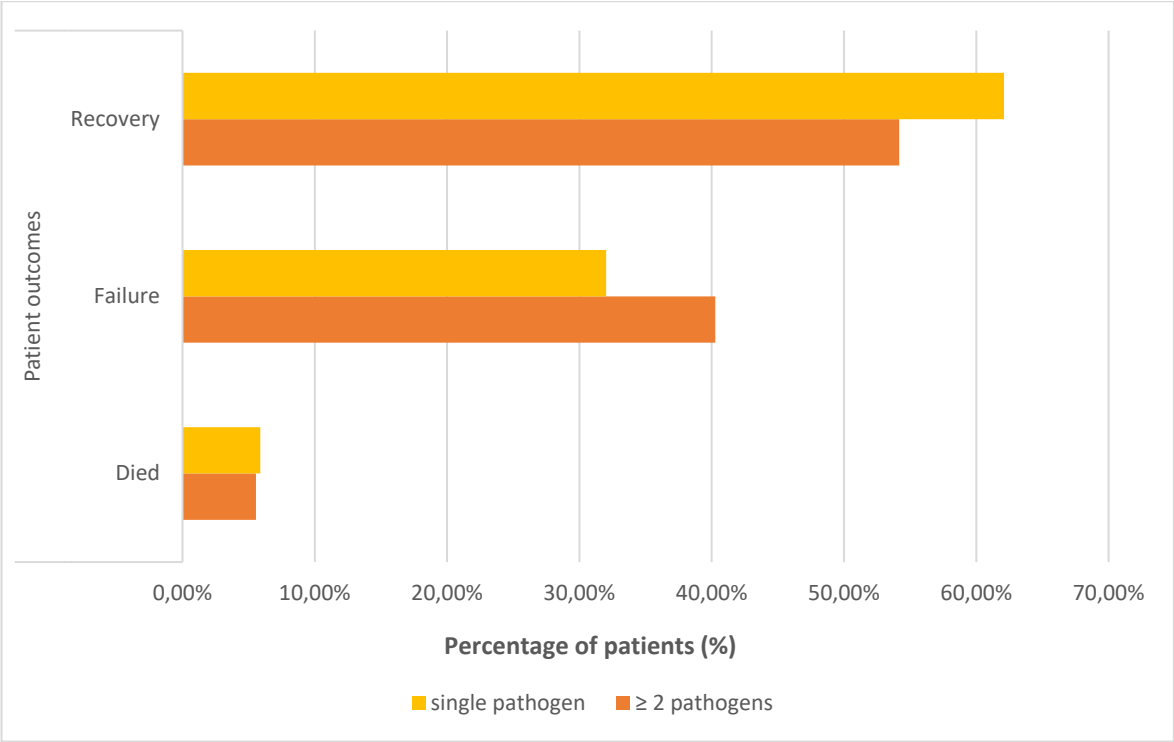


Figure 3-9. Patient outcomes in groups with single pathogens versus those with two or more pathogens.

3.2.4 The selection of bacteria and antibiotics for simulations

This phase investigated the demographic characteristics, microbiological profiles, antibiotic treatments, and clinical outcomes of 225 hospitalised patients with gram-negative bacterial infections at two sites. The population had a median age of 59 years and included both adult and paediatric patients. Renal impairment was common, reflected by elevated serum creatinine levels, and most patients presented with signs of systemic inflammation, including high C-reactive protein and procalcitonin levels.

Among the pathogens identified, *K. pneumoniae* was the most frequently isolated organism, followed *by A. baumannii*, *E. coli*, and *P. aeruginosa*. Notably, one-third of the patients were co-infected with two or more bacteria, and the likelihood of single-pathogen infections increased with patient age. A wide range of antibiotics was administered, with meropenem and imipenem-cilastatin being the most commonly prescribed agents. The choice of antibiotic varied by age group, but patients aged 18–65 received the majority of all agents. Combination therapy was used in approximately one-third of cases, while the remaining patients received monotherapy.

Clinical outcomes demonstrated that nearly 60% of patients recovered, while approximately 35% experienced treatment failure and 6% died. Recovery rates were higher among those who received single-pathogen-targeted therapy or monotherapy, but these groups also included more clinically stable patients. In contrast, combination therapy and multiple-pathogen infections were associated with higher rates of failure, reflecting the likely greater severity of illness in these patients.

These findings highlight the importance of early, appropriate antimicrobial selection tailored to patient characteristics and infection severity. The data also underscore the potential value of PK/PD-informed dosing strategies. The results show that *K. pneumoniae* emerged as the most frequently isolated pathogen and meropenem was the most commonly prescribed antibiotic in the clinical cohort, a focused PK/PD simulation study to predict the efficacy of meropenem and ceftazidime/avibactam combination to treat *K. pneumoniae* was conducted (Phase III). Phase III study aimed to evaluate the efficacy of meropenem in combination with ceftazidime/avibactam against *K. pneumoniae*, particularly in the context of multidrug resistance and varying renal function. By leveraging real-world patient data and incorporating population pharmacokinetic models, this simulation-based analysis sought to optimise dosing regimens and assess the potential of combination therapy to achieve PK/PD targets, thereby providing a mechanistic rationale to support or refine clinical treatment strategies observed in Phase II.

The Table 3-4 summarises data from 23 patients who received combination therapy with meropenem and ceftazidime/avibactam. The cohort included both male (n = 14) and female (n = 9) patients, with ages ranging from 15 to 91 years and body weights from 40 to 80 kg. The estimated CrCl varied widely, from 15.1 to 136.5 mL/min. Dosing regimens for meropenem ranged from 500 mg to 2000 mg, administered at intervals from every 6 to 12 hours over 3-hour infusions. Similarly, ceftazidime/avibactam doses were mostly standardised at 2500 mg, administered every 8 to 24 hours over 2-hour infusions. One patient (ID097) received an adaptive meropenem regimen that transitioned from 1000 mg q12h to q8 h; thus in the simulations, this patient was presented separately as ID097_1 and ID097_2. 39.1% of patients experienced treatment failure due to reports of worsening conditions, and 17.3% were reported as fatalities.

Table 3-4. Demographic and clinical characteristics of 23 patients receiving meropenem and ceftazidime/avibactam, including dosing regimens and estimated CrCl

No	Patient_ID	Weight (Kg)	Age (Years)	Gender	CrCl (ml/min)	Meropenem	Ceftazidime/ avibactam	Patient outcomes
1	ID004	80	46	M	41.1	1000mg q6h 3h	2500mg q24h 2h	Recovery
2	ID014	60	60	M	15.1	1000mg q8h 3h	2500mg q12h 2h	Recovery
3	ID028	65	38	M	37.3	1000mg q12h 3h	2500mg q8h 2h	Recovery
4	ID073	55	15	M	128.8	1000mg q8h 3h	2500mg q8h 2h	Failure
5	ID088	56	75	M	23.6	1000mg q8h 3h	2500mg q12h 2h	Recovery
6	ID092	47	44	F	46.3	1000mg q8h 3h	2500mg q8h 2h	Recovery
7	ID097	51	72	F	136.5	1000mg q12h (3 days) then 1000mg q8h (3 days), 3h	2500mg q8h 2h	Died
8	ID112	45	79	F	17.4	1000mg q12h 3h	2500mg q24h 2h	Recovery
9	ID117	49	82	F	23.8	2000mg q12h 3h	2500mg q24h 2h	Failure
10	ID120	65	48	M	22	2000mg q8h 3h	2500mg q8h 2h	Died
11	ID121	48	91	F	34.7	1000mg q12h 3h	2500mg q8h 2h	Failure
12	ID128	64	64	M	74.6	1000mg q8h 3h	2500mg q8h 2h	Recovery
13	ID152	48	69	F	111.8	1000mg q8h 3h	2500mg q8h 2h	Recovery
14	ID159	57	45	M	20.6	2000mg q12h 3h	2500mg q12h 2h	Failure
15	ID179	80	46	M	23.5	2000mg q12h 3h	2500mg q24h 2h	Failure
16	ID185	49	87	M	19.5	2000mg q12h 3h	2500mg q12h 2h	Died
17	ID191	70	21	M	57.2	1000mg q8h 3h	2500mg q8h 2h	Recovery
18	ID224	51	83	F	37.7	500mg q12h 3h	2500mg q24h 2h	Recovery

No	Patient_ID	Weight (Kg)	Age (Years)	Gender	CrCl (ml/min)	Meropenem	Ceftazidime/ avibactam	Patient outcomes
19	ID240	72	59	M	27.3	1000mg q12h 3h	2500mg q12h 2h	Failure
20	ID248	56	69	M	21.5	1000mg q12h 3h	2500mg q12h 2h	Failure
21	ID267	54	19	F	203	1000mg q12h 3h	2500mg q8h 2h	Failure
22	ID270	40	91	M	19.4	1000mg q12h 3h	2500mg q8h 2h	Died
23	ID273	60	59	M	19.3	2000mg q8h 3h	2500mg q24h 2h	Failure

3.3 PK/PD modelling and simulations

Phase III of this project aimed to develop a PK/PD model for meropenem and ceftazidime/avibactam combination. Published data collected from Phase I were used to derive the optimal dosing regimens that increase the likelihood of achieving the PK/PD targets for patients in practice.

The first step in model development was to define the structural pharmacokinetic model, representing the disposition of the drug within the body. Given the physical and chemical characteristics of both meropenem and ceftazidime/avibactam, one-, two-compartment models with first-order elimination were sequentially evaluated.

- Meropenem: Previous literature suggests that meropenem disposition is often best captured by a two-compartment model, reflecting both central plasma distribution and peripheral tissue compartments (Usman et al., 2017; O'Jeanson, 2021; Boonpeng et al., 2022; Li et al., 2023). However, a one-compartment model was tested initially as the simplest representation (Dhaese et al., 2018). Model fit was compared using likelihood-based criteria and goodness-of-fit plots.
- Ceftazidime/avibactam: Although previous population PK studies have often described ceftazidime and avibactam with two-compartment models, in this analysis a one- structure or two-compartment would be applied for ceftazidime/avibactam following the selection of PopPK model for meropenem. This decision was made to ensure consistency with the meropenem model, thereby facilitating the integration of both agents into combined PK/PD simulations. While this approach may not fully capture the distributional kinetics reported in some external studies, it provided a harmonised framework for evaluating dosing regimens and combination therapy.

Each model was parameterised in terms of Cl and Vd. A separate PopPK model was not developed for avibactam, as it does not possess antibacterial activity but rather acts as a beta-lactamase inhibitor to protect ceftazidime from beta-lactamase degradation. In this thesis, avibactam exposure was incorporated into the ceftazidime/avibactam model framework to account for its supportive role, while ceftazidime concentrations were used as the primary driver of antibacterial PK/PD effects.

3.3.1 Population PK model of meropenem

3.3.1.1 Pharmacokinetics of meropenem

Meropenem, a broad-spectrum carbapenem, is widely used in both study hospitals. It is given empirically before microbiology results and then continued or tailored once results are available, often in combination with another antibiotic for severe infections. After administration, meropenem is distributed throughout various body tissues and fluids, including the urinary tract, interstitial fluid, bone, bile, lungs, muscle tissue, and heart tissue, and it effectively penetrates the cerebrospinal fluid. As a hydrophilic agent, meropenem has a Vd of approximately 0.3 L/kg, along with low plasma protein binding (approximately 2%). The drug is predominantly excreted by the kidneys, with roughly 70% eliminated unchanged and about 30% in an inactive form. It possesses a short half-life ($t_{1/2}$) of about one hour. In critically ill patients, alterations in pharmacokinetics may lead to an increased volume of distribution due to oedema or changes in kidney excretory function. This can result in an elevated clearance of meropenem in the non-renal fraction, ultimately decreasing the concentration of the drug in the bloodstream.

Meropenem is a time-dependent antibiotic; longer concentrations above the MIC enhance therapy effectiveness. Its key PD target is *f*T>MIC. Meropenem is primarily eliminated from the body through the kidneys. When a patient experiences augmented renal clearance (ARC), this can significantly decrease the plasma concentration of meropenem (Udy et al., 2013). Consequently, the duration for which the drug concentration remains above MIC decreases, leading to a reduced effectiveness of the antibiotic. This is particularly problematic when dealing with bacteria that exhibit a high MIC, as it increases the risk of treatment failure (Hobbs et al., 2015; Murínová et al., 2024). Moreover, in patients with elevated clearance rates, the Vd of the drug may rise due to the presence of oedema or fluid shifts. This further contributes to the decline in meropenem plasma concentration, making it even more challenging to achieve the therapeutic levels required for effective treatment.

3.3.1.2 Preparation for the dataset

The patient data, including patient demographics such as age, gender, weight, and clearance creatinine and antibiotic therapy such as dosing information

and available antibiotic concentrations, were collected from six included studies of the systematic review (Kuti et al., 2004; Bulik et al., 2010; Delfino et al., 2018; Gatti et al., 2021; O'Jeanson, 2021; Cojutti et al., 2022). In these six studies, observed plasma concentrations of meropenem were collected from individual patients at specific timepoints to support popPK modelling. For most patients, sampling occurred at two key post-dose timepoints: approximately 48.5 hours and 55.5 hours after the initiation of therapy (day 2 onwards). These timepoints were selected to represent the steady-state phase, capturing both mid-dosing interval and near-trough concentrations during repeated dosing. The consistent timing across patients based on the information of doses (in mg), frequency of dosing intervals (in hours), and infusion duration (in hours) was required for the comparability and model robustness, though the limited number of timepoints per subject may restrict the precision of individual parameter estimation. The dataset for the development of the population pharmacokinetic model for meropenem was prepared from 23 included patients and antibiotic data from six of the abovementioned studies. An example of the Monolix-required formatted dataset is as follows:

Table 3-5. Patient-level dataset used for meropenem population PK modelling (Monolix)

ID	TIME	CP	EVID	DOSE	WT	AGE	SEX	CRCL
1	0	0	1	1000	58	88	1	53.2
1	48.5	56.1	0	0	58	88	1	53.2
1	55.5	2.2	0	0	58	88	1	53.2
2	0	0	1	1000	78	77	0	70.8
2	48.5	35.6	0	0	78	77	0	70.8
2	55.5	7.9	0	0	78	77	0	70.8

ID: the patients from six studies were numbered

(The completed data can be found in the Phase3_meropenem in Appendix 3)

- TIME: time of dose or measurement (hours), modified from the information of dosing regimens, i.e. 3g of meropenem every 8 hours, starting from 0.
- CP: measured plasma concentrations (mg/L).
- EVID: event identifier, 0=measurement, 1=dose.
- DOSE: the dose amount of meropenem (mg).
- WT: weight of patients included in the studies (kg).

- AGE: age of patients included in the studies (year).
- SEX: 1=male, 0=female
- CRCL: creatinine clearance (mL/min)

3.3.1.3 Structural models

A one-compartment structural model was selected to describe the population pharmacokinetic model of meropenem and ceftazidime/avibactam. Although both agents are known to exhibit two-compartment kinetics in healthy volunteers, the use of a simplified model was deemed appropriate given the characteristics of the available clinical data, which primarily consisted of sparse sampling (i.e., peak and trough concentrations) and TDM data from real-world patients extracted from published studies. The one-compartment model could still provide a stable and robust fit to the observed data, and adequately described the concentration–time profiles without evidence of systematic bias in diagnostic plots. Additionally, simpler structural models are commonly used in clinical PK/PD studies and model-informed precision dosing, especially when the primary aim is to characterise drug exposure and optimise dosing rather than to capture detailed distribution kinetics. Therefore, using a one-compartment model in this context was methodologically sound and clinically relevant.

3.3.1.4 Covariate models

To evaluate the influence of covariates on the meropenem PK parameters, the following potential covariates were tested: demographic variables (sex, age, weight), renal function (serum creatinine [SCr], estimated via the Cockcroft-Gault equation). Most data were tested as time-varying covariates, except fixed variables, such as sex, age, and weight, which were considered time-independent.

3.3.1.5 Development of the population PK model for meropenem

The PopPK model for meropenem was developed using nonlinear mixed-effects modelling in Monolix. Model development began with the specification of a one-compartment structural model with intravenous infusion input and first-order elimination. The model was estimated using the SAEM algorithm. The model was

built using a special computer method called the SAEM algorithm, which helps estimate drug behaviour in the body. This method works by repeatedly guessing and refining the best values for the model, even when the data are incomplete or noisy. It is useful for complex models and is commonly used in drug research to ensure the predictions are as accurate as possible. Therefore, the SAEM algorithm was employed for simulation in clinical practice.

Initial parameter values were selected based on literature and physiological plausibility. Based on literature values and prior knowledge in adult critically ill populations, the initial estimate for Cl was set to 10 L/h, reflecting the typical renal clearance of meropenem in patients with normal renal function. The Vd was initialised at 20 L, representing an intermediate value consistent with reported ranges in critically ill adults (15–25 L), accounting for potential fluid shifts. These values were used to guide the estimation algorithm toward physiologically plausible regions of the parameter space. Interindividual variability (IIV) for Cl and V was implemented using exponential random effects with initial standard deviations corresponding to 25% coefficient of variation (i.e., ω_CL = 0.25 and ω_V = 0.25). These values reflect common variability in antimicrobial PK across patient populations and ensure sufficient model flexibility during early exploration. A combined residual error model was initially selected to capture both proportional and additive measurement errors. The proportional error parameter (a) was set to 0.2 based on typical assay variability, and the additive component (b) was initialised at 0.3. This was later simplified to an additive-only error model after evaluation of residual plots and bootstrap diagnostics indicated that proportional error contributed minimally to unexplained variability.

Covariate screening was conducted using stepwise inclusion of log-transformed variables, including CrCl, body weight (WT), and age (AGE), based on physiological rationale and prior pharmacokinetic knowledge. While initial models included all three covariates, subsequent bootstrap analysis revealed high uncertainty in the WT and AGE effects, with relative standard errors (R.S.E.) exceeding 100% and 95% confidence intervals that crossed zero. Therefore, the final model retained CRCL as the sole covariate on clearance, justified by its known mechanistic role in renal elimination and consistent positive direction across models. The final covariate structure was:

$$\log(Cl) = \log(\theta_{Cl}) + \beta_{CrCl} \times \log\left(\frac{CrCl}{75}\right)$$
$$\log(V) = \log(\theta_V)$$

(Equation 11)

The initial parameter estimates (Table 3-6) were: CL_pop = 3.90 L/h, beta_CL_logtCRCL = 0.30, beta_CL_logtWT = 0.15; and V_pop = 12.19 L, beta_V_logtAGE = –0.66. Inter-individual variability was moderate for CL (ω_CL = 0.23, CV = 23.5%) and low for V (ω_V = 0.077, CV = 7.7%). The residual variability was adequately captured using an additive error model with b = 0.31. Diagnostic plots confirmed a good agreement between observed and individual predicted concentrations, with data closely aligned along the identity line and no major systemic bias. The VPC further demonstrated the model's predictive accuracy, as the observed concentration medians were well captured within the simulated 90% prediction intervals across time points. Slight underprediction was observed at the upper concentration range and during the distribution phase, likely reflecting variability in peak concentrations.

Table 3-6. Base population PK model for meropenem

Parameter	**Value**	**Cond. Mode Shrinkage (%)**	**Brief interpretation**
Fixed Effects			
CL_pop (L/h)	3.9	6.04	A bit low compared with literature (8–10 L/h)
beta_CL_logtCRCL	0.3		Moderate CrCl effect — renal clearance clearly modelled
beta_CL_logtWT	0.15		Small but consistent allometric effect
V_pop (L)	12.19	75.51	Reasonable for meropenem in critically ill adults
beta_V_logtAGE	-0.66		Strong and plausible: older patients = smaller central volume
Standard Deviation of the Random Effects			
	Value	C.V.(%)	
omega_CL	0.23	23.5	Ideal variability — neither under- nor over-fitted
omega_V	0.077	7.74	Very low — volume well explained by AGE
Error Model Parameters			

Parameter	Value	Cond. Mode Shrinkage (%)	Brief interpretation
b	0.31		Good — all residual variability handled with additive term alone

A non-parametric bootstrap with 300 replicates was performed to evaluate the precision and stability of parameter estimates in the base model. While the core structural parameters (CL_pop and V_pop) demonstrated low bias and narrow confidence intervals, the covariate effects of weight on clearance (beta_CL_logtWT) and age on volume (beta_V_logtAGE) showed very poor precision. Specifically, beta_CL_logtWT had a R.S.E of 356.6%, and its 95% confidence interval ranged from –0.98 to 0.88. Similarly, beta_V_logtAGE had an R.S.E of 168.8% and a confidence interval spanning –1.26 to 0.69. In both cases, the wide confidence intervals encompassed zero, and the bootstrap-derived bias values were substantial. These findings suggested that the dataset did not support the reliable estimation of these covariate effects. As a result, both covariates were removed from the final model, and only logCrCl was retained on clearance due to its physiological relevance and more stable estimate. After rerun the model and bootstrapping (n=300, final estimates, nonparametric, CI 95%) with only logCrCl classified as covariate, the final model was presented at Table 3-7.

Table 3-7. Final meropenem PopPK model used for subsequent simulations

Parameter	REFERENCE	MEAN	S.E.	R.S.E. (%)	P2.5	MEDIAN	P97.5	Bias (%)
Fixed Effects								
CL_pop (L/h)	3.87	3.97	0.33	8.25	3.37	3.97	4.63	2.69
beta_CL_logtCRCL	0.12	0.094	0.13	135.96	-0.2	0.092	0.3	-19.3
V_pop (L)	12.09	12.48	1.02	8.2	10.57	12.37	14.52	3.16
Standard Deviation of the Random Effects								
omega_CL	0.3	0.28	0.06	20.97	0.16	0.29	0.4	-4.5
omega_V	0.13	0.1	0.045	44.81	0.038	0.088	0.22	-21.09
Error Model Parameters								
b	0.24	0.22	0.067	30.39	0.08	0.22	0.35	-8.32

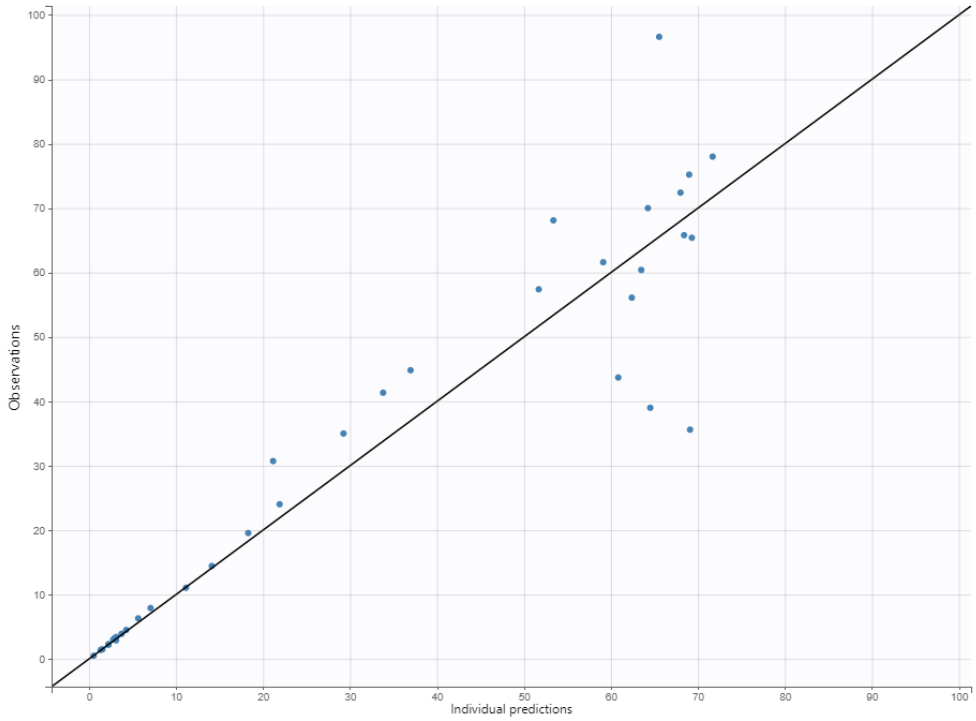


Figure 3-10. Observations vs predictions (final model of meropenem)

The plot (Figure 3-10) displays observed concentrations (y-axis) against individual predictions (IPRED, x-axis), overlaid with the line of identity (y = x). This GOF diagnostic assesses the agreement between observed and model-predicted values for each subject, accounting for individual random effects (η). In the final model of meropenem, most data points fall close to the line of identity, indicating good predictive performance across the range of concentrations. Some moderate scatter is seen at higher concentrations (>60 mg/L), including slight underprediction of peak values in a few individuals. However, the spread is symmetric and no major systematic bias is apparent. These findings support the adequacy of the structural model and suggest that the individual-level variability was well captured.

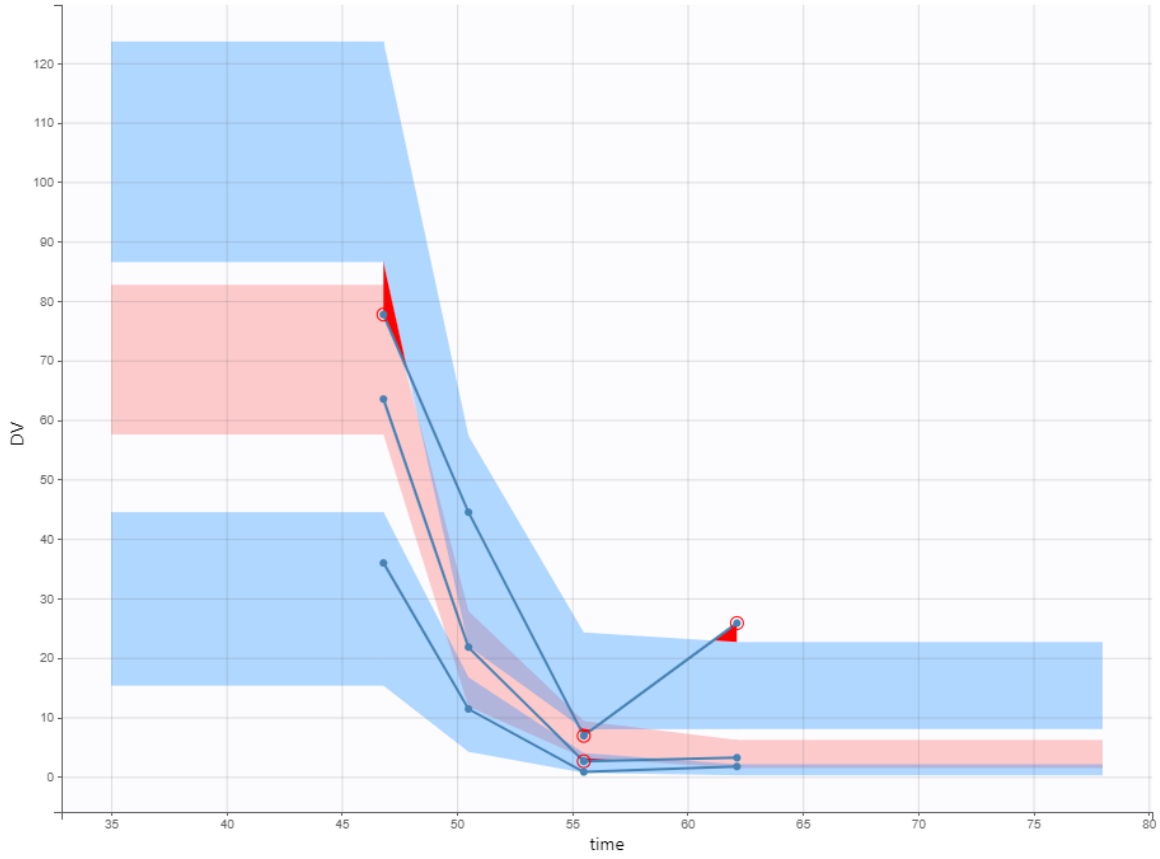


Figure 3-11. Visual predictive check of final model of meropenem.

The visual predictive check (VPC, Figure 3-11) plot displays the observed meropenem concentrations (red points and median line) overlaid on the prediction intervals from 1,000 Monte Carlo simulations based on the final population PK model. The red shaded area represents the 90% prediction interval for the simulated median concentration, while the blue shaded area represents the 90% prediction interval for the observed concentrations. The observed median (red line) closely tracks within the red shaded zone across all time points, indicating that the model adequately captures the central tendency of the data. Furthermore, the majority of observed concentrations fall within the blue prediction envelope, confirming that the model successfully reproduces the variability observed in the population.

A few deviations are noted at early time points (≈45 min), where individual concentrations slightly exceed the 90% upper prediction bound. This may reflect interindividual variability in C_{max} or infusion timing, which is common in real-world data. However, these deviations are not systematic and do not indicate structural model misspecification.

Model evaluation included diagnostic GOF plots, VPC, and a nonparametric bootstrap with 300 replicates. The final parameter estimates were: CL_pop = 3.97 L/h, beta_CL_logtCRCL = 0.094, and V_pop = 12.48 L. Interindividual variability was moderate for CL (ω_CL = 0.28, RSE = 20.97%) and low for V (ω_V = 0.13, RSE=44.81%). The residual variability was described by an additive error model (b = 0.22), capturing homoscedastic residual error across the concentration range. The bootstrap confirmed the robustness of the core structural parameters, with low bias and tight 95% confidence intervals. However, the covariate effect of CRCL on CL exhibited higher uncertainty (R.S.E. = 135.96%, 95% CI: –0.20 to 0.30), and was retained based on clinical plausibility and model consistency.

3.3.2 Population PK model of ceftazidime/avibactam

Given that the overall objective of the analysis was to integrate PK/PD models for meropenem and ceftazidime/avibactam, NCA was not pursued for ceftazidime/avibactam. A mechanistic population PK approach was required to support the integration with an E_{max}-based PD model. Furthermore, a robust NCA could not be reliably developed for meropenem due to the poor estimation of λz and terminal phase exposure. To ensure methodological consistency and compatibility with PK/PD modelling, the same approach was applied to develop a population PK model for ceftazidime/avibactam.

Preparation for the dataset:

The patient data, including patient creatinine clearance and antibiotic therapy such as dosing information and available antibiotic concentrations, were collected from four included studies of the systematic review (Wenzler et al., 2017; Soukup et al., 2019; Teng et al., 2022; Zhang et al., 2022; Gatti et al., 2023). Observed plasma concentrations of ceftazidime/avibactam were collected from 15 individual patients across four studies. The sampling timepoints ranged from early post-infusion measurements to late steady-state or trough phases, reflecting peak, trough and maintenance exposures. Timepoints were set up in hours after the start of infusion based on the information on doses and frequency of doses (dosing intervals) available in each study. They were then standardised to prepare for model analysis. Most observations were categorised as either peak, trough, or steady-state levels, aligned with TDM protocols. Infusion durations varied between 0.08 h (≈5 min, cases

were administered by IV bolus) and 8 hours, depending on the dosing strategy applied in each study. These timepoints and classifications provide valuable anchors for validating population PK models and simulating individualised dosing profiles under real-world Clinical conditions. The dataset for the development of the pharmacokinetic model, which is prepared from the patient and antibiotic data, can be found in the Phase3_Cefta.avi in Appendix 3.

Development of the Population PK model for ceftazidime/avibactam

A population pharmacokinetic model for ceftazidime/avibactam was then developed using the aforementioned dataset, the majority of whom had moderate renal impairment (median CrCl ~40.5 ml/min). A one-compartment model with first-order elimination was applied, incorporating CrCl as a covariate on Cl. The model was estimated using the SAEM algorithm.

Initial parameter values were selected based on literature and physiological plausibility. The Cl_pop was initially fixed at 3.0 L/h, consistent with prior reports in critically ill patients, where typical CL ranges from 2.5 to 4.5 L/h, depending on CrCl and modality (Sime et al., 2015). V_pop was set to 18.0 L – typically published values for ceftazidime/avibactam in healthy adults (15–20 L) (Pfizer Inc., 2023). A CrCl–Cl relationship was modelled using a log-transformed covariate with a coefficient (beta) fixed at 0.5, aligning with typical values for renally eliminated beta-lactams, where beta is reported between 0.3 and 0.7 (Sadilová et al., 2020).

Random effects on Cl and Vd were included using exponential models (herein log-normal distributions), with initial ω values of 0.3 and 0.2, respectively. The residual unexplained variability was modelled using a combined additive and proportional error structure (combined1), with initial estimates of a = 1.0 and b = 0.3.

Initial parameter estimates are summarised in the Table 3-8. The estimated Cl_pop was 3.84 L/h, and V_pop was 13.13 L. The model revealed high conditional mode shrinkage, particularly for CL (93.35%) and V (78.53%), indicating limited information in the data to reliably estimate individual post hoc parameters, which is considered a common limitation in sparse TDM data. Inter-individual variability (IIV) was moderate, with ω_CL = 0.26 (CV 26.06%) and ω_V = 0.29 (CV 29.91%). The additive residual error component (a = 48.75) was the primary source of residual variability, whereas the proportional component (b = 0.00043) contributed minimally and could potentially be removed in future model refinements.

Table 3-8. Parameters of the base model of ceftazidime/avibactam

Parameter	Value		Cond. Mode Shrinkage (%)	Brief interpretation
Fixed Effects				
CL_pop (L/h)	3.84		93.35	Typical clearance, but shrinkage was very high (problematic)
beta_CL_logtCRCL	1.27			
V_pop (L)	13.13		78.53	Shrinkage was high (problematic)
Standard Deviation of the Random Effects				
	Value	C.V.(%)		
omega_CL	0.26	26.06		Moderate IIV on CL
omega_V	0.29	29.91		Moderate IIV on V
Error Model Parameters				
a	48.75			Primary source of error
b	0.00043			Negligible contribution — could potentially be dropped

However, given the initial PopPK model for ceftazidime/avibactam demonstrated high conditional mode shrinkage for both clearance (CL, 93.35%) and volume of distribution (V, 78.53%), concerns were raised regarding the stability and robustness of individual parameter estimates. Shrinkage values exceeding 30–40% typically indicate insufficient data to reliably estimate inter-individual variability, especially for individual post hoc predictions. Additionally, the residual error was largely dominated by the additive component (a = 48.75), and the proportional error (b = 0.00043) showed negligible contribution. To address these issues and assess model stability, a nonparametric bootstrap analysis (n=300 replicates, final estimates and nonparametric mode were applied) was performed. Results were as follows:

Table 3-9. Bootstrap results of the PopPK model for ceftazidime/avibactam

Parameter	REFERENCE	MEAN	S.E.	R.S.E. (%)	P2.5	MEDIAN	P97.5	Bias (%)
Fixed Effects								
CL_pop	3.84	5.21	2.56	49.14	1.78	4.54	11.21	35.61
beta_CL_logtCRCL	1.27	1.63	0.81	50	-0.17	1.8	2.82	28.14
V_pop	13.13	9.1	4.49	49.28	0.87	10.83	15.19	-30.68
Standard Deviation of the Random Effects								
omega_CL	0.26	0.22	0.11	52.22	0.05	0.2	0.46	-15.32
omega_V	0.29	0.43	0.53	122.49	0.066	0.17	1.94	47.82
Error Model Parameters								
a	48.75	38.15	12.15	31.83	7.81	42.26	52	-21.75
b	0.00043	0.023	0.066	285.26	0	0.000000000000001 4	0.26	5297.76

These results (Table 3-9) indicated substantial uncertainty in the volume parameter (V_pop) and a highly unstable estimate of the interindividual variability in volume (ω_V). In addition, the proportional residual error term (b) was imprecisely estimated, supporting the decision to simplify the error model to an additive-only structure. Therefore, the modification of the error mode (switched to additive-only) was performed.

The final PopPK for ceftazidime/avibactam included a one-compartment IV structure with CrCl as a covariate on Cl (Table 3-10). The estimated typical Cl (Cl_pop) was 5.0 L/h and the volume of distribution (V_pop) was 12.69 L. The effect of CrCl on Cl was modelled using a log-transformed covariate, with beta_CL_logtCRCL estimated at 1.51. Although beta_CL_logtCRCL = 1.51 suggests a strong influence of CrCl on Cl, this value is higher than typically reported for beta in renally cleared antibiotics, which often range from 0.5 to 1.2 (US FDA, 2020). The elevated estimate may reflect model sensitivity to CrCl in a population with predominantly impaired renal function.

Interindividual variability was retained only on Cl (ω_CL= 0.23; CV = 23.19%), while the variability on volume was excluded due to instability and overparameterisation in earlier models. Residual unexplained variability was described by a combined

error model. The additive component (a = 47.75) accounted for most of the residual variance, while the proportional component (b = 0.01) was negligible. These estimates were supported by diagnostic plots and bootstrapped parameter confidence intervals.

Table 3-10. Final PopPK model for ceftazidime/avibactam.

Parameter	**Value**		**Cond. Mode**
			Shrinkage (%)
Fixed Effects			
CL_pop	5		91.63
beta_CL_logtCRCL	1.51		
V_pop	12.69		NaN
Standard Deviation of the Random Effects			
	Value	C.V.(%)	
omega_CL	0.23	23.19	
Error Model Parameters			
a	47.75		
b	0.01		

In the Monolix output for the model, the shrinkage value for the fixed effect parameter V_pop of Ceftazidime/avibactam was reported as NaN (Not a Number). This indicates that shrinkage could not be computed for this parameter, most likely due to the absence of an associated interindividual variability term (i.e., no estimated random effect omega_V). Shrinkage quantifies the extent to which individual parameter estimates are “shrunk” toward the population mean in the presence of limited information. Since V_pop was modelled without variability across individuals, shrinkage is not applicable and therefore not calculated. This is consistent with standard population pharmacokinetic modelling practices, where shrinkage is only estimated for parameters with defined random effects.

A boostrap (n=300, final estimates, nonparametric, CI 95%) was rerun, using the final parameters of PopPK model (Table 3-10). The bootstrap analysis confirmed the overall robustness of the Population PK model for ceftazidime. Estimates for Cl (Cl_pop 5.31 L/h) and volume (V_pop = 10.75 L) were consistent with the original SAEM output and demonstrated moderate precision. The effect of CrCl on Cl

(beta_CL_logtCRCL) was retained in the final model, although its 95% confidence interval included zero (–0.28 to 3.29), and its R.S.E. exceeded 50%, suggesting limited statistical significance. Interindividual variability on Clearance showed high uncertainty (R.S.E. = 83.4%), while residual unexplained variability was well characterised by an additive error model (a = 43.76, R.S.E. = 15.2%). Despite limitations in covariate precision, the model adequately described observed concentrations and was used for subsequent simulations.

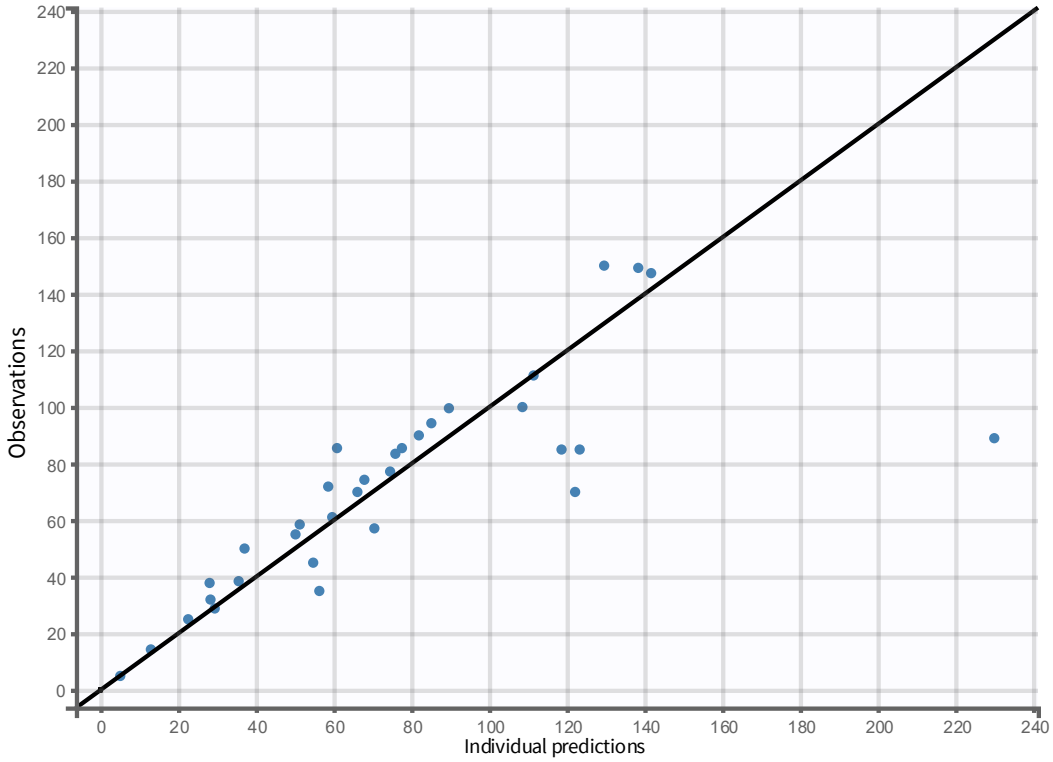


Figure 3-12. Observations vs predictions (final model of ceftazidime/avibactam)

The scatter plot (Figure 3-12) compares the individual predicted concentrations (x-axis) with the observed concentrations (y-axis) of ceftazidime/avibactam. The solid diagonal line represents the line of identity (i.e., where predicted values perfectly match observed ones). The points are scattered around the line with visible deviations, particularly at lower and higher concentration ranges. While some predictions align well with the observed data, several points deviate substantially, suggesting residual variability and model imprecision in certain individuals. This plot indicates that the model captures the general trend but may under- or overestimate concentrations for some patients.

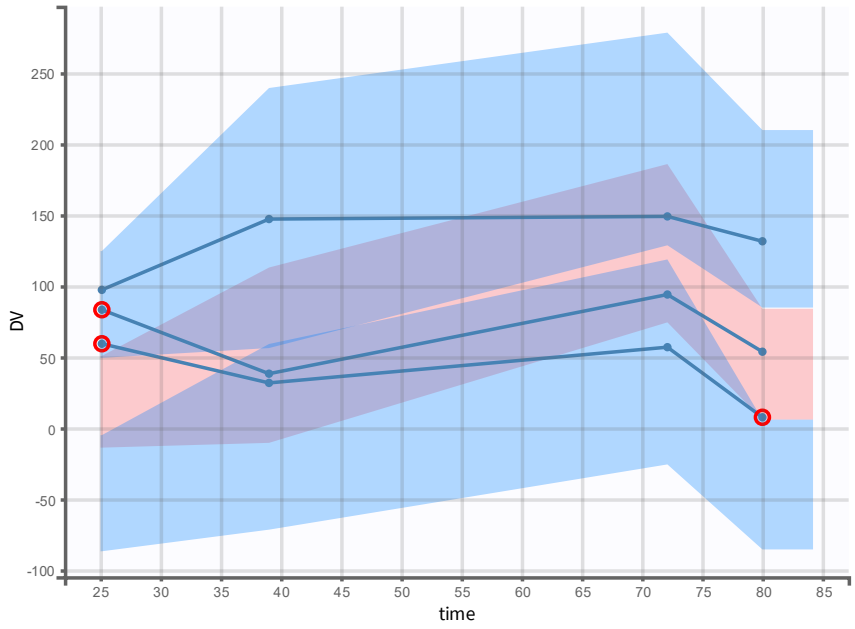


Figure 3-13. Visual predictive check of base model of ceftazidime/avibactam

The VPC (Figure 3-13) illustrates the predictive performance of the population pharmacokinetic model for ceftazidime/avibactam. The shaded regions represent the 50% (inner pink area) and 90% (outer blue area) prediction intervals derived from model simulations, while the solid lines indicate the median and percentile trends of simulated concentrations over time. The observed data points (red-circled dots) mostly fall within the 90% prediction interval, with the observed median values closely aligning with the simulated median line. These results suggest that the model might provide an adequate prediction of central tendency and variability, although minor deviations at later timepoints may reflect sparse sampling or inter-individual variability.

Table 3-11. Final PK parameters of ceftazidime/avibactam used for subsequent simulations

Parameter	REFERENCE	MEAN	S.E.	R.S.E. (%)	P2.5	MEDIAN	P97.5	Bias (%)
Fixed Effects								
CL_pop	5	5.31	2.74	51.63	1.74	4.64	11.1	6.26
beta_CL_logtCRCL	1.51	1.66	0.85	50.85	-0.28	1.78	3.29	10.09
V_pop	12.69	10.75	3	27.91	2.28	11.59	14.43	-15.28
Standard Deviation of the Random Effects								

Parameter	REFERENCE	MEAN	S.E.	R.S.E. (%)	P2.5	MEDIAN	P97.5	Bias (%)
omega_CL	0.23	0.25	0.21	83.37	0.057	0.21	0.7	9.09
Error Model Parameters								
a	47.75	43.76	6.65	15.19	28.17	44.57	54.32	-8.35

The final PopPK model for ceftazidime/avibactam included log-transformed creatinine clearance (logtCRCL) as a covariate on clearance and assumed a proportional residual error structure. Bootstrap analysis with 300 replicates revealed moderate to high variability in parameter estimates. While the central tendency for clearance (CL_pop = 5.00 L/h) and volume of distribution (V_pop = 12.69 L) was consistent with previous findings, the covariate effect of logtCRCL on clearance (beta = 1.51) showed a wide 95% confidence interval (–0.28 to 3.29) and high relative standard error (51%), indicating limited precision. The variability in clearance (ω_CL = 0.23) was also imprecisely estimated. In contrast, the residual error term (a = 47.75) was more stable with a narrower confidence interval. These findings suggest that while the model structurally fits the data, the covariate effect of renal function requires further evaluation in larger or more diverse datasets.

3.3.3 Simulations

3.3.3.1 Simulations of concentrations

Data sources and pharmacokinetic parameters PopPK parameters were derived from previously developed one-compartment models for meropenem and ceftazidime/avibactam using Monolix. The models included fixed effects for Cl and Vd, covariate effects of CrCl on Cl, and random effects (ω) to quantify inter-individual variability (IIV). The final parameter estimates were as follows:

- **Meropenem** (from the model, as can be seen in the Table 3-7):
    - Cl_pop = 3.97 L/h
    - beta_Cl_logtCRCL = 0.094
    - V_pop = 12.48 L
    - ω_Cl = 0.3, ω_V = 0.13

- **Ceftazidime/Avibactam** (from the model, as can be seen in the Table 3-11):
    - Cl_pop = 5 L/h
    - beta_Cl_logtCRCL = 1.66
    - V_pop = 12.69 L
    - ω_Cl = 0.23

These values were used to simulate individual posterior distributions of PK parameters for virtual patients. Posterior distributions of Cl and Vd were generated using log-normal sampling to ensure biological plausibility. A total of 500 virtual patients were simulated per drug. The standard deviation on the log scale (sdlog) was determined using estimated ω values from the Monolix models (ω = 0.3 for meropenem Cl, ω = 0.23 for ceftazidime Cl). These distributions reflect variability across a clinically relevant population.

Model assumptions and covariate effects Cl for both drugs was adjusted using patient-specific CrCl available from the findings of Phase II. Covariate models followed the functional forms from the Monolix outputs:

- Meropenem:

$$Cl_i = Cl_{pop} \times \varepsilon(\beta_{logtCrCl} \times \log(CrCl)) \qquad \textit{(Equation 12)}$$

- Ceftazidime/Avibactam:

$$Cl_i = Cl_{pop} \times (\frac{CrCl}{100})^{\beta} \qquad \textit{(Equation 13)}$$

Posterior distributions of CL and V were sampled using log-normal distributions to ensure positive values. The standard deviation on the log scale (sdlog) was derived from the estimated ω values.

Based on the covariates and the clinical use of antibiotics, antibiotic concentrations were simulated using function Desolve powered by R and Monte Carlo Simulations. Due to the limited data on antibiotic concentration observed for data input, which were extracted from studies in Phase I, the models for meropenem and ceftazidime-avibactam were developed only. The formula for a one-compartment model:

$$C(t) = \frac{Dose}{V} \times e^{-kt} \qquad \textit{(Equation 14)}$$

where $k = \frac{Cl}{V}$

Time Points (time_points): The time interval for simulations was 72 hours, by 1 hour

Population Pharmacokinetics:

- Meropenem: mero_V_pop, mero_Cl_pop
- Ceftazidime/avibactam: cefta_V_pop, cefta_Cl_pop

Individual simulations:

- $V_{mero,i}$ = Vpop (fixed, as no covariates for V)
- $Cl_{mero,i}$ = Clpop x exp(beta_Cl_logtCRCL x log($CrCl_i$))
- $V_{cefta,i}$ = Vpop (fixed, as no covariates for V)
- $Cl_{Cefta,i}$ = Clpop x $(CrCl_i/100)^{beta\_CL\_logtCRCL}$

Each drug was simulated over a 72-hour period using an Euler-based differential equation solver with a time step of 1 h. For each of the 500 posterior samples, individual concentration-time profiles were generated. The differential equation used was:

$$\frac{dC}{dt} = \frac{Rate_{in}}{V} - \frac{Cl}{V} \times C \qquad \textit{(Equation 15)}$$

Dosing Regimens: For each simulation, the actual dose and dosing interval of meropenem and ceftazidime/avibactam prescribed to 23 patients in Phase II were input.

Table 3-12. Maintained doses of meropenem according to CrCl

CrCl (mL/min)	Meropenem dosing adjustment according to FDA, Sanford Guide, and EUCAST dosing guidelines
>50	1000 – 2000 mg q8h (3h infusion for critically ill)
26–50	1000 mg q12h or 2000 g q12h (prolonged infusion)
10–25	500 – 1000 mg q12h
<10	500 – 1000 mg q24h

Table 3-13. Maintained doses of ceftazidime/avibactam according to CrCl

CrCl (mL/min)	Ceftazidime/avibactam dosing adjustment according to the instruction of use (Pfizer Inc., 2023)
>50	2500 mg q8h
31–50	1250 mg q8h
16–30	940 mg q12h
6–15	940 mg q12h
≤5	940 mg q24h

PTA Evaluation:

For both drugs, the PTA was calculated across a range of MIC values (0.25 to 64 mg/L) using the predefined PK/PD target of 100% fT>MIC and 100% *f*T>4×MIC (results from Phase I) during the 72 hours. PTA was defined as the percentage of profiles achieving the target at each MIC. Results were visualised as a PTA according to MIC curve on a log scale.

PTA indicates the likelihood of achieving 100% *f*T > 4xMIC based on 1000 virtual patients for each patient data from Phase II and Monte Carlo simulations and was calculated using the Equation of Drusano, G. L. (2004) (Drusano, 2004):

$$PTA = \frac{Number\ of\ simulations\ that\ met\ the\ target}{Total\ number\ of\ simulations} \times 100 \qquad \textit{(Equation 16)}$$

Regimens with PTAs of at least 90% were considered optimal. Each patient was simulated with adjusted dosing regimens of meropenem and ceftazidime/avibactam using the PoP PK parameters only.

MIC determination:

Table 3-14. MIC breakpoints established by EUCAST and CLSI for meropenem and ceftazidime/avibactam.

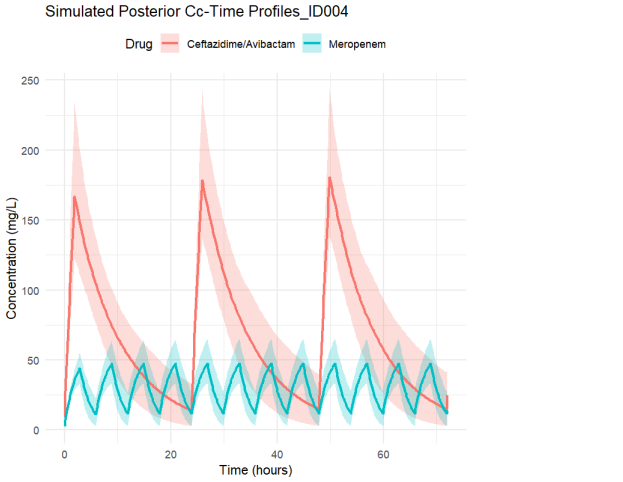
Antibiotics / Pathogens	**Meropenem**	**Ceftazidime/avibactam**
	Standard dose: 1000mg q8h	Standard dose: 2500mg q8h over 2-hour infusion
Enterobacteriaceae (which includes *K. pneumoniae* and *E. coli*)	EUCAST: S ≤2 mg/L; R >8 mg/L CLSI: S ≤1 mg/L; I = 2; R ≥4 mg/L	EUCAST and CLSI use harmonised breakpoints: S ≤8 mg/L; R >8 mg/L
S: Susceptible - I: Susceptible with increased exposure - R: Resistant (all units were converted to mg/L) Source: EUCAST version 14.0, CLSI M100 (CLSI, 2024; EUCAST, 2024)		

As the retrospective data on MIC values of bacterial strains collected at both hospitals were already interpreted in accordance with CLSI (for CRH) and EUCAST (for COCH) to inform the prescribers whether bacteria remained sensitive or resistant to antibiotics, it was not feasible to determine the precise MIC value for each patient during data collection. Furthermore, both hospitals employed the identification and antimicrobial susceptibility testing known as VITEK®-2 to conduct antibiotic resistance testing, which provided information on MIC values above or below a certain threshold rather than specific ones. Consequently, based on EUCAST and CLSI, a MIC value of 4 mg/L was selected as the basis for determining PTA with a PK/PD target value of 100% *f*T>4 x MIC (MIC was 4 mg/L) for meropenem and MIC 8 mg/L with a PK/PD target value of 100% fT>MIC for ceftazidime/avibactam, which were defined by the findings of Phase I.

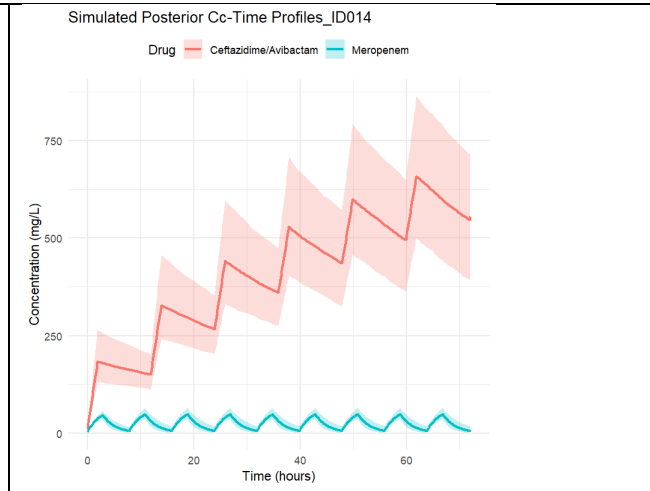
A range of MIC values (0.25, 0.5, 1, 2, 4, 6, 8, 16, 32, and 64 mg/L) was used to evaluate the PTA of real-world dosing regimens based solely on the PopPK models of both antibiotics due to the missing specific value of MICs.

The results of the simulated concentrations for each patient were presented in Table 3-15.

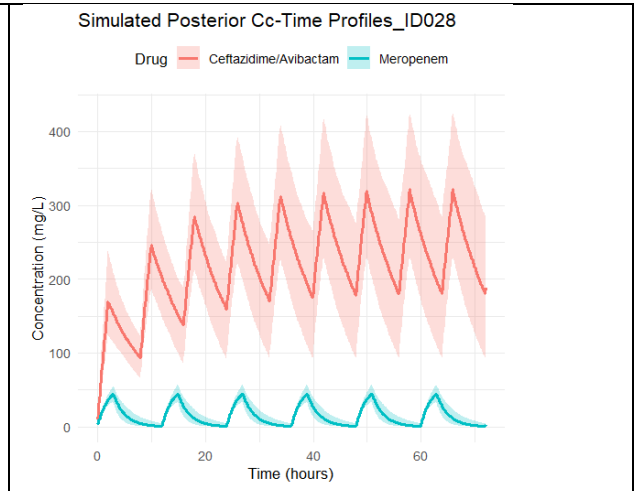
Table 3-15. Simulated concentrations of meropenem and ceftazidime/avibactam using data from patients collected during Phase II



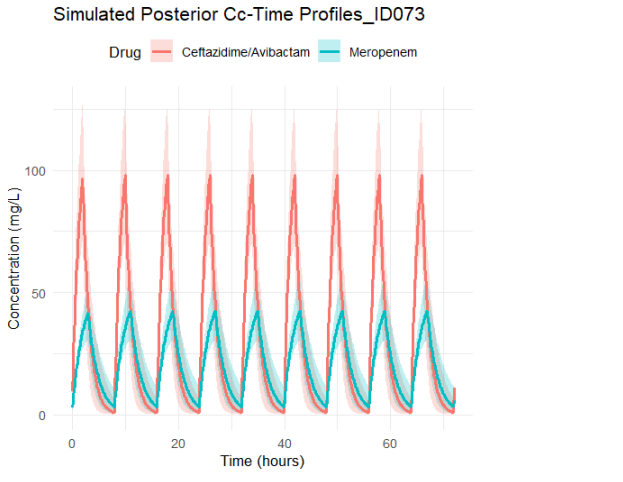
ID	Age	CrCl	Antibiotics	dosing regimens
ID004	46	41.1	Meropenem	1000mg q6h 3h
ID004	46	41.1	Cefta/avi	2500mg q24h 2h



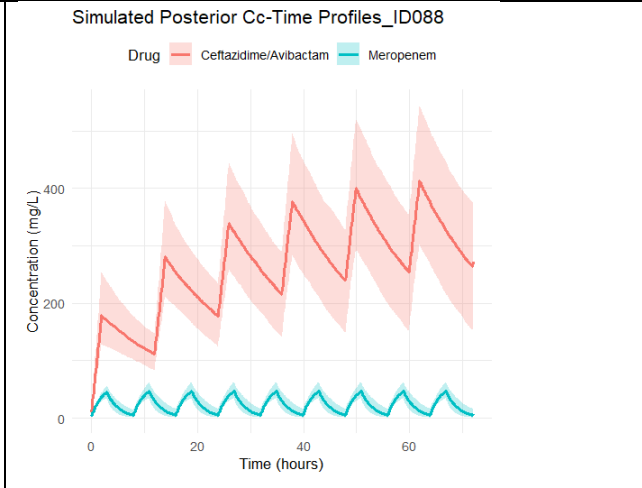
ID	Age	CrCl	Antibiotics	dosing regimens
ID014	60	15.1	Meropenem	1000mg q8h 3h
ID014	60	15.1	Cefta/avi	2500mg q12h 2h



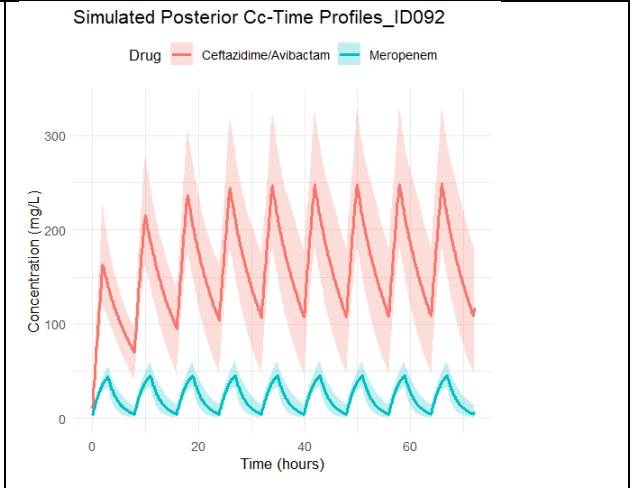
ID	Age	CrCl	Antibiotics	dosing regimens
ID028	38	37.3	Meropenem	1000mg q12h 3h
ID028	38	37.3	Cefta/avi	2500mg q8h 2h



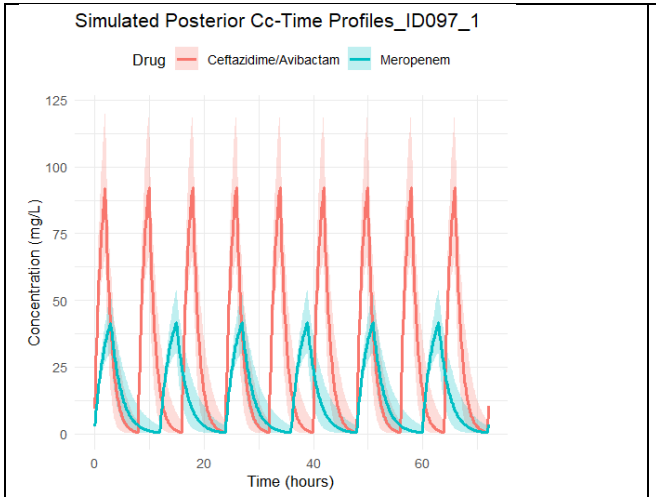
ID	Age	CrCl	Antibiotics	dosing regimens
ID073	15	128.8	Meropenem	1000mg q8h 3h
ID073	15	128.8	Cefta/avi	2500mg q8h 2h



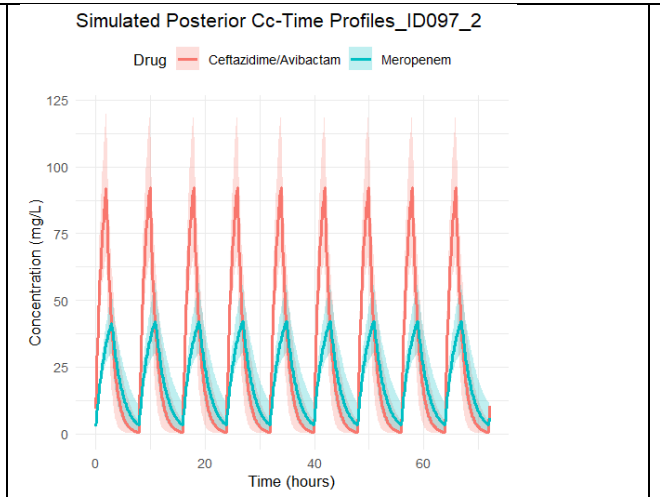
ID	Age	CrCl	Antibiotics	dosing regimens
ID088	75	23.6	Meropenem	1000mg q8h 3h
ID088	75	23.6	Cefta/avi	2500mg q12h 2h



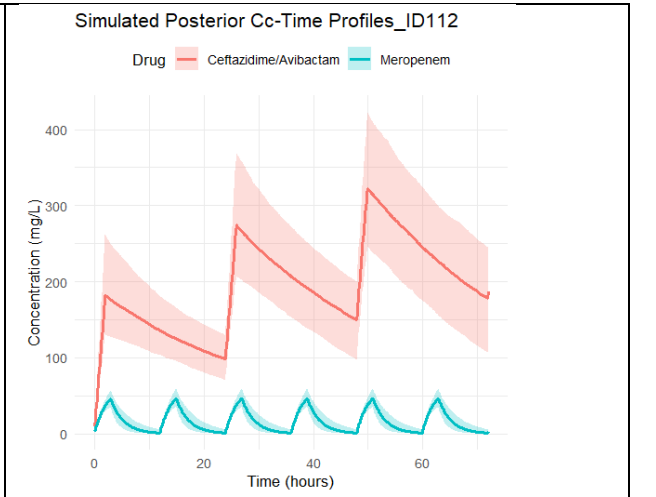
ID	Age	CrCl	Antibiotics	dosing regimens
ID092	44	46.3	Meropenem	1000mg q8h 3h
ID092	44	46.3	Cefta/avi	2500mg q8h 2h



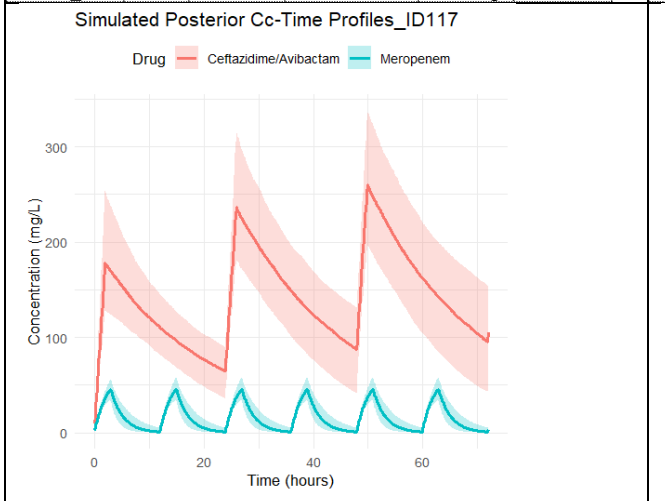
ID	Age	CrCl	Antibiotics	dosing regimens
ID097_1	72	136.5	Meropenem	1000mg q12h 3h
ID097_1	72	136.5	Cefta/avi	2500mg q8h 2h



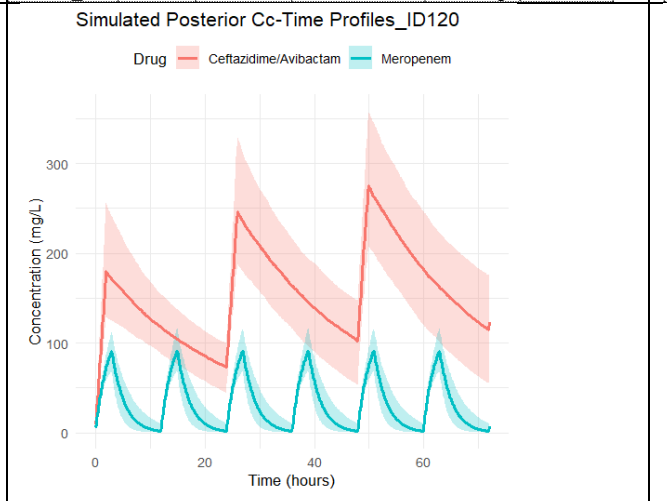
ID	Age	CrCl	Antibiotics	dosing regimens
ID097_2	72	136.5	Meropenem	1000mg q8h 3h
ID097_2	72	136.5	Cefta/avi	2500mg q8h 2h



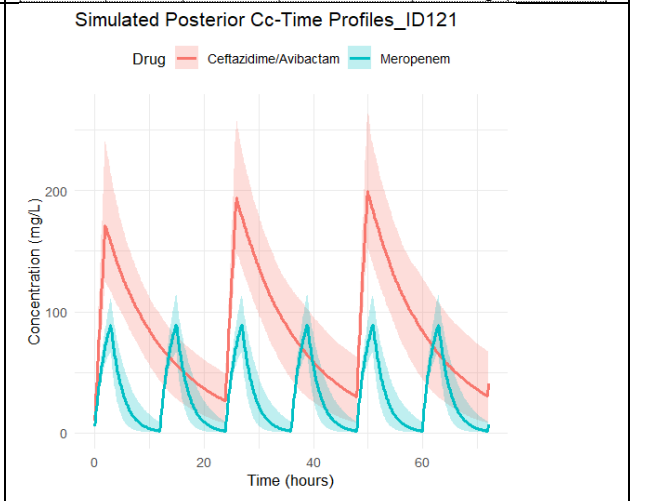
ID	Age	CrCl	Antibiotics	dosing regimens
ID112	79	17.4	Meropenem	1000mg q12h 3h
ID112	79	17.4	Cefta/avi	2500mg q24h 2h



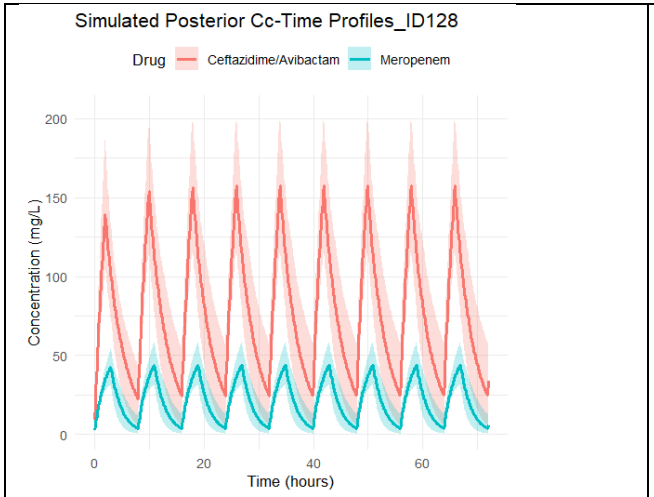
ID	Age	CrCl	Antibiotics	dosing regimens
ID117	82	23.8	Meropenem	2000mg q12h 3h
ID117	82	23.8	Cefta/avi	2500mg q24h 2h



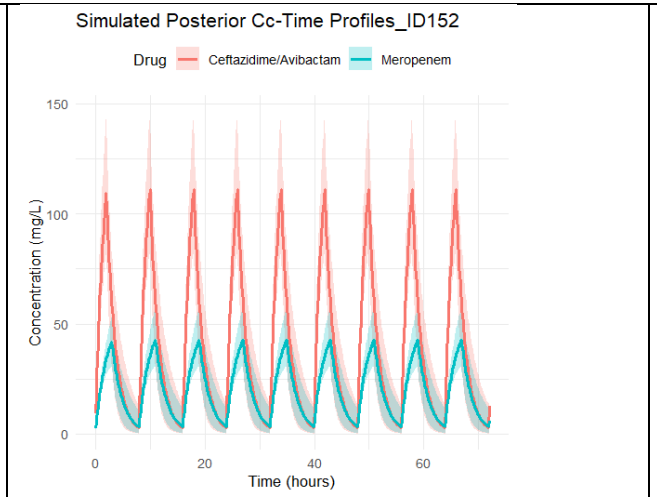
ID	Age	CrCl	Antibiotics	dosing regimens
ID120	48	22	Meropenem	2000mg q8h 3h
ID120	48	22	Cefta/avi	2500mg q8h 2h



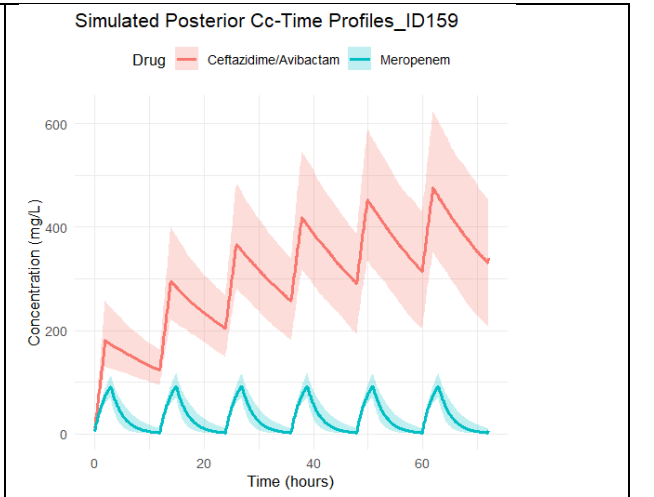
ID	Age	CrCl	Antibiotics	dosing regimens
ID121	91	34.7	Meropenem	1000mg q12h 3h
ID121	91	34.7	Cefta/avi	2500mg q8h 2h



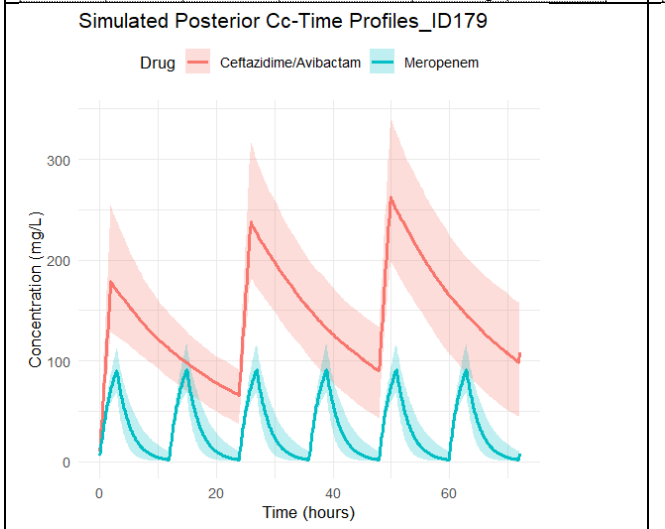
ID	Age	CrCl	Antibiotics	dosing regimens
ID128	64	74.6	Meropenem	1000mg q8h 3h
ID128	64	74.6	Cefta/avi	2500mg q8h 2h



ID	Age	CrCl	Antibiotics	dosing regimens
ID152	69	111.8	Meropenem	1000mg q8h 3h
ID152	69	111.8	Cefta/avi	2500mg q8h 2h

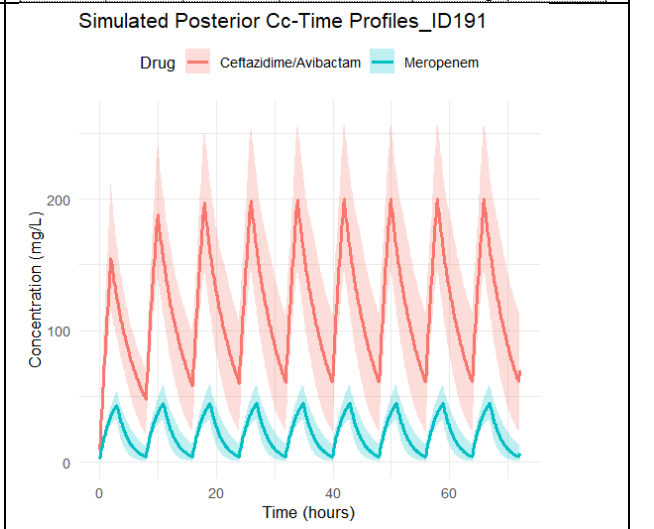


ID	Age	CrCl	Antibiotics	dosing regimens
ID159	45	20.6	Meropenem	2000mg q12h 3h
ID159	45	20.6	Cefta/avi	2500mg q12h 2h

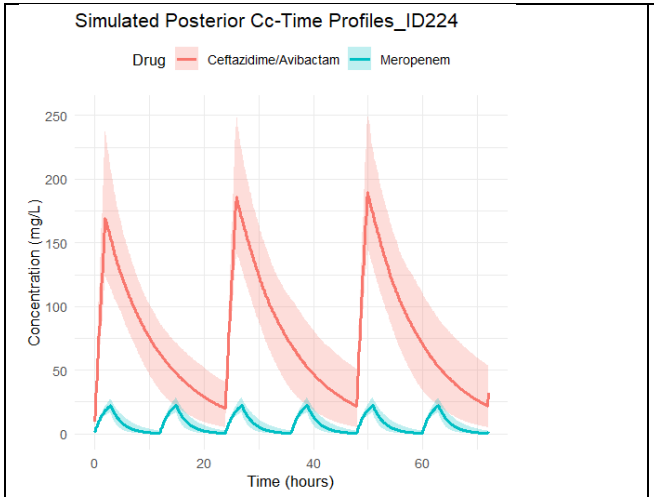


ID	Age	CrCl	Antibiotics	dosing regimens
ID179	46	23.5	Meropenem	2000mg q12h 3h
ID179	46	23.5	Cefta/avi	2500mg q24h 2h

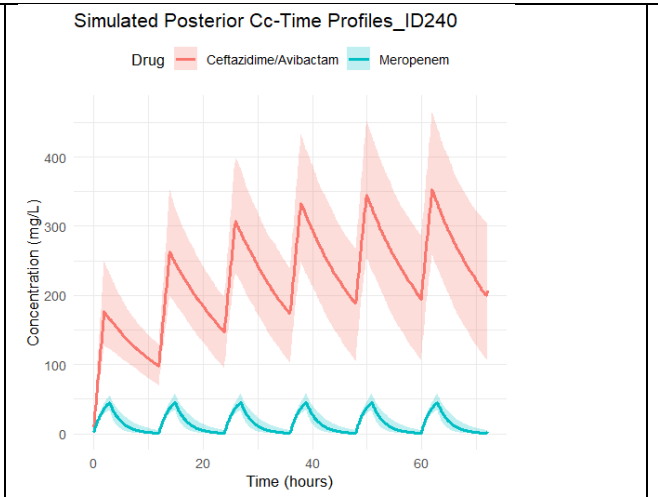
ID	Age	CrCl	Antibiotics	dosing regimens
ID185	87	19.5	Meropenem	2000mg q12h 3h
ID185	87	19.5	Cefta/avi	2500mg q12h 2h



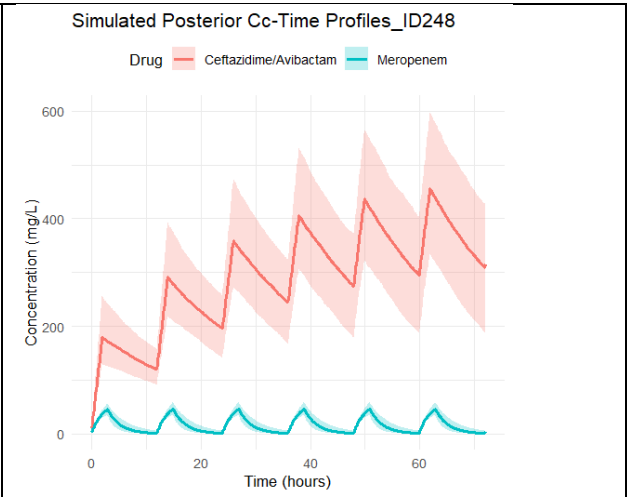
ID	Age	CrCl	Antibiotics	dosing regimens
ID191	21	57.2	Meropenem	1000mg q8h 3h
ID191	21	57.2	Cefta/avi	2500mg q8h 2h



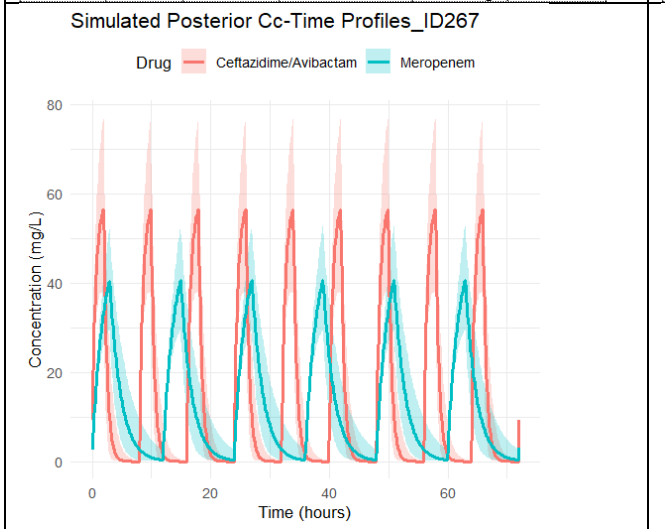
ID	Age	CrCl	Antibiotics	dosing regimens
ID224	83	37.7	Meropenem	500mg q12h 3h
ID224	83	37.7	Cefta/avi	2500mg q24h 2h



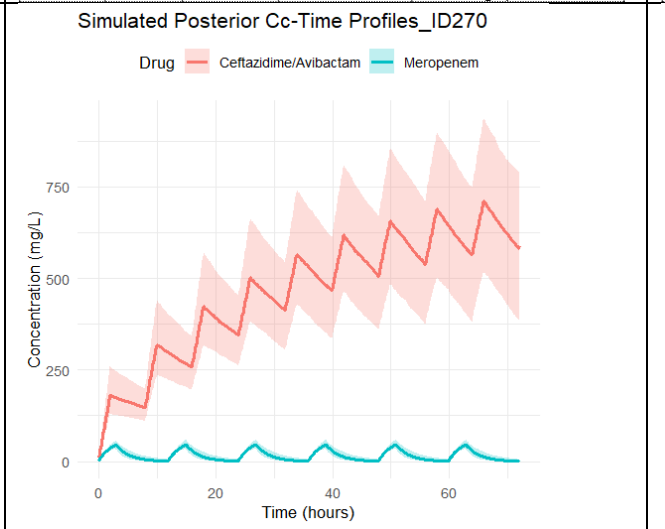
ID	Age	CrCl	Antibiotics	dosing regimens
ID240	59	27.3	Meropenem	1000mg q12h 3h
ID240	59	27.3	Cefta/avi	2500mg q12h 2h



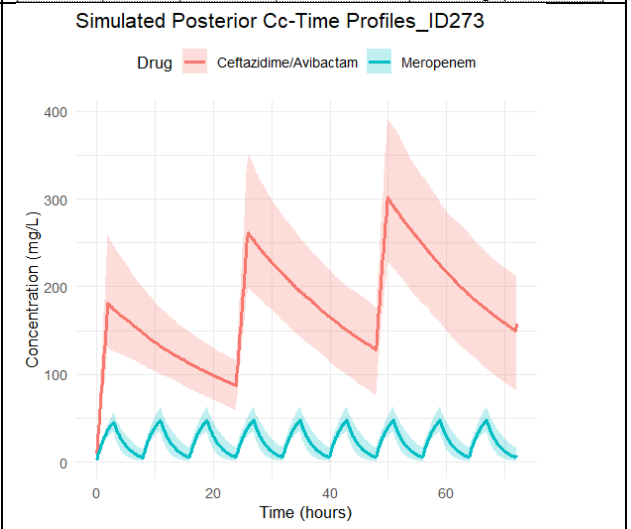
ID	Age	CrCl	Antibiotics	dosing regimens
ID248	69	21.5	Meropenem	1000mg q12h 3h
ID248	69	21.5	Cefta/avi	2500mg q12h 2h



ID	Age	CrCl	Antibiotics	dosing regimens
ID267	19	203	Meropenem	1000mg q12h 3h
ID267	19	203	Cefta/avi	2500mg q8h 2h



ID	Age	CrCl	Antibiotics	dosing regimens
ID270	91	19.4	Meropenem	1000mg q12h 3h
ID270	91	19.4	Cefta/avi	2500mg q8h 2h

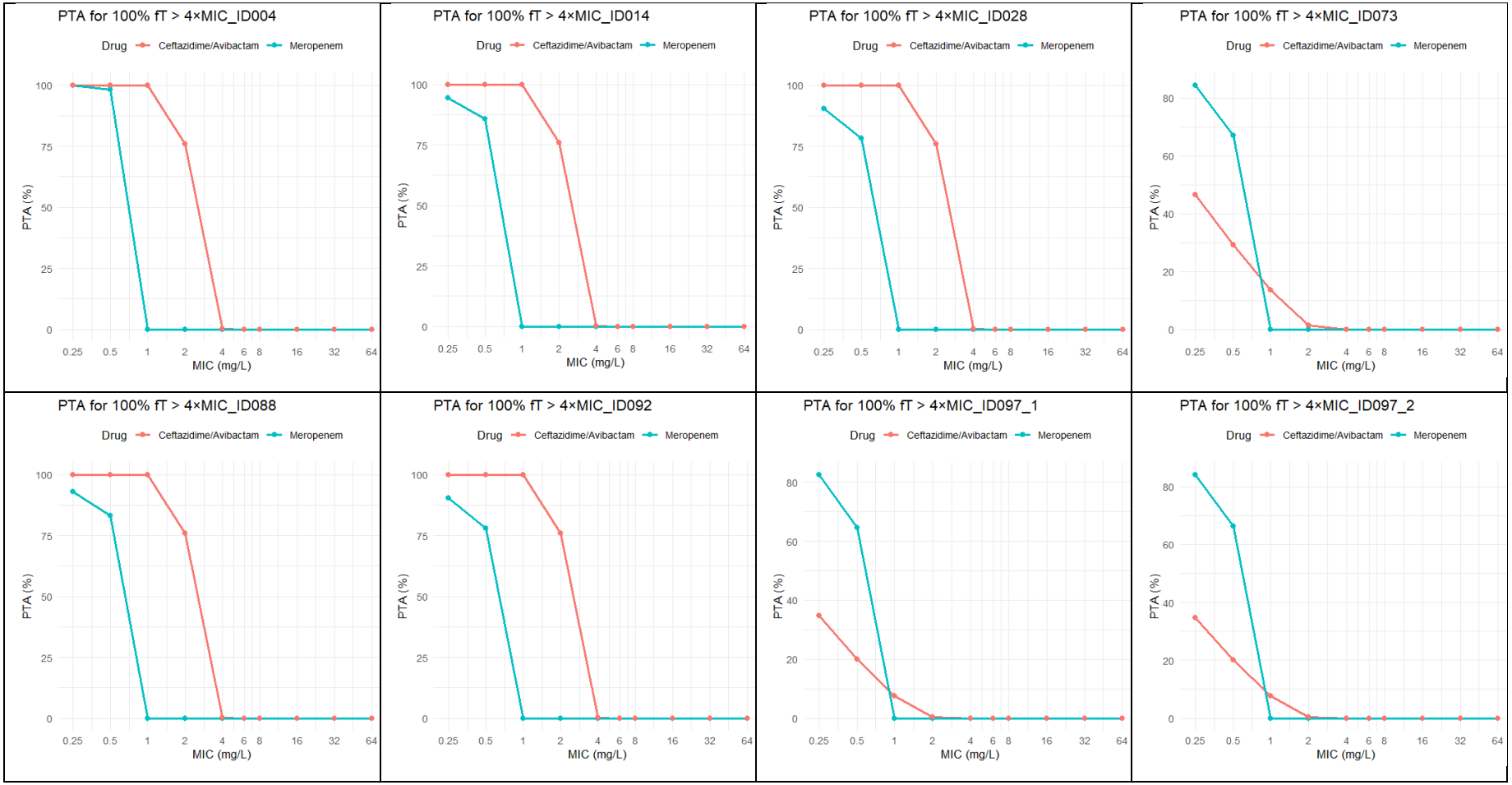


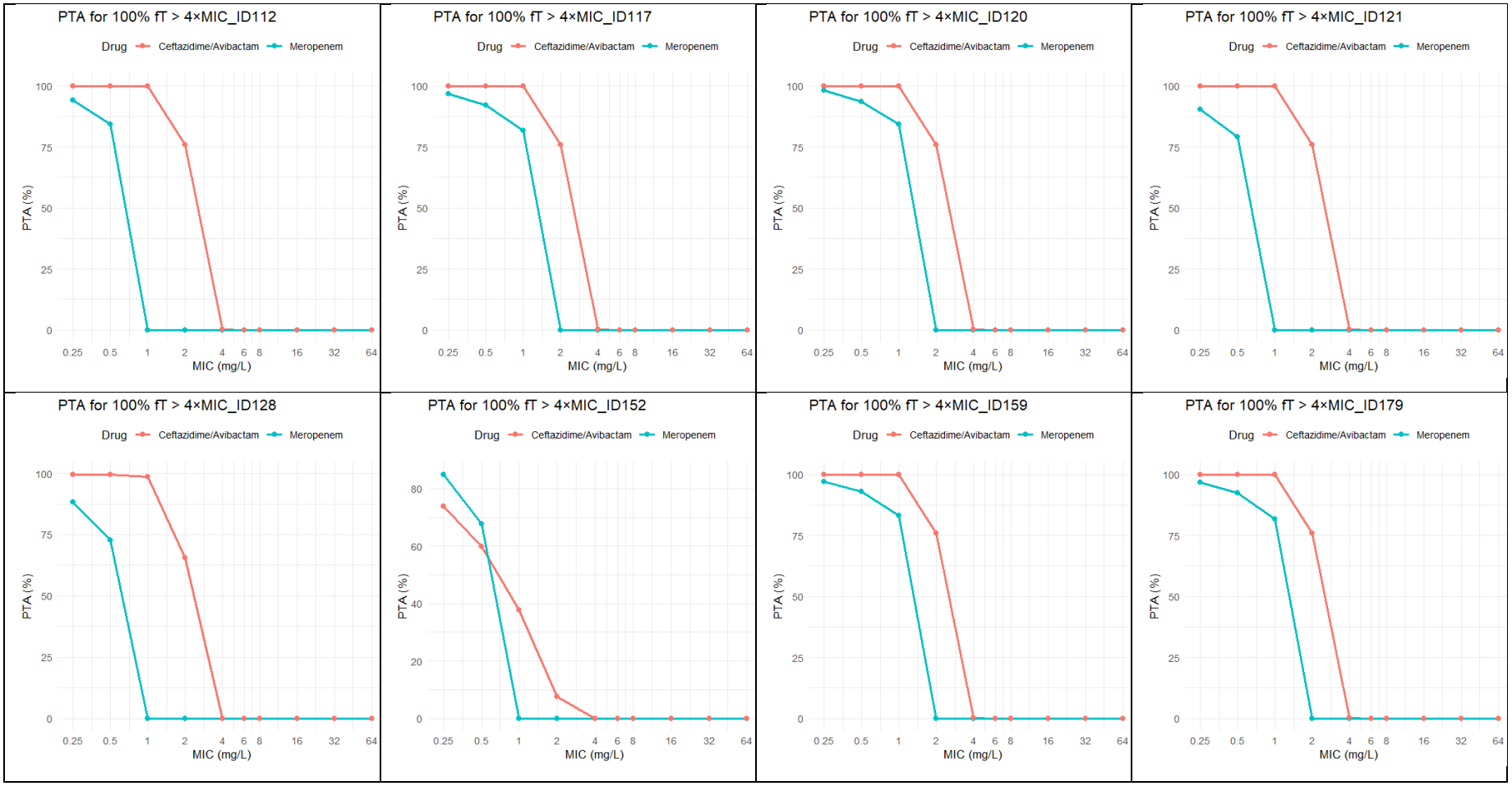
ID	Age	CrCl	Antibiotics	dosing regimens
ID273	59	19.3	Meropenem	2000mg q8h 3h
ID273	59	19.3	Cefta/avi	2500mg q24h 2h

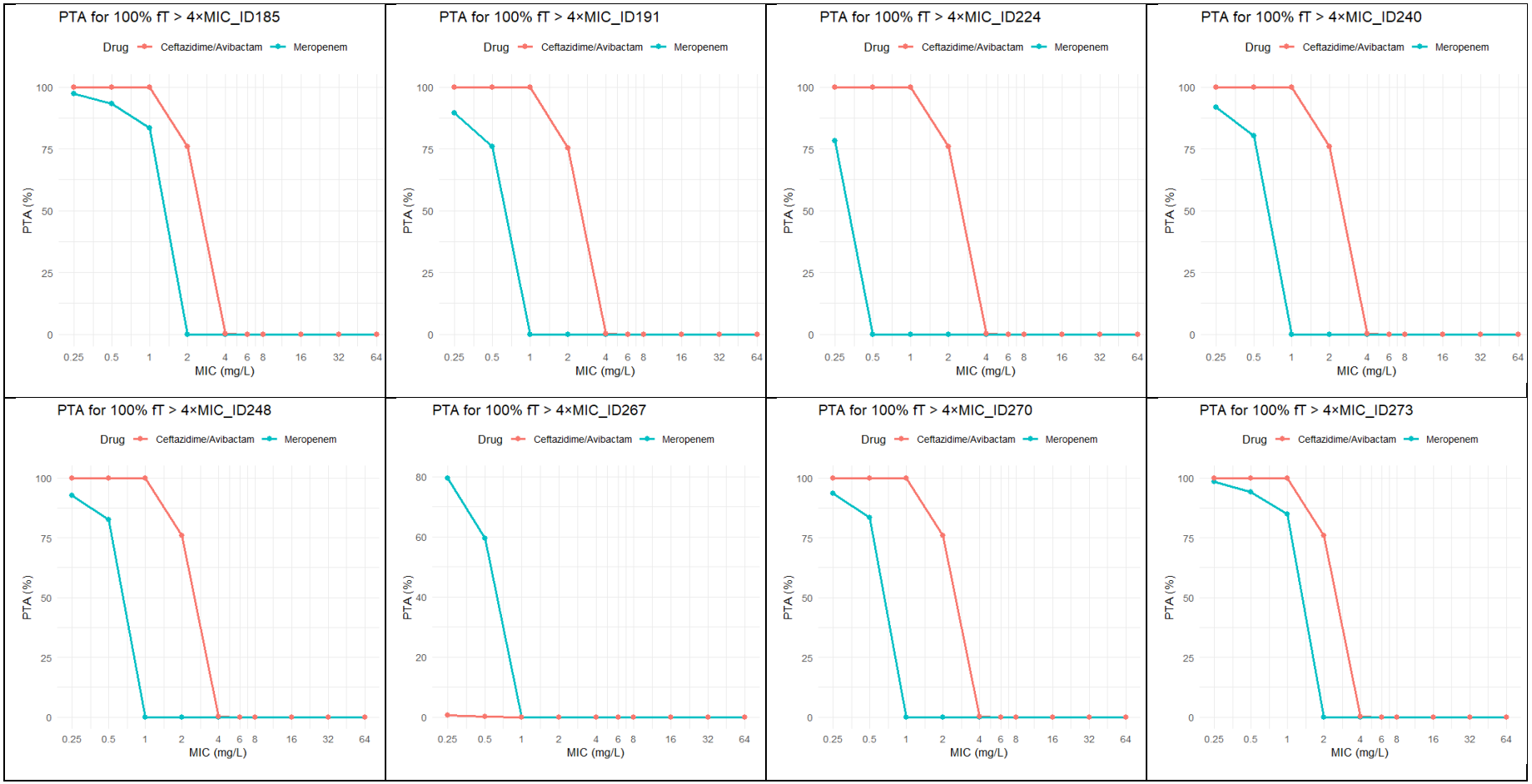
3.3.3.2 Simulations of the probability of target attainment

Table 3-16 shows the PTA for meropenem and ceftazidime/avibactam across a range of MIC values. The PTA represents the percentage of simulated dosing scenarios in which the free drug concentration remained above four times the MIC for the entire dosing interval. Both drugs demonstrated a steep decline in PTA at higher MICs, indicating a reduced likelihood of achieving pharmacodynamic targets against more resistant pathogens. Particularly, across the cohort, meropenem achieved 100% PTA at MICs ≤0.25 mg/L in most patients and maintained acceptable PTA up to 0.5–1 mg/L in patients with preserved renal function. However, a steep decline in PTA occurred beyond MIC 1–2 mg/L, highlighting the limited utility of meropenem monotherapy against organisms with reduced susceptibility (MIC ≥4 mg/L). In contrast, ceftazidime/avibactam, consistently failed to reach 90% PTA for 100% *f*T > 4×MIC in all patients at MICs ≥2 mg/L. For most patients, PTA dropped sharply beyond MIC = 1 mg/L. Notably, several patients (i.e., ID073, ID097_1, ID267) exhibited low PTA across all MIC levels, possibly reflecting impaired drug Cl, subtherapeutic concentrations, or Vd extremes.

Table 3-16. PTA of simulated concentrations for patients in Phase II using population PK models of meropenem and ceftazidime/avibactam across a range of MICs




PTA for 100% fT > 4×MIC_ID112
PTA for 100% fT > 4×MIC_ID117
PTA for 100% fT > 4×MIC_ID120
PTA for 100% fT > 4×MIC_ID121
PTA for 100% fT > 4×MIC_ID128
PTA for 100% fT > 4×MIC_ID152
PTA for 100% fT > 4×MIC_ID159
PTA for 100% fT > 4×MIC_ID179
Drug
Ceftazidime/Avibactam
Meropenem
PTA (%)
MIC (mg/L)
0.25
0.5
1
2
4
6
8
16
32
64


PTA for 100% fT > 4×MIC_ID185
PTA for 100% fT > 4×MIC_ID191
PTA for 100% fT > 4×MIC_ID224
PTA for 100% fT > 4×MIC_ID240
PTA for 100% fT > 4×MIC_ID248
PTA for 100% fT > 4×MIC_ID267
PTA for 100% fT > 4×MIC_ID270
PTA for 100% fT > 4×MIC_ID273
Drug
Ceftazidime/Avibactam
Meropenem
PTA (%)
MIC (mg/L)
0.25
0.5
1
2
4
6
8
16
32
64

3.3.3.3 Simulations of PK/PD to predict the optimised dosing regimens

To develop a PK/PD model for the combination of meropenem and ceftazidime/avibactam in the treatment of *K. pneumoniae*, without prior knowledge of their effects on the bacteria, a stepwise approach was implemented. Initially, the specific PK parameters were determined from two PopPK models for meropenem and ceftazidime/avibactam (described in Chapter 4), which were utilised. Subsequently, a simple E_{max} model based on the Hill equation as detailed in 2.3.4 was applied to develop a pharmacodynamic model for each antibiotic separately, as follows (Gesztelyi et al., 2012):

$$E_{mero} = E_{max}^{mero} \times \frac{C_{mero}}{IC_{50}^{mero} + C_{mero}} \qquad \textit{(Equation 17)}$$

where:

- E_{max}^{mero}: the maximum bacterial killing effect of meropenem
- IC_{50}^{mero}: concentration of meropenem that produces 50% of E_{max}, it refers to the concentration at which $E = \frac{Emax}{2}$
- C_{mero}: free concentration of meropenem

The research conducted by Zheng et al. (2024) showed the effect of ceftazidime/avibactam on MIC reduction in a murine model of infection with KPC-producing *K. pneumoniae* when combined with meropenem (Zheng et al., 2024). Using data on how the presence of ceftazidime/avibactam affected meropenem's IC50 resulted from this research, a PK/PD model was developed.

$$IC_{50}^{combination} = IC_{50}^{mero} \times (1 - \frac{\alpha \times C_{Cefta.avi}}{K_d + C_{Cefta.avi}}) \qquad \textit{(Equation 18)}$$

where:

- *α:* Maximum reduction in IC50 in the presence of ceftazidime/avibactam
- *Kd:* Ceftazidime/avibactam's concentration that produces half-maximal reductions in IC50
- $C_{Cefta.avi}$*:* free concentration of ceftazidime/avibactam

The reduction factor was determined based on the decrease in meropenem concentration at which the IC50 was achieved. In the study by Zheng et al. (2024), the IC50 of meropenem decreased from 8.179±2.745 to 0.033±0.005 mg/L, while the E_{max} reduced to 63% to show a high effectiveness on bactericidal activity.

$$Reduction\ factor = \frac{0.033 \pm 0.005}{8.179\ \pm 2.745} \sim 0.004 \qquad \textit{(Equation 19)}$$

So, in the presence of ceftazidime/avibactam, the E_{max} model (PD model) of meropenem was as follows:

$$E_{combination} = E_{max}^{combination} \times \frac{C_{mero}}{IC_{50}^{combination} + C_{mero}} \qquad \textit{(Equation 20)}$$

Simulated dosing regimens:

A grid of dosing regimens (as can be seen at Table 3-12 and Table 3-13) was evaluated, including meropenem doses of 500, 1000, and 2000 mg every 6, 8, or 12 hours infused over 3 hours, and ceftazidime/avibactam doses of 940, 1250, and 2500 mg every 8, 12, or 24 hours infused over 3 hours. For each regimen, concentration-time profiles were simulated using Euler's method over a 72-hour horizon, with a time step of 1 hour.

MIC and PTA Definition

To align with clinical breakpoints for KPC-producing *K. pneumoniae*, we defined a MIC-derived PD target effect threshold, calculated as the predicted E(t) value when plasma concentrations of meropenem and ceftazidime/avibactam were 4 mg/L and 8 mg/L, respectively (CLSI 2024). PTA was defined as the percentage of posterior PK simulations in which 100% of the dosing interval maintained $E(t)>E_{MIC}$, where E(t) > 1 was selected as the threshold for meaningful bactericidal activity. This approach provided an integrated measure of pharmacodynamic effect based on the total concentration of both agents.

Output and Visualisation

For each patient, PTA values for all tested regimens were recorded and exported to Excel. A heatmap was generated to visualise the PTA distribution across combinations of meropenem and ceftazidime/avibactam doses and intervals, stratified by regimen. Regimens achieving ≥90% PTA were considered optimal and highlighted in yellow. The analysis was conducted using R version 4.4.2 with ggplot2, writexl, and readxl packages.

Figure 3-14 shows the PTA for multiple dosing regimens of meropenem and ceftazidime/avibactam, using PD targets including 100% fT>MIC, 100% fT>4×MIC, and a model-derived E(t) > 1 threshold. The results showed that the E(t) > 1 target was consistently achieved across all patients and dosing combinations, indicating that the combined pharmacodynamic effect of both antibiotics may offer sufficient coverage when synergy is assumed. In contrast, meropenem met the 100% fT>MIC threshold only under high-dose regimens (i.e., 2000 mg every 6 hours), and even then, PTA values were suboptimal in patients with higher CrCl. Ceftazidime/avibactam, simulated at an MIC of 8 mg/L, failed to achieve adequate PTA under any regimen. When the more aggressive target of 100% *f*T>4×MIC was applied, both antibiotics showed marked reductions in PTA, with ceftazidime/avibactam consistently performing below therapeutic thresholds and meropenem achieving only marginal success under the most intensive regimens. These findings were consistent across the spectrum of renal function, although patients with reduced clearance demonstrated slightly better PTA profiles.

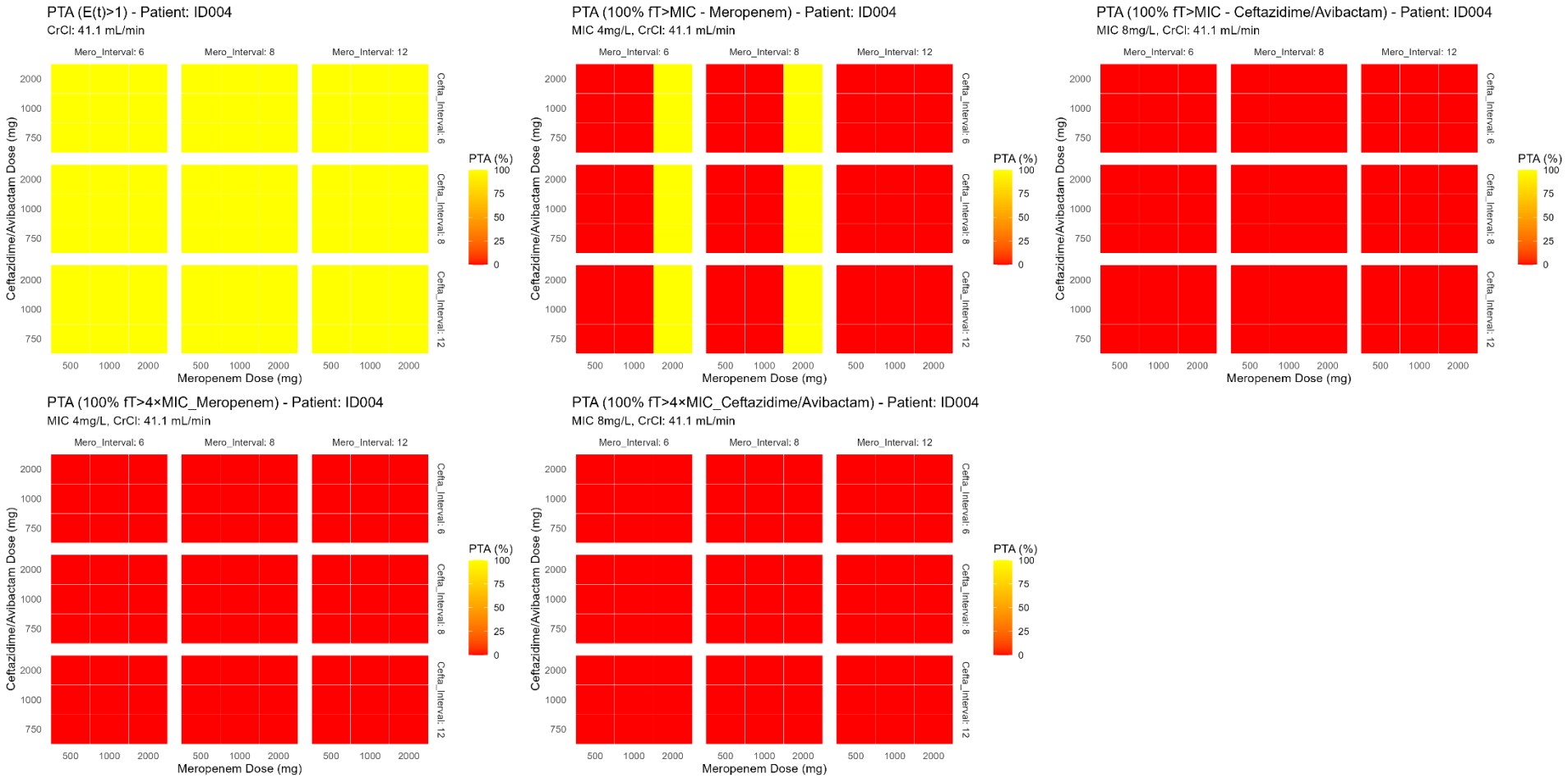


Figure 3-14. Example of PTA of simulated concentrations for patient ID004 in Phase II from PK/PD modelling and simulations

Across all 23 simulated patients, including the representative case of Patient ID004 shown in Figure 3-14, none of the evaluated dosing regimens achieved the PD targets of 100% *f*T>MIC or 100% *f*T>4×MIC for either meropenem (MIC = 4 mg/L) or ceftazidime/avibactam (MIC = 8 mg/L). This was consistent across all combinations of meropenem (500–2000 mg every 6–12

hours over 3-h infusion) and ceftazidime/avibactam (750–2000 mg every 8–12 hours over 3-h infusion). However, when the effect-based PD model E(t)>1 was applied to the same patients, the PTA reached 100% across all simulated regimens, including those that failed traditional fT-based thresholds. The remaining of 22 patients' results were presented in Appendix 3 (PK.PD_simulatedCc_22 Patients.docx).

Also, across all dosing regimens, PK/PD target attainment did not differ between the simultaneous-infusion scenario and the sequential-administration scenario.

Chapter 4 Discussion

This thesis developed a predictive PK/PD modelling framework by constructing separate PopPK models for meropenem and ceftazidime/avibactam based on published clinical data, and subsequently integrating them with an E-max based PD model to characterise their combined antibacterial effect. Using this joint PK/PD model, Monte Carlo simulations were performed to estimate the probability of PK/PD target attainment across a wide range of MICs. The simulations explored multiple dosing regimens, infusion strategies, and patient renal function profiles, thereby providing quantitative insights into the likelihood that critically ill patients would achieve therapeutic targets. In doing so, this study not only demonstrates the feasibility of MIPD for complex antibiotic combinations but also addresses key challenges in the management of MDR GNB, particularly within resource-limited hospital environments where MIPD remains underutilised. The research was structured into three phases: a systematic review, real-world data collection, and PK/PD modelling and simulations.

In Phase I, a systematic review was conducted to ascertain the PK/PD target values that patients in clinical practice would need to achieve, in order to produce a successful outcome of the antibiotic therapy; this is essential for optimising antibiotic use. However, the results were insufficient to establish a statistically significant association between PK/PD target attainment and favourable patient outcomes. The review, however, highlighted the variability of target values in practice. The result of the meta-analysis indicated that the labelled dosing regimens, whose dosages were determined mainly based on *in vitro* findings and data from healthy participants or non-critically ill patients, may not be adequate for critically ill populations to reliably achieve PK/PD targets. This suggests that modifications of dosage and administration strategies, tailored to the PK/PD properties of specific antibiotics, could improve the likelihood of achieving target attainment, which is vital to therapeutic success in clinical practice.

This variability in PK/PD targets and the limitations of standard dosing regimens are particularly important in the broader context of antimicrobial resistance. A better understanding of exposure targets that can suppress resistance amplification is needed to guide the dosing of currently available agents in clinical settings. PK/PD targets provide useful insights for informing antimicrobial dosing strategies aimed at

preventing resistance emergence (Abdelraouf et al., 2017). Exposure targets required for the suppression of resistance were seen as higher than those for efficacy and might not be clinically feasible when introduced widely without considering application in certain patients. The PK/PD target is defined as the minimal PK/PD value that is associated with a high probability of successful treatment (Abdelraouf et al., 2017). However, the non-clinical PK/PD target value was not considered to have been applied in most eligible studies of this systematic review. 58% of the investigated population in 44 included articles used the predefined PK/PD indices derived from *in vitro* studies. The others were modified from the basic theories. The findings of PK/PD targets for beta-lactams, cefiderocol, aminoglycosides, and fosfomycin are given below:

4.1 Aminoglycosides

For this antimicrobial class, the PK/PD target for preventing the emergence of resistance depends on the specific molecule used, on the administration modality and on isolates. Consequently, when amikacin or gentamycin was used, a Cmax/MIC ratio ≥ 8 was used to evaluate the probability of suppressing resistance against several Gram-negative strains such as *P. aeruginosa*, MDR *A. baumannii*, or *E. coli*. The dosage of amikacin in the study of Corcione et al. (2021) was twice that suggested by EUCAST, but the studied patients did not achieve the target Cmax/MIC above 8. In contrast, the dosage of gentamicin was not increased but the patients achieved the PK/PD targets. There were only two cases treated with amikacin in this systematic review, and the findings were not sufficient to conclude the target value ratio.

4.2 Beta-lactams (including carbapenems and cephalosporins)

Overall, beta-lactams are a class of antibiotics characterised by a very short half-life (about 4h for some cephalosporins). Even though the most important parameter associated with efficacy (both clinical outcome and/or killing) for this class of antimicrobials is *f*T>MIC, and other parameters derived from this parameter such as *f*Ctrough/MIC, *f*Cmin/MIC and Css/MIC are applied in patients undergoing CVVH, patients with multi-drug resistant infections. However, the value of both *f*T>MIC and others was not fixed for each beta-lactam; it was dependent on several factors, such

as microorganisms, MIC values, and duration of exposure. The higher burden required a higher ratio value. For instance, when piperacillin-tazobactam, meropenem, and ceftazidime were not given as a bolus but as a prolonged infusion, 40% *f*T>MIC was no longer suitable to achieve efficacy, 100% fT>MIC or even 100% *fT*> 4 to 5-fold MIC were required. Higher ratios (>20-fold MIC) have been reported for ertapenem for preventing the development of resistance in *P. aeruginosa, ESBL E. cloacae* in patients with prosthetic joint infection. Meropenem was the most frequently observed antibiotic in the systematic review, which was selected for susceptible strains and multidrug-resistant ones. The recommended 40% *f*T>MIC may no longer be suitable in patients with severe pharmacokinetic alterations or with multidrug-resistant infections. Instead, 100% *f*T>MIC or 100% *f*T> 4 – 5 x MIC seem to be a valid range.

4.3 Cefiderocol

Cefiderocol, a siderophore cephalosporin, was one of the most prescribed antibiotics for multidrug-resistant bacteria. Similarly, the PK/PD index was *f*T>MIC, but the value varied greatly across various studies in this systematic review. The target suggested by EUCAST, which was 77% *f*T>MIC, was revised to 100% *f*T>4 x MIC for 16 patients in studies by Gatti et al. (2021) and Mornese Pinna et al. (2022). Only 43% of them achieved this target with the labelled dosing regimens. A lower value, which was about 80% to above 99% *f*T>MIC, was achieved in 57 patients prescribed with Cefiderocol to treat *P. aeruginosa, A. baumanii,* XDR *A. baumannii*. On the other hand, a lower value than 77% was recorded in five patients in the studies conducted by König et al. (2021). Although the 75% *f*T>MIC was achieved, the bactericidal effect was not observed in four out of five patients.

4.4 Fosfomycin

Fosfomycin is commonly used in the community to treat urinary tract infections. In hospitals, it is typically reserved for infections caused by MDR organisms and may be given intravenously at total daily doses up to 24 g. Outside the urinary tract, resistance is relatively frequent (≈20%). Suppression of resistance is associated with achieving an AUC/MIC ratio greater than 83. However, a target of just about

40.8 could help to achieve the bactericidal effect. Fosfomycin should only be given in combination with chemotherapy and at high daily doses.

The variability in PK/PD targets emphasises the need for patient-specific dosing strategies that consider individual pharmacokinetics and the severity of infections. As can be seen from the findings, the PK/PD target should not be a fixed number; it should instead be a range of values, i.e. 50-100% *f*T>MIC to the cases of beta-lactam rather than 40% *f*T>MIC. The PK/PD indices derived from *in vitro* studies can be applied to human patients, but the value may need to be adjusted for specific cases to optimise dosing for individual patients. Maintaining antibiotic concentrations above the MIC by prolonging the duration of infusion, as seen in beta-lactams and cefiderocol, enhances their efficacy. However, the evidence from real-world patient studies is mainly based on case reports, which makes the results less reliable. Additionally, the correlation between patient outcomes and the achievement of PK/PD targets has not been firmly established. Further studies investigating the effectiveness of dosing optimisation based on PK/PD value should be made.

In summary, while the technical execution of PK measurements was robust across the dataset, concerns regarding confounding, participant selection, and selective reporting were common. These limitations resulted in the majority of studies being categorised as having moderate or serious overall risk of bias, underscoring the need for more rigorous methodological approaches in future observational PK/PD research.

Phase II involved the retrospective collection of real-world data from hospitals in Vietnam and the UK, collecting patient demographics, microbiological characteristics, and antibiotic usage. Data from over 225 included anonymised patients from two sites highlighted the features of the clinical use of antibiotics and the challenges in treating MDR GNB. The findings emphasised the need for improved antibiotic stewardship and personalised dosing strategies to enhance treatment outcomes and reduce resistance. Real-world data from this phase of the project highlight the critical gaps in current practices and provide a foundation for refining PK/PD-based dosing regimens in low-constrained healthcare settings.

Of the 225 included patients, a single pathogen was identified in 153 patients (68%). The other cases were infected with at least two bacteria (72 cases, 32%). The most

commonly isolated microorganisms were *K. pneumoniae* (116 cases, 75.8%), *A. baumannii (15 cases, 9.8%) and E. coli* (11 cases, 7.1%) and *P. aeruginosa* (11 cases, 7.1%) (Figure 3-9). The risk of co-infection with two GNB in both hospitals is around 30%. However, the treatment failure rate is approximately 40% higher compared to cases involving only one strain of bacteria.

The MIC values derived from the antibiotic culture results revealed that four types of bacteria exhibited resistance to at least one of the nine antibiotics studied. In clinical settings, it is often unavoidable to use antibiotics without the accompanying results from bacterial cultures and antibiotic susceptibility tests. This necessity arises primarily because bacterial identification can take up to 48 hours post-culture, and there are instances where culture may not be feasible if the bacteria fail to grow. Additionally, there has been the evolution of ESBLs produced by strains, which have become resistant to multiple older antibiotics.

4.5 The use of antibiotics in clinical settings

Although the sample size estimation using Daniel's formula indicated that approximately 308 participants were required, the final dataset comprised 277 eligible patients. This discrepancy is explained by the fact that patient recruitment was limited to a predefined 12-month study period (January–December 2023). Within this timeframe, only 277 patients met all eligibility criteria, including confirmed gram-negative infections, complete clinical records, and adequate documentation of antimicrobial therapy and outcomes.

Furthermore, the principal aim of this study was not to estimate a population proportion but to explore PK/PD patterns and clinical characteristics. For such analytic observational studies, data completeness and internal validity outweigh marginal increases in sample size. Therefore, the final dataset of 277 well-documented cases provides adequate statistical power and retains the methodological integrity required for the analyses presented.

In hospital settings, empirical antibiotic use is initially guided by biomarkers such as CRP and PCT. Regimens are typically revised within 3 to 5 days based on clinical response and biomarker trends, often involving dosage adjustments or the addition of new agents. Despite the potential benefits of PK/PD optimisation for antibiotic dosing, it has not been adopted in the two investigated hospitals. Notably,

quantification results for the antibiotics were virtually nonexistent at both hospitals involved in the study. While these antibiotics are broad-spectrum and effective against both gram-negative and gram-positive bacteria, their dosing was mainly aligned with standard usage guidelines. Neither hospital monitored antibiotic blood levels to evaluate the PK/PD target attainment of their dosing regimens. Instead, both institutions relied on Vitek2 devices to perform bacterial identification and antibiotic susceptibility testing, which offer range values rather than precise measurements. Furthermore, no established protocols were in place for quantifying antibiotic blood levels, making the dosing optimisation based on PK/PD more complex.

In low- to middle-income countries, the implementation of PK/PD modelling and simulations to optimise antibiotic dosing regimens remains limited. This is primarily attributed to insufficient infrastructure for measuring antibiotic concentrations and software for developing PK/PD models aligned with target values. In clinical settings, adjustments to antibiotic dosing are generally made based on the patient's liver and kidney function, as well as the Clinical judgment of the prescribers.

More than half of the patients in Phase II received a minimum of two antibiotics to treat multidrug-resistant gram-negative organisms. The secondary antibiotic combination could include one of the nine antibiotics investigated in the study, specifically those that were paired with at least two antibiotics targeting gram-negative organisms. Alternatively, it could be combined with one or more additional antibiotics that target either gram-negative or gram-positive organisms. The median hospitalisation duration was 35 days (range 5 to 177), with a cure rate of 59%, a treatment failure rate of 34%, and a mortality rate of 5%.

4.6 The use of PK/PD modelling and simulations to predict the optimal dosing regimens

This research applied a mechanism-based PK/PD modelling approach to predict the efficacy of antibiotics, as demonstrated in several prior studies. For example, Matsumoto et al. (2014) developed a PK/PD model using *in vitro* time-kill data and published pharmacokinetic parameters to characterise the pharmacodynamic properties of tebipenem pivoxil and cefditoren pivoxil against *S. pneumoniae* and *H. influenzae*, successfully predicting their clinical bacteriological efficacy in pediatric

patients with acute otitis media (Matsumoto et al., 2014). Similarly, Peña et al. (2004) employed an *in vivo* PK/*in vitro* PD approach combined with E_{max} modelling to evaluate the antibacterial activity of cefaclor against respiratory pathogens, showing that a twice-daily modified-release formulation achieved comparable efficacy to the conventional thrice-daily regimen despite lower bioavailability (Peña et al., 2004). Mohamed et al. (2012) developed a semimechanistic PK/PD model based on *in vitro* time-kill data to describe gentamicin's bactericidal activity and adaptive resistance against *E. coli*, demonstrating that extended-interval dosing (36–48 hours) was as effective as 24-hour dosing in neonates, with greater bacterial killing observed in preterm infants due to gentamicin's longer half-life (Mohamed et al., 2012). Additionally, Lim et al. (2014) constructed integrated PK and PD models using clinical pharmacokinetic data and *in vitro* time-kill experiments for vancomycin against MRSA, showing that model-based simulations could reliably predict bacterial responses and support dosing optimisation in accordance with clinical guidelines (Lim et al., 2014). More recently, a study by Bian et al. (2019) developed a semimechanistic PK/PD model to evaluate the efficacy of colistin-based combination therapy against carbapenem-resistant *A. baumannii*, using checkerboard assays, static time-kill studies, and an *in vitro* dynamic PK/PD system. Their model showed that the combination of meropenem (2 g/day via 3-h infusion) and colistin (≥2 mg/L) achieved synergistic killing, even against isolates with high meropenem MICs (≥32 mg/L), and provided valuable PK/PD evidence for optimising combination regimens in hospital-acquired pneumonia (Bian et al., 2019).

Building upon this established modelling framework, the present study aimed to predict optimal dosing regimens for the use of meropenem and ceftazidime/avibactam in combination using readily published clinical data extracted from literature and simulation-based integration of population pharmacokinetic and pharmacodynamic models. To the best of our knowledge, no prior study has applied this PK/PD modelling strategy to characterise or optimise the clinical use of this specific antibiotic combination, despite its growing relevance in treating multidrug-resistant gram-negative infections. The use of PK/PD modelling and simulations has been encouraged to improve the efficacy and prevent the emergence of bacterial resistance (De Araujo et al., 2011; Nielsen et al., 2011; Nielsen et al., 2013; Onita et al., 2025).

Both meropenem and ceftazidime/avibactam are first-line agents in the management of MDR GNB. Meropenem remains a cornerstone carbapenem, but resistance mediated by carbapenemases such as KPC and NDM limits its efficacy. Ceftazidime/avibactam restores activity against many KPC-producing organisms but has variable activity against MBLs and is susceptible to resistance emergence.

Optimising dosing of these agents is considerable given the paucity of novel antibiotics in development. For meropenem, the key question is how to extend efficacy in patients with high CI or borderline MICs. For ceftazidime/avibactam, the challenge lies in balancing adequate ceftazidime exposure with sustained avibactam levels to prevent enzymatic degradation. PopPK-PD models provide a means to address these clinical challenges systematically.

Although some previously published population PK models for ceftazidime and avibactam have employed two-compartment structures, in this thesis an one-compartment model was selected. This decision was guided by the need to integrate the ceftazidime/avibactam model with the meropenem model, which was best described by a one-compartment structure. Using models of consistent structural complexity facilitated the development of combination PK/PD simulations and avoided difficulties in aligning exposure profiles between drugs. While this simplification may sacrifice some detail in distributional kinetics, it ensured internal consistency across the modelling framework and provided robust predictions for the dosing regimens evaluated.

Recent *in vitro* evidence has increasingly supported the use of combination therapy to enhance the efficacy of ceftazidime/avibactam and suppress resistance in carbapenem-resistant *K. pneumoniae*. A comprehensive systematic review and meta-analysis by Assefa et al. (2025) reported high synergy rates when ceftazidime/avibactam was combined with several antibiotics. Notably, the combination with meropenem showed high synergy in Etest and checkerboard assays (effect size = 1.00), with no observed antagonism, and moderate synergy in time-kill studies (effect size = 0.50). These results highlight the potential role of dual beta-lactam therapy in managing infections caused by resistant *K. pneumoniae* strains, particularly through complementary PBP-binding and restoration of carbapenem susceptibility in some KPC mutants. Although the most robust *in vitro* synergy was seen with ceftazidime/avibactam plus aztreonam, especially for metallo-beta-lactamase-producing strains, the combination with meropenem

remains a promising strategy for isolates with non-MBL resistance mechanisms. This supports the rationale for developing a PK/PD model to optimise this dual therapy, particularly in settings where therapeutic options are limited.

In Phase III, population PK models for meropenem and ceftazidime/avibactam were developed and validated using data from Phase II. Simulations revealed that standard dosing regimens achieved suboptimal PTA in patients with high MICs, particularly those in critical care settings. Alternative dosing strategies, including prolonged infusions and adjusted dosing based on patient-specific factors such as renal function, were proposed and demonstrated significantly improved PTA in simulations. The integration of these models with an E_{max} model allowed for robust predictions of clinical efficacy, addressing the variability observed in real-world practice. The findings underscore the potential of MIPD to enhance antibiotic therapy effectiveness by personalising dosing regimens. Simplified PK/PD modelling approaches, using readily available patient demographic data and simulations, showed promise in overcoming barriers to implementation, particularly in resource-limited settings.

The models were then combined to leverage the collective behaviours of both meropenem and ceftazidime/avibactam. For hydrophilic antibiotics that are eliminated mainly unmodified by the renal route, such as meropenem, a high correlation between creatinine clearance and drug clearance was observed in different patient populations. This set of simulations evaluated the PTA for 100% *f*T > 4×MIC using only popPK model parameters for meropenem and ceftazidime/avibactam. The analysis focused on individualised simulations across a cohort of patients with varying renal function, applying CrCl-based dose adjustments and infusion strategies. Both meropenem and ceftazidime/avibactam were administered according to the correspondent dosing regimen the patient was prescribed in phase II.

With the simulations based on PopPK parameters only, meropenem achieved PTA ≥90% for MIC values up to 0.25–0.5 mg/L. However, its PTA dropped sharply beyond 1 mg/L, especially under stringent 100% *f*T > 4×MIC conditions. In contrast, ceftazidime/avibactam failed to achieve PTA ≥90% in all patients at MIC ≥2 mg/L. Several patients (i.e., ID073, ID097_1, ID267) exhibited low PTA values even at MIC ≤1 mg/L, highlighting the limitations of monotherapy exposure in some clinical contexts. These results represent purely pharmacokinetic target attainment, and do

not yet incorporate pharmacodynamic interaction or synergy between the two agents. Specifically, the E_{max}-based PK/PD model, which accounts for additive or synergistic bacterial killing, was not applied in this analysis. Therefore, while both meropenem and ceftazidime/avibactam may fall short of traditional PK targets when simulated independently, their combined pharmacodynamic effect may still produce effective bacterial killing, as explored in the E(t) models. This finding emphasises the need to go beyond single-agent PK metrics and incorporate full PK/PD interaction modelling, particularly when treating infections caused by pathogens with elevated MIC values (i.e., ≥4 mg/L). It also supports the rationale for combination therapy and individualised dosing regimens when managing multidrug-resistant gram-negative infections.

Many standard dosing regimens failed to achieve an optimal PTA, particularly for critically ill patients with altered pharmacokinetics or infections with high MICs. The results from the PK/PD model consistently predict lower PTA for higher MIC values, showing the potential that it aligns better with real-world challenges compared to that simulated from PK models. Sensitivity to parameter changes and clinical relevance can also guide the choice. These findings suggest that even high-dose and prolonged-infusion regimens may not provide sufficient antimicrobial exposure to meet stringent pharmacodynamic thresholds when MIC values are elevated. This result was particularly evident when using a combined MIC-based threshold defined by CLSI breakpoints for KPC-Kp: 4 mg/L for meropenem and 8 mg/L for ceftazidime/avibactam. Whether evaluated using the E_{max} model or standard PK targets, the regimens tested failed to achieve PTA ≥ 90% in most simulated patients. The implication is that, based on current EUCAST breakpoints and pharmacodynamic modelling, standard or even maximised doses of this antibiotic combination may be insufficient for optimal bacterial killing in this patient population.

The application of the E_{max} model in this analysis warrants closer interpretation. In the context of this study, E_{max} was defined as the maximal antibacterial effect, where higher values of E(t) represent stronger bacterial killing. A total E_{max} of 1.63 was used to represent the additive effect of meropenem and ceftazidime/avibactam, acknowledging potential synergy. However, in some pharmacodynamic literature, particularly in studies defining effect in terms of net bacterial growth, a lower E_{max} (i.e, 0.5 to 1) can represent enhanced bacterial killing, as it reflects reduced growth potential. In contrast, this research adopted the more conventional definition, where

increased E_{max} denotes greater kill potential. Therefore, when simulated concentrations failed to maintain E(t) above the MIC-derived threshold, it indicated that the drug exposure was likely insufficient to suppress bacterial growth or achieve bacterial killing to a meaningful degree.

Interestingly, while all regimens achieved 100% PTA using the E(t) > 1.0 target, simulations based on traditional PK/PD indices—100% *f*T > MIC and 100% *f*T > 4×MIC (assuming MICs of 4 mg/L for meropenem and 8 mg/L for ceftazidime/avibactam) consistently yielded 0% PTA across all tested regimens. This discrepancy highlights a fundamental difference in the interpretation of antimicrobial activity between effect-based models and concentration-based thresholds. In the E_{max} framework, meaningful bacterial killing can occur at total drug concentrations below the MIC, particularly when IC50 is low and drug synergy is accounted for. In contrast, fT-based targets demand unbroken coverage above MIC or 4×MIC, which may be overly strict in this context and not account for combined drug effects. These findings support the utility of E_{max} models in evaluating antibiotic combinations, especially when synergy and post-antibiotic effects are suspected. Although the predefined PK/PD targets were not achieved, the clinical outcomes among the 23 patients indicated that over half of the study population experienced favourable clinical responses (Table 3-4). This suggests that factors beyond strict PK/PD target attainment may contribute to therapeutic success in real-world settings.

In clinical practice, not all patients permit simultaneous infusions of both agents. Many patients, commonly those were not on central venous catheters (CVCs) or who have single peripheral line, can receive the antibiotics sequentially, with one infusion finishing before the next can be started.

The staggered-dose simulation performed in this study was designed to mimic exactly this scenario. Notably, our results showed that whether the two antibiotics were infused concurrently or sequentially make no meaningful difference to PK/PD target attainment against KPC-producing *K. pneumoniae*.

Despite the inability to meet PK/PD thresholds in simulation, this outcome should not be interpreted as definitive evidence against the clinical utility of meropenem and ceftazidime/avibactam for KPC-producing pathogens. These agents are commonly used in clinical practice, and real-world outcomes often depend on a

broader range of factors, including host immunity, infection site, bacterial burden, and potential synergy that may not be fully captured in PK/PD models. Moreover, the high MIC breakpoints used in this simulation may be conservative estimates that fail to reflect the MICs of many clinical KPC isolates, which often remain at or below susceptibility thresholds.

4.7 Prospects for future work

4.7.1 Limitations of current models

This study developed a PK/PD simulation framework to evaluate the effectiveness of meropenem and ceftazidime/avibactam combination therapy against KPC-producing Klebsiella pneumoniae using individualised patient characteristics and pharmacokinetic models. The goal was to identify optimised dosing strategies capable of achieving pharmacodynamic targets in patients with multidrug-resistant gram-negative infections. The simulations incorporated both conventional PK/PD targets—100% *f*T>MIC and 100% *f*T>4×MIC, and a model-derived E(t) > 1 threshold that accounted for the additive or synergistic effects of drug combinations. Despite these strengths, the modelling framework has several limitations. First, the population PK parameters were drawn from published models and not fitted to observed concentration data from the study population. This may limit the precision of predictions, particularly in critically ill patients with altered pharmacokinetics. Second, while the E_{max} model captured additive effects, it did not account for potential time-dependent synergy, immune system contributions, or drug distribution at the site of infection. Third, the use of fixed MIC values based on CLSI breakpoints (4 mg/L for meropenem, 8 mg/L for ceftazidime/avibactam) may not reflect the true MIC distribution of local *K. pneumoniae* isolates, especially given that actual MICs from hospital laboratories were often censored or reported categorically (i.e., susceptible or resistant). Finally, the model assumed perfect adherence to simulated dosing regimens, which may not hold in clinical practice.

4.7.2 Future directions

The results clearly demonstrated that conventional dosing regimens were often insufficient to reach stringent PK/PD targets in many patients, particularly when high MIC values were assumed. In contrast, when the E(t) model was applied, the

combination therapy consistently achieved 100% PTA for E(t) > 1 across all patients, including those with high MICs and augmented renal clearance. This suggests that additive or synergistic pharmacodynamic interactions may provide effective bacterial suppression even when traditional targets are unmet. These findings highlight the limitations of relying solely on *f*T>MIC-based targets, especially in combination therapies, and underscore the utility of effect-based PK/PD modelling in guiding precision dosing.

Building on these findings, future studies should focus on prospectively validating the effect-based models in clinical settings, accounting for evolving MIC distributions, and linking simulation outcomes to clinical endpoints. The modelling approach developed in this thesis can also be extended to explore other antibiotic combinations, such as those involving polymyxins, tigecycline, or Fosfomycin, to support the optimisation of combination therapy in MDR-GNB infections.

4.8 Concluding remarks

This thesis addressed key challenges in optimising antibiotic therapy for MDR-GNB infections through a three-phase research programme. The systematic review in Phase I highlighted inconsistencies in PK/PD target definitions and underscored the need for more clinically relevant thresholds. Phase II revealed substantial variability in antibiotic dosing practices across two hospital settings, indicating a gap between guideline recommendations and real-world application. In response, Phase III developed and applied population pharmacokinetic models integrating patient-specific parameters to simulate PTA and evaluate the combined effect of meropenem and ceftazidime/avibactam using an E(t)-based PD model. The combination of meropenem and ceftazidime/avibactam was selected for modelling and simulation in this thesis due to its promising synergistic activity observed in preclinical *in vitro* studies, particularly against carbapenemase-producing *K. pneumoniae* strains, which have been challenging to treat. Several *in vitro* experiments and time-kill assays have demonstrated enhanced bactericidal effects when these agents are used together, suggesting potential clinical utility in overcoming resistance mechanisms. Despite this theoretical advantage, there remains a significant gap in the clinical literature regarding the efficacy of this combination in practice. No PK/PD studies or clinical trials had, at the time of this

research, systematically assessed the joint effect of meropenem and ceftazidime/avibactam in critically ill patients. Given the increasing prevalence of MDR GNB and the limitations of monotherapy in such cases, this combination was a rational starting point for simulation-based exploration. The modelling approach adopted in this thesis provides a foundational framework to investigate the potential benefit of dual beta-lactam therapy under patient-specific conditions. It is anticipated that future work will extend this methodology to other antibiotic combinations to further support precision dosing strategies in severe infections caused by resistant organisms.

References

Abdelraouf, K., Linder, K.E., Nailor, M.D.et al. (2017) Predicting and preventing antimicrobial resistance utilizing pharmacodynamics: part II Gram-negative bacteria. *Expert Opin. Drug Metab. Toxicol.*, 13 (7), 705-714.

Abhilash, B., Tripathi, C.D., Gogia, A.R.et al. (2015) Pharmacokinetic/pharmacodynamic profiling of imipenem in patients admitted to an intensive care unit in India: A nonrandomized, cross-sectional, analytical, open-labeled study. *Indian J. Crit. Care Med.*, 19 (10), 587-592.

Abhilash, B., Tripathi, C.D., Gogia, A.R.et al. (2016) Variability in plasma concentration of cefotaxime in critically ill patients in an Intensive Care Unit of India and its pharmacodynamic outcome: A nonrandomized, prospective, open-label, analytical study. *J. Pharmacol. Pharmacother.*, 7 (1), 15-21.

Adediran, T., Harris, A.D., Johnson, J.K.et al. (2020) Epidemiologic and microbiologic characteristics of hospitalized patients co-colonized with multiple species of carbapenem-resistant enterobacteriaceae in the United States. *Open Forum Infectious Diseases* [online], 7 (10), ofaa386

Ambrose, P.G., Bhavnani, S.M. and Owens, R.C. (2003) Clinical pharmacodynamics of quinolones. *Infectious Disease Clinics*, 17 (3), 529-543.

Aoki, Y., Urakami, T., Magarifuchi, H.et al. (2011) The importance of pharmacokinetic consultation of cefepime treatment for Pseudomonas aeruginosa bacteremia: a case report of severe thermal burn injury. *J Infect Chemother*, 17 (3), 407-411.

Asín-Prieto, E., Rodríguez-Gascón, A. and Isla, A. (2015) Applications of the pharmacokinetic/pharmacodynamic (PK/PD) analysis of antimicrobial agents. *Journal of Infection and Chemotherapy*, 21 (5), 319-329.

Balasubramanian, R., Van Boeckel, T.P., Carmeli, Y.et al. (2023) Global incidence in hospital-associated infections resistant to antibiotics: An analysis of point prevalence surveys from 99 countries. *PLoS Med.*, 20 (6), e1004178.

Barrett, J.S. (2018) *mrgsolve: Simulate from ODE-Based Models* [online]

Bayot ML, B.B. (2024) *Antimicrobial Susceptibility Testing* [online]

Bian, X., Liu, X., Chen, Y.et al. (2019) Dose Optimization of Colistin Combinations against Carbapenem-Resistant Acinetobacter baumannii from Patients with Hospital-Acquired Pneumonia in China by Using an In Vitro Pharmacokinetic/Pharmacodynamic Model. *Antimicrob. Agents Chemother.*, 63 (4), 04.

Bjarnsholt, T., Kirketerp-Møller, K., Jensen, P.Ø.et al. (2008) Why chronic wounds will not heal: a novel hypothesis. *Wound Repair Regen.*, 16 (1), 2-10.

Blot, S., Ruppé, E., Harbarth, S.et al. (2022) Healthcare-associated infections in adult intensive care unit patients: Changes in epidemiology, diagnosis, prevention and contributions of new technologies. *Intensive Crit. Care Nurs.*, 70, 103227.

Bognár, B., Spohn, R. and Lázár, V. (2024) Drug combinations targeting antibiotic resistance. *npj Antimicrobials and Resistance*, 2 (1), 29.

Boonpeng, A., Jaruratanasirikul, S., Jullangkoon, M.et al. (2022) Population pharmacokinetics/pharmacodynamics and clinical outcomes of meropenem in critically ill patients. *Antimicrob. Agents Chemother.*, 66 (11), e00845-00822.

Borenstein, M., Hedges, L.V., Higgins, J.P.et al. (2021) *Introduction to meta-analysis*. John Wiley & Sons.

Bulik, C.C., Quintiliani, R., Samuel Pope, J.et al. (2010) Pharmacodynamics and tolerability of high-dose, prolonged infusion carbapenems in adults with cystic fibrosis – A review of 3 cases. *Respir. Med. CME*, 3 (3), 146-149.

Bulman, Z.P., Wicha, S.G., Nielsen, E.I.et al. (2022) Research priorities towards precision antibiotic therapy to improve patient care. *The Lancet Microbe*, 3 (10), e795-e802.

Burki, T.K. (2018) Superbugs: an arms race against bacteria. *The Lancet Respiratory Medicine*, 6 (9), 668.

Burns, P.B., Rohrich, R.J. and Chung, K.C. (2011) The levels of evidence and their role in evidence-based medicine. *Plast. Reconstr. Surg.*, 128 (1), 305-310.

Butcher (2008) Differential and Difference Equations. In: (ed.) *Numerical Methods for Ordinary Differential Equations.* pp. 1-49.

CLSI (2024) *Performance standards for antimicrobial susceptibility testing*. 34th edition ed. Wayne, PA: Clinical and Laboratory Standards Institute (CLSI).

Cojutti, P.G., Fornaro, G., Gatti, M.et al. (2022) Successful Treatment of Bacteremia and Ventilator-Associated Pneumonia Caused by KPC/OXA-48-like Klebsiella pneumoniae Co-Producer with a Continuous Infusion of High-Dose Meropenem Plus Fosfomycin Guided by Real-Time Therapeutic Drug Monitoring. *Infect. Dis. Rep.*, 14 (1), 88-92.

Corcione, S., De Nicolò, A., Lupia, T.et al. (2021) Observed concentrations of amikacin and gentamycin in the setting of burn patients with gram-negative bacterial infections: Preliminary data from a prospective study. *Therapie*, 76 (5), 409-414.

Craig, W.A. (1998) Pharmacokinetic/Pharmacodynamic Parameters: Rationale for Antibacterial Dosing of Mice and Men. *Clin. Infect. Dis.*, 26 (1), 1-12.

Craig, W.A. (2001) Does the dose matter? *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 33 Suppl 3, S233-237.

Curtin, F. (2017) Meta-analysis combining parallel and crossover trials using generalised estimating equation method. *Res Synth Methods*, 8 (3), 312-320.

Cusack, T.P., Ashley, E.A., Ling, C.L.et al. (2019) Time to switch from CLSI to EUCAST? A Southeast Asian perspective. *Clin. Microbiol. Infect.*, 25 (7), 782-785.

Chabert, P., Provoost, J., Cohen, S.et al. (2022) Pharmacokinetics, efficacy and tolerance of cefoxitin in the treatment of cefoxitin-susceptible extended-spectrum beta-lactamase producing Enterobacterales infections in critically ill patients: a retrospective single-center study. *Ann Intensive Care*, 12 (1), 90.

Chacko, J., Pawar, S., Seppelt, I.et al. (2023) Multidrug-Resistant Gram-Negative Bacteria in the ICU: Do We Have Answers? In: Chacko, J., Pawar, S., Seppelt, I. and Brar, G. (ed.) *Controversies in Critical Care.* Singapore: Springer Nature Singapore. pp. 355-364.

Chen, N. (2020) The antibiotic paradox: why companies can't afford to create life-saving drugs. *Lancet*, 395.

Cheng, I.-L., Chen, Y.-H., Lai, C.-C.et al. (2018) Intravenous colistin monotherapy versus combination therapy against carbapenem-resistant gram-negative bacteria

infections: meta-analysis of randomized controlled trials. *Journal of Clinical Medicine*, 7 (8), 208.

Chongcharoenyanon, T., Wacharachaisurapol, N., Anugulruengkitt, S.et al. (2021) Comparison of piperacillin plasma concentrations in a prospective randomised trial of extended infusion versus intermittent bolus of piperacillin/tazobactam in paediatric patients. *Int. J. Infect. Dis.*, 108, 102-108.

D'Arcy, D.M., Casey, E., Gowing, C.M.et al. (2012) An open prospective study of amikacin pharmacokinetics in critically ill patients during treatment with continuous venovenous haemodiafiltration. *BMC Pharmacol Toxicol*, 13, 14.

Daniel, W. (1999) Biostatistics: A Foundation for analysis in the health sciences, 7th edR Wiley. *New York*, 141-142.

Darwich, A.S., Ogungbenro, K., Hatley, O.J.et al. (2017a) Role of pharmacokinetic modeling and simulation in precision dosing of anticancer drugs. *Translational Cancer Research*, S1512-S1529.

Darwich, A.S., Ogungbenro, K., Vinks, A.A.et al. (2017b) Why Has Model-Informed Precision Dosing Not Yet Become Common Clinical Reality? Lessons From the Past and a Roadmap for the Future. *Clin. Pharmacol. Ther.*, 101 (5), 646-656.

De Araujo, B.V., Diniz, A., Palma, E.C.et al. (2011) PK-PD modeling of β-lactam antibiotics: in vitro or in vivo models? *J. Antibiot. (Tokyo)*, 64 (6), 439-446.

De la Rosa-Riestra, S., Martínez Pérez-Crespo, P.M., Pérez Rodríguez, M.T.et al. (2024) Mortality impact of further delays in active targeted antibiotic therapy in bacteraemic patients that did not receive initial active empiric treatment: Results from the prospective, multicentre cohort PROBAC. *Int. J. Infect. Dis.*, 145, 107072.

Del Valle-Moreno, P., Suarez-Casillas, P., Mejías-Trueba, M.et al. (2023) Model-Informed Precision Dosing Software Tools for Dosage Regimen Individualization: A Scoping Review. *Pharmaceutics*, 15 (7), 1859.

Delfino, E., Fucile, C., Del Bono, V.et al. (2018) Pharmacokinetics of high-dose extended-infusion meropenem during pulmonary exacerbation in adult cystic fibrosis patients: a case series. *New Microbiol.*, 41 (1), 47-51.

Derendorf, H. and Meibohm, B. (1999) Modeling of Pharmacokinetic/Pharmacodynamic (PK/PD) Relationships: Concepts and Perspectives. *Pharm. Res.*, 16 (2), 176-185.

Dhaese, S.A.M., Farkas, A., Colin, P.et al. (2018) Population pharmacokinetics and evaluation of the predictive performance of pharmacokinetic models in critically ill patients receiving continuous infusion meropenem: a comparison of eight pharmacokinetic models. *J. Antimicrob. Chemother.*, 74 (2), 432-441.

Dibbets, A.C., Koldeweij, C., Osinga, E.P.et al. (2024) Barriers and Facilitators for Bringing Model-Informed Precision Dosing to the Patient's Bedside: A Systematic Review. *Clin. Pharmacol. Ther.*

Drawz, S.M. and Bonomo, R.A. (2010) Three decades of β-lactamase inhibitors. *Clin. Microbiol. Rev.*, 23 (1), 160-201.

Drusano, G.L. (2004) Antimicrobial pharmacodynamics: critical interactions of'bug and drug'. *Nature Reviews Microbiology*, 2 (4), 289-300.

Drusano, G.L. and Craig, W.A. (2012) Antibacterial Chemotherapy. In: Goldman, L. and Schafer, A. I. (ed.) *Goldman's Cecil Medicine.* Philadelphia: W.B. Saunders. pp. 1803-1815.

Dudley, M.N. (1991) Pharmacodynamics and pharmacokinetics of antibiotics with special reference to the fluoroquinolones. *The American journal of medicine*, 91 (6A), 45S-50S.

Eisert, A., Lanckohr, C., Frey, J.et al. (2021) Comparison of two empirical prolonged infusion dosing regimens for meropenem in patients with septic shock: A two-center pilot study. *Int. J. Antimicrob. Agents*, 57 (3), 106289.

Estes, K.S. and Derendorf, H. (2010) Comparison of the pharmacokinetic properties of vancomycin, linezolid, tigecyclin, and daptomycin. *Eur. J. Med. Res.*, 15 (12), 533.

EUCAST (2024) *Breakpoint tables for interpretation of MICs and zone diameters* [online]

European Agency for the Evaluation of Medicinal Products (2000) Points to consider on pharmacokinetics and pharmacodynamics in the development of antibacterial medicinal products. Document CPMP/EWP/2655/99. [online]

FDA (2013) Codevelopment of two or more new investigational drugs for use in combination. [online]

Ferri, M., Ranucci, E., Romagnoli, P.et al. (2017) Antimicrobial resistance: A global emerging threat to public health systems. *Crit. Rev. Food Sci. Nutr.*, 57 (13), 2857-2876.

Food Drug Administration (2003) Guidance for industry: exposure-response relationships-study design, data analysis, and regulatory applications. *http://www. fda. gov/cber/gdlns/exposure. pdf*.

Fox, W., Ellard, G.A. and Mitchison, D.A. (1999) Studies on the treatment of tuberculosis undertaken by the British Medical Research Council tuberculosis units, 1946–1986, with relevant subsequent publications. *The International Journal of Tuberculosis and Lung Disease*, 3 (10), S231-S279.

Franzetti, F., Antonelli, M., Bassetti, M.et al. (2010) Consensus document on controversial issues for the treatment of hospital-associated pneumonia. *Int. J. Infect. Dis.*, 14 Suppl 4, S55-65.

Fresan, D., Luque, S., Benítez-Cano, A.et al. (2023) Pharmacokinetics/pharmacodynamics and therapeutic drug monitoring of ceftazidime/avibactam administered by continuous infusion in patients with MDR Gram-negative bacterial infections. *J. Antimicrob. Chemother.*, 78 (3), 678-683.

Gabrielsson, J. and Weiner, D. (2001) Pharmacokinetic and pharmacodynamic data analysis: concepts and applications.

Gallo, J.M. (2010) Pharmacokinetic/ pharmacodynamic-driven drug development. *Mt. Sinai J. Med.*, 77 (4), 381-388.

Gandra, S., Alvarez-Uria, G., Turner, P.et al. (2020) Antimicrobial Resistance Surveillance in Low- and Middle-Income Countries: Progress and Challenges in Eight South Asian and Southeast Asian Countries. *Clin. Microbiol. Rev.*, 33 (3).

Gatti, M., Cojutti, P.G., Pascale, R.et al. (2021) Assessment of a PK/PD Target of Continuous Infusion Beta-Lactams Useful for Preventing Microbiological Failure and/or Resistance Development in Critically Ill Patients Affected by Documented Gram-Negative Infections. *Antibiotics (Basel)*, 10 (11).

Gatti, M., Giannella, M., Rinaldi, M.et al. (2022) Pharmacokinetic/Pharmacodynamic Analysis of Continuous-Infusion Fosfomycin in Combination with Extended-Infusion Cefiderocol or Continuous-Infusion Ceftazidime-Avibactam in a Case Series of Difficult-to-Treat Resistant Pseudomonas aeruginosa Bloodstream Infections and/or Hospital-Acquired Pneumonia. *Antibiotics (Basel)*, 11 (12).

Gatti, M., Rinaldi, M., Gaibani, P.et al. (2023) A descriptive pharmacokinetic/pharmacodynamic analysis of continuous infusion ceftazidime-avibactam for treating DTR gram-negative infections in a case series of critically ill patients undergoing continuous veno-venous haemodiafiltration (CVVHDF). *J. Crit. Care*, 76, 154301.

Gesztelyi, R., Zsuga, J., Kemeny-Beke, A.et al. (2012) The Hill equation and the origin of quantitative pharmacology. *Archive for History of Exact Sciences*, 66 (4), 427-438.

Gómez-Junyent, J., Rigo-Bonnin, R., Benavent, E.et al. (2020) Efficacy and Therapeutic Drug Monitoring of Continuous Beta-Lactam Infusion for Osteoarticular Infections Caused by Fluoroquinolone-Resistant Pseudomonas aeruginosa: A Prospective Cohort Study. *Eur. J. Drug Metab. Pharmacokinet.*, 45 (5), 587-599.

Goutelle, S., Conrad, A., Pouderoux, C.et al. (2021) Pharmacokinetic/Pharmacodynamic Dosage Individualization of Suppressive Beta-Lactam Therapy Administered by Subcutaneous Route in Patients With Prosthetic Joint Infection. *Front Med (Lausanne)*, 8, 583086.

Greppmair, S., Brinkmann, A., Roehr, A.et al. (2023) Towards model-informed precision dosing of piperacillin: multicenter systematic external evaluation of pharmacokinetic models in critically ill adults with a focus on Bayesian forecasting. *Intensive Care Med.*, 49 (8), 966-976.

Greve, J.M. and Cowan, J.A. (2022) Tackling antimicrobial stewardship through synergy and antimicrobial peptides. *RSC Medicinal Chemistry*, 13 (5), 511-521.Guillamet, M.C.V., Damulira, C., Atkinson, A.et al. (2023) Addition of aminoglycosides reduces recurrence of infections with multidrug-resistant Gram-negative bacilli in patients with sepsis and septic shock. *Int. J. Antimicrob. Agents*, 62 (4), 106913.Haddad, M.J., McComsey, G., Radix, A. and Natella Rakhmanina, W.R., 2024. IDSA GUIDELINES [online]

Hanretty, A.M., Kaur, I., Evangelista, A.T.et al. (2018) Pharmacokinetics of the Meropenem Component of Meropenem-Vaborbactam in the Treatment of KPC-Producing Klebsiella pneumoniae Bloodstream Infection in a Pediatric Patient. *Pharmacotherapy*, 38 (12), e87-e91.Heffernan, A.J.S., F. B. Lipman, J. Roberts, J. A. (2018) Individualising Therapy to Minimize Bacterial Multidrug Resistance. *Drugs*, 78 (6), 621-641.

Heil, E.L., Lowery, A.V., Thom, K.A.et al. (2015) Treatment of multidrug-resistant pseudomonas aeruginosa using extended-infusion antimicrobial regimens. *Pharmacotherapy*, 35 (1), 54-58.Hobbs, A.L., Shea, K.M., Roberts, K.M.et al. (2015) Implications of Augmented Renal Clearance on Drug Dosing in Critically Ill Patients: A Focus on Antibiotics. *Pharmacotherapy*, 35 (11), 1063-1075.Hochhaus, G., Barrett, J.S. and Derendorf, H. (2000) Evolution of Pharmacokinetics and Pharmacokinetic/Dynamic Correlations during the 20th Century. *The Journal of Clinical Pharmacology*, 40 (9), 908-917.

Hoiby, N., Ciofu, O. and Bjarnsholt, T. (2010) Pseudomonas aeruginosa biofilms in cystic fibrosis. *Future Microbiol.*, 5 (11), 1663-1674.

Jacobs, M.R. (2003) How can we predict bacterial eradication? *Int. J. Infect. Dis.*, 7 Suppl 1, S13-20.

Jaruratanasirikul, S., Sriwiriyajan, S. and Punyo, J. (2005) Comparison of the pharmacodynamics of meropenem in patients with ventilator-associated pneumonia following administration by 3-hour infusion or bolus injection. *Antimicrob. Agents Chemother.*, 49 (4), 1337-1339.

Kahlmeter, G., Brown, D., Goldstein, F.et al. (2006) European Committee on Antimicrobial Susceptibility Testing (EUCAST) technical notes on antimicrobial susceptibility testing. [online]

Kahlmeter, G., Giske Christian, G., Kirn Thomas, J.et al. (2019) Point-Counterpoint: Differences between the European Committee on Antimicrobial Susceptibility Testing and Clinical and Laboratory Standards Institute Recommendations for Reporting Antimicrobial Susceptibility Results. *J. Clin. Microbiol.*, 57 (9), 10.1128/jcm.01129-01119.

Kantasiripitak, W., Van Daele, R., Gijsen, M.et al. (2020) Software Tools for Model-Informed Precision Dosing: How Well Do They Satisfy the Needs? *Front. Pharmacol.*, 11, 620.

Kerantzas, C. and Jacobs, W. (2017) Origins of combination therapy for tuberculosis: lessons for future antimicrobial development and application. mBio 8: e01586-16. *DOI: https://doi. org/10.1128/mBio*, 01586-01516.

Kluwe, F., Michelet, R., Mueller-Schoell, A.et al. (2021) Perspectives on Model-Informed Precision Dosing in the Digital Health Era: Challenges, Opportunities, and Recommendations. *Clin. Pharmacol. Ther.*, 109 (1), 29-36.

Kobic, E., Abouelhassan, Y., Singaravelu, K.et al. (2022) Pharmacokinetic Analysis and In Vitro Synergy Evaluation of Cefiderocol, Sulbactam, and Tigecycline in an Extensively Drug-Resistant Acinetobacter baumannii Pneumonia Patient Receiving Continuous Venovenous Hemodiafiltration. *Open Forum Infect Dis*, 9 (10), ofac484.

Kobic, E., Gill, C.M., Mochon, A.B.et al. (2021) Cefiderocol Pharmacokinetics in a Patient Receiving Continuous Venovenous Hemodiafiltration. *Open Forum Infect Dis*, 8 (7), ofab252.

König, C., Both, A., Rohde, H.et al. (2021) Cefiderocol in Critically Ill Patients with Multi-Drug Resistant Pathogens: Real-Life Data on Pharmacokinetics and Microbiological Surveillance. *Antibiotics (Basel)*, 10 (6).

Kuriyama, T., Karasawa, T. and Williams, D.W. (2014) Chapter Thirteen - Antimicrobial Chemotherapy: Significance to Healthcare. In: Percival, S. L., Williams, D. W., Randle, J. and Cooper, T. (ed.) *Biofilms in Infection Prevention and Control.* Boston: Academic Press. pp. 209-244.

Kuti, J.L. (2016) OPTIMIZING ANTIMICROBIAL PHARMACODYNAMICS: A GUIDE FOR YOUR STEWARDSHIP PROGRAM. *Revista Médica Clínica Las Condes*, 27 (5), 615-624.

Kuti, J.L., Moss, K.M., Nicolau, D.P.et al. (2004) Empiric treatment of multidrug-resistant Burkholderia cepacia lung exacerbation in a patient with cystic fibrosis: application of pharmacodynamic concepts to meropenem therapy. *Pharmacotherapy*, 24 (11), 1641-1645.

Lalonde, R.L., Kowalski, K.G., Hutmacher, M.M.et al. (2007) Model-based Drug Development. *Clin. Pharmacol. Ther.*, 82 (1), 21-32.

Larkins-Ford, J. and Aldridge, B.B. (2023) Advances in the design of combination therapies for the treatment of tuberculosis. *Expert Opin Drug Discov*, 18 (1), 83-97.

Lea-Henry, T.N., Carland, J.E., Stocker, S.L.et al. (2018) Clinical Pharmacokinetics in Kidney Disease: Fundamental Principles. *Clin. J. Am. Soc. Nephrol.*, 13 (7), 1085-1095.

Li, L., Sassen, S.D., Ewoldt, T.M.et al. (2023) Meropenem model-informed precision dosing in the treatment of critically ill patients: Can we use it? *Antibiotics*, 12 (2), 383.

Li, X.-Z. and Nikaido, H. (2009) Efflux-mediated drug resistance in bacteria: an update. *Drugs*, 69, 1555-1623.

Lim, H.S., Chong, Y.P., Noh, Y.H.et al. (2014) Exploration of optimal dosing regimens of vancomycin in patients infected with methicillin-resistant Staphylococcus aureus by modeling and simulation. *J. Clin. Pharm. Ther.*, 39 (2), 196-203.

Linder, K.E.N., David P. Nailor, Michael D. (2016) Predicting and preventing antimicrobial resistance utilizing pharmacodynamics: Part I gram positive bacteria. *Expert Opin. Drug Metab. Toxicol.*, 12 (3), 267-280.

Liu, X., Zheng, H., Zhang, W.et al. (2016) Tracking Cefoperazone/Sulbactam Resistance Development In vivo in A. baumannii Isolated from a Patient with Hospital-Acquired Pneumonia by Whole-Genome Sequencing. *Front. Microbiol.*, 7, 1268.

Maier, C., de Wiljes, J., Hartung, N.et al. (2022) A continued learning approach for model-informed precision dosing: Updating models in clinical practice. *CPT: Pharmacometrics & Systems Pharmacology*, 11 (2), 185-198.

Matsumoto, K., Sugano, T., Sato, N.et al. (2014) Prediction of clinical bacteriological efficacy of oral antibiotics using a mechanism-based pharmacokinetic-pharmacodynamics modeling. *Jpn. J. Antibiot.*, 67 (1), 33-47.

Maynard, M., Drusano, G.L., Vicchiarelli, M.et al. (2021) Polymyxin B pharmacodynamics in the hollow-fiber infection model: What you see may not be what you get. *Antimicrob. Agents Chemother.*, 65 (8).

Meibohm, B. and Derendorf, H. (1997) Basic concepts of pharmacokinetic/pharmacodynamic (PK/PD) modelling. *Int. J. Clin. Pharmacol. Ther.*, 35 (10), 401-413.

Menna, P., Salvatorelli, E., Mattei, A.et al. (2018) Modified Colistin Regimen for Critically Ill Patients with Acute Renal Impairment and Continuous Renal Replacement Therapy. *Chemotherapy*, 63 (1), 35-38.

Meyer, C.T., Wooten, D.J., Paudel, B.B.et al. (2019) Quantifying drug combination synergy along potency and efficacy axes. *Cell systems*, 8 (2), 97-108. e116.

Mohamed, A.F., Nielsen, E.I., Cars, O.et al. (2012) Pharmacokinetic-pharmacodynamic model for gentamicin and its adaptive resistance with predictions of dosing schedules in newborn infants. *Antimicrob. Agents Chemother.*, 56 (1), 179-188.

Morales Castro, D., Dresser, L., Granton, J.et al. (2023) Pharmacokinetic Alterations Associated with Critical Illness. *Clin. Pharmacokinet.*, 62 (2), 209-220.

Mornese Pinna, S., Corcione, S., De Nicolò, A.et al. (2022) Pharmacokinetic of Cefiderocol in Critically Ill Patients Receiving Renal Replacement Therapy: A Case Series. *Antibiotics (Basel)*, 11 (12).

Mouton, J.W., Brown, D.F.J., Apfalter, P.et al. (2012) The role of pharmacokinetics/pharmacodynamics in setting clinical MIC breakpoints: The EUCAST approach. *Clin. Microbiol. Infect.*, 18 (3), E37-E45.

Mouton, J.W., Dudley, M.N., Cars, O.et al. (2002) Standardization of pharmacokinetic/pharmacodynamic (PK/PD) terminology for anti-infective drugs. *Int. J. Antimicrob. Agents*, 19 (4), 355-358.

Mudenda, S., Chabalenge, B., Daka, V.et al. (2023) Global Strategies to Combat Antimicrobial Resistance: A One Health Perspective. *Pharmacology & Pharmacy*, 14 (8), 271-328.

Murínová, I., Švidrnoch, M., Gucký, T.et al. (2024) Meropenem population pharmacokinetics and model-based dosing optimisation in patients with serious bacterial infection. *European Journal of Hospital Pharmacy*, 31 (3), 253-258.

Murray, C.J.L.e.a. (2022) Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet*, 399 (10325), 629-655.

Nielsen, E.I., Cars, O. and Friberg, L.E. (2011) Pharmacokinetic/pharmacodynamic (PK/PD) indices of antibiotics predicted by a semimechanistic PKPD model: a step toward model-based dose optimization. *Antimicrob. Agents Chemother.*, 55 (10), 4619-4630.

Nielsen, E.I. and Friberg, L.E. (2013) Pharmacokinetic-pharmacodynamic modeling of antibacterial drugs. *Pharmacol. Rev.*, 65 (3), 1053-1090.

Nikaido, H. (2003) Molecular basis of bacterial outer membrane permeability revisited. *Microbiol. Mol. Biol. Rev.*, 67 (4), 593-656.

Ny, P., Nieberg, P. and Wong-Beringer, A. (2015) Impact of carbapenem resistance on epidemiology and outcomes of nonbacteremic Klebsiella pneumoniae infections. *Am. J. Infect. Control*, 43 (10), 1076-1080.

NHLBI (2013) *Quality Assessment Tool for Case Series Studies* [online]

O'Jeanson, A.L., Romaric Le Souder, Cosette Djebli, Nassim Khier, Sonia (2021) Population Pharmacokinetics and Pharmacodynamics of Meropenem in Critically Ill Patients: How to Achieve Best Dosage Regimen According to the Clinical Situation. *Eur. J. Drug Metab. Pharmacokinet.*, 46 (5), 695-705.

O'Neill, J. (2016) Tackling drug-resistant infections globally: final report and recommendations.

Olbrisch, K., Kisch, T., Thern, J.et al. (2019) After standard dosage of piperacillin plasma concentrations of drug are subtherapeutic in burn patients. *Naunyn Schmiedebergs Arch. Pharmacol.*, 392 (2), 229-241.

Olofsson, S.K. and Cars, O. (2007) Optimizing drug exposure to minimize selection of antibiotic resistance. *Clin. Infect. Dis.*, 45 Suppl 2, S129-136.

Onita, T., Ishihara, N. and Yano, T. (2025) PK/PD-Guided Strategies for Appropriate Antibiotic Use in the Era of Antimicrobial Resistance. *Antibiotics*, 14 (1), 92.

Page, M.J., McKenzie, J.E., Bossuyt, P.M.et al. (2021) The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*, 372, n71.

Paul, M., Lador, A., Grozinsky-Glasberg, S.et al. (2014) Beta lactam antibiotic monotherapy versus beta lactam-aminoglycoside antibiotic combination therapy for sepsis. *Cochrane Database Syst. Rev.* (1).

Paul, M., Soares-Weiser, K. and Leibovici, L. (2003) β lactam monotherapy versus β lactam-aminoglycoside combination therapy for fever with neutropenia: systematic review and meta-analysis. *BMJ*, 326 (7399), 1111.

Peck, C. and Cross, J. (2007) “Getting the dose right”: facts, a blueprint, and encouragements. *Clin. Pharmacol. Ther.*, 82 (1), 12-14.

Peleg, A.Y. and Hooper, D.C. (2010) Hospital-acquired infections due to gram-negative bacteria. *N. Engl. J. Med.*, 362 (19), 1804-1813.

Peña, A.d.l., Gräbe, A., Rand, K.H.et al. (2004) PK–PD modelling of the effect of cefaclor on four different bacterial strains. *Int. J. Antimicrob. Agents*, 23 (3), 218-225.

Pereira, L.C., de Fatima, M.A., Santos, V.V.et al. (2022a) Pharmacokinetic/Pharmacodynamic Modeling and Application in Antibacterial and Antifungal Pharmacotherapy: A Narrative Review. *Antibiotics-Basel*, 11 (8), 20.

Pereira, L.C., Fátima, M.A., Santos, V.V.et al. (2022b) Pharmacokinetic/Pharmacodynamic Modeling and Application in Antibacterial and Antifungal Pharmacotherapy: A Narrative Review. *Antibiotics (Basel)*, 11 (8).

Pfizer Inc. (2023) Avycaz (ceftazidime and avibactam) injection, for intravenous use – Prescribing information.

Pilmis, B., Mizrahi, A., Mory, C.et al. (2021) How to optimize administration of cefoxitin for the treatment of extended spectrum producing Enterobacteriaceae-related infection? *Eur. J. Clin. Microbiol. Infect. Dis.*, 40 (7), 1393-1397.

Póvoa, P. (2008) Serum markers in community-acquired pneumonia and ventilator-associated pneumonia. *Curr. Opin. Infect. Dis.*, 21 (2), 157-162.

Philpott, C.D., Droege, C.A., Droege, M.E.et al. (2019) Pharmacokinetics and Pharmacodynamics of Extended-Infusion Cefepime in Critically Ill Patients Receiving Continuous Renal Replacement Therapy: A Prospective, Open-Label Study. *Pharmacotherapy*, 39 (11), 1066-1076.

R Core Team (2023) *R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria.* https://www.R-project.org/ [computer program]

R Core Team (2024) *R: A language and environment for statistical computing* (4.4.2) [computer program]

Ramirez, M.S. and Tolmasky, M.E. (2010) Aminoglycoside modifying enzymes. *Drug resistance updates*, 13 (6), 151-171.

Reed, M.D. (2000) Optimal antibiotic dosing. The pharmacokinetic-pharmacodynamic interface. *Postgrad. Med.*, 108 (7 Suppl Contemporaty), 17-24.

Rodríguez-Gascón, A., Solinís, M. and Isla, A. (2021) The Role of PK/PD Analysis in the Development and Evaluation of Antimicrobials. *Pharmaceutics*, 13 (6).

Routledge, P.A. and Hutchings, A.D. (2013) Chapter 9.22 - Therapeutic Drug Monitoring (TDM). In: Wild, D. (ed.) *The Immunoassay Handbook (Fourth Edition).* Oxford: Elsevier. pp. 945-962.

Rybak, M.J. (2006) The Pharmacokinetic and Pharmacodynamic Properties of Vancomycin. *Clin. Infect. Dis.*, 42 (Supplement_1), S35-S39.

Sackett, D.L. (1989) Rules of evidence and clinical recommendations on the use of antithrombotic agents. *Chest*, 95 (2 Suppl), 2s-4s.

Sadilová, K., Halačová, M. and Černý, D. (2020) Pharmacokinetic aspects of beta-lactam antibiotic therapy in intensive care unit patients: A one-center experience with TDM Farmakokinetické aspekty terapie beta-laktamovými antibiotiky u kriticky nemocných pacientů: Zkušenosti jednoho centra s TDM.

Sanghavi, K., Ribbing, J., Rogers, J.A.et al. (2024) Covariate modeling in pharmacometrics: General points for consideration. *CPT Pharmacometrics Syst Pharmacol*, 13 (5), 710-728.

Shah, S., Nicolau, D.P., McManus, D.et al. (2021) A Novel Dosing Strategy of Ceftolozane/Tazobactam in a Patient Receiving Intermittent Hemodialysis. *Open Forum Infect Dis*, 8 (6), ofab238.

Sharif, A.J.O.a.S. (2025) *Drug Distribution* [online]

Sime, F.B., Roberts, M.S. and Roberts, J.A. (2015) Optimization of dosing regimens and dosing in special populations. *Clin. Microbiol. Infect.*, 21 (10), 886-893.

Simoni, A., Schwartz, L., Junquera, G.Y.et al. (2024) Current and emerging strategies to curb antibiotic-resistant urinary tract infections. *Nature Reviews Urology*, 21 (12), 707-722.

Sinnollareddy, M.G., Roberts, M.S., Lipman, J.et al. (2012) β-lactam pharmacokinetics and pharmacodynamics in critically ill patients and strategies for dose optimization: a structured review. *Clin. Exp. Pharmacol. Physiol.*, 39 (6), 489-496.

Soetaert, K., Petzoldt, T., & Setzer, R. W., (2010) Solving differential equations in R: Package deSolve. *Journal of Statistical Software*, 33 (9), 1-25.

Sophonsri, A., Kelsom, C., Lou, M.et al. (2023) Risk factors and outcome associated with coinfection with carbapenem-resistant Klebsiella pneumoniae and carbapenem-resistant Pseudomonas aeruginosa or Acinetobacter baumanii: a descriptive analysis. *Frontiers in Cellular and Infection Microbiology*, 13.

Sorlí, L., Luque, S., Li, J.et al. (2019) Colistin for the treatment of urinary tract infections caused by extremely drug-resistant Pseudomonas aeruginosa: Dose is critical. *J. Infect.*, 79 (3), 253-261.

Soukup, P., Faust, A.C., Edpuganti, V.et al. (2019) Steady-state ceftazidime-avibactam serum concentrations and dosing recommendations in a critically ill patient being treated for Pseudomonas aeruginosa pneumonia and undergoing continuous venovenous hemodiafiltration. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, 39 (12), 1216-1222.

Sterne, J.A., Hernán, M.A., Reeves, B.C.et al. (2016) ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *BMJ*, 355, i4919.

Sterne, J.A.C., Savović, J., Page, M.J.et al. (2019) RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ*, 366, l4898.

Su, W., Song, S., Liu, J.et al. (2024) Population pharmacokinetics and individualized dosing of tigecycline for critically ill patients: a prospective study with intensive sampling. *Front. Pharmacol.*, Volume 15 - 2024.

Sulis, G., Sayood, S. and Gandra, S. (2023) How can we tackle the overuse of antibiotics in low- and middle-income countries? *Expert Rev. Anti Infect. Ther.*, 21 (11), 1189-1201.

Tabah, A., Buetti, N., Staiquly, Q. et al (2023) Epidemiology and outcomes of hospital-acquired bloodstream infections in intensive care unit patients: the EUROBACT-2 international cohort study. *Intensive Care Med.*, 49 (2), 178-190.

Tacconelli, E., Carrara, E., Savoldi, A.et al. (2018) Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect. Dis.*, 18 (3), 318-327.

Tamma, P.D., Cosgrove, S.E. and Maragakis, L.L. (2012) Combination therapy for treatment of infections with gram-negative bacteria. *Clin. Microbiol. Rev.*, 25 (3), 450-470.

Tamma, P.D., Heil, E.L., Justo, J.A.et al. (2024) Infectious Diseases Society of America 2024 guidance on the treatment of antimicrobial-resistant gram-negative infections. *Clin. Infect. Dis.*, ciae403.

Tängdén, T., Lundberg, C.V., Friberg, L.E.et al. (2020) How preclinical infection models help define antibiotic doses in the clinic. *Int. J. Antimicrob. Agents*, 56 (2), 106008.

Teng, X.Q., Qu, Q., Luo, Y.et al. (2022) Therapeutic Drug Monitoring of Ceftazidime-Avibactam Concentrations in Carbapenem-Resistant K. pneumoniae-Infected Patients With Different Kidney Statuses. *Front. Pharmacol.*, 13.

Tilanus, A. and Drusano, G. (2023) Optimizing the Use of Beta-Lactam Antibiotics in Clinical Practice: A Test of Time. *Open Forum Infectious Diseases*, 10 (7).

Torella, J.P., Chait, R. and Kishony, R. (2010) Optimal drug synergy in antimicrobial treatments. *PLoS Comput. Biol.*, 6 (6), e1000796.

Tsai, D., Lipman, J. and Roberts, J.A. (2015) Pharmacokinetic/pharmacodynamic considerations for the optimization of antimicrobial delivery in the critically ill. *Curr. Opin. Crit. Care*, 21 (5), 412-420.

Tsuji, B.T., Pogue, J.M., Zavascki, A.P.et al. (2019) International Consensus Guidelines for the Optimal Use of the Polymyxins: Endorsed by the American College of Clinical Pharmacy (ACCP), European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Infectious Diseases Society of America (IDSA), International Society for Anti-infective Pharmacology (ISAP), Society of Critical Care

Medicine (SCCM), and Society of Infectious Diseases Pharmacists (SIDP). *Pharmacotherapy*, 39 (1), 10-39.

Tyers, M. and Wright, G.D. (2019) Drug combinations: a strategy to extend the life of antibiotics in the 21st century. *Nature Reviews Microbiology*, 17 (3), 141-155.

Udy, A.A., Roberts, J.A. and Lipman, J. (2013) Clinical implications of antibiotic pharmacokinetic principles in the critically ill. *Intensive Care Med.*, 39, 2070-2082.

Ullah, I., Ali, E. and Fakhar ud, D. (2020) Bioavailability of Antibiotics and Their Toxicity. In: Hashmi, M. Z. (ed.) *Antibiotics and Antimicrobial Resistance Genes: Environmental Occurrence and Treatment Technologies.* Cham: Springer International Publishing. pp. 211-238.

Ulldemolins, M., Roberts, J.A., Rello, J.et al. (2011) The effects of hypoalbuminaemia on optimizing antibacterial dosing in critically ill patients. *Clin. Pharmacokinet.*, 50 (2), 99-110.

US FDA (2020) Pharmacokinetics in patients with impaired renal function—study design, data analysis, and impact on dosing. *Washington, DC: US Food and Drug Administration*.

Usman, M., Frey, O.R. and Hempel, G. (2017) Population pharmacokinetics of meropenem in elderly patients: dosing simulations based on renal function. *Eur. J. Clin. Pharmacol.*, 73 (3), 333-342.

Utrup, T.R., Mueller, E.W., Healy, D.P.et al. (2010) High-dose ciprofloxacin for serious gram-negative infection in an obese, critically ill patient receiving continuous venovenous hemodiafiltration. *Ann. Pharmacother.*, 44 (10), 1660-1664.

Van den Broek, A.K., Prins, J.M., Visser, C.E.et al. (2021) Systematic review: the bioavailability of orally administered antibiotics during the initial phase of a systemic infection in non-ICU patients. *BMC Infect. Dis.*, 21 (1), 285.

Van Ingen, J.-E., Eric F. Levin, Adrah Totten, Sarah E. Boeree, Martin J. Mouton, Johan W. Aarnoutse, Rob E. Heifets, Leonid B. Peloquin, Charles A. Daley, Charles L. (2012) The pharmacokinetics and pharmacodynamics of pulmonary Mycobacterium avium complex disease treatment. *Am. J. Respir. Crit. Care Med.*, 186 (6), 559-565.

Veiga, R.P. and Paiva, J.-A. (2018) Pharmacokinetics–pharmacodynamics issues relevant for the clinical use of beta-lactam antibiotics in critically ill patients. *Critical Care*, 22 (1), 233.

Wenzler, E.A.-O., Bunnell, K.L., Bleasdale, S.C.et al. (2017) Pharmacokinetics and Dialytic Clearance of Ceftazidime-Avibactam in a Critically Ill Patient on Continuous Venovenous Hemofiltration. LID - 10.1128/AAC.00464-17 [doi] LID - e00464-17. (1098-6596 (Electronic)).

WHO (2023) *High-level messaging on the HAI and AMR burden* [online]

Wieringa, A., Ter Horst, P.G.J., Wagenvoort, G.H.J.et al. (2022) Target attainment and pharmacokinetics of cefotaxime in critically ill patients undergoing continuous kidney replacement therapy. *J. Antimicrob. Chemother.*, 77 (12), 3421-3426.

Wright, G.D. (2007) The antibiotic resistome: the nexus of chemical and genetic diversity. *Nature reviews microbiology*, 5 (3), 175-186.

Wu, Y.E., Xu, H.Y., Shi, H.Y.et al. (2020) Carbapenem-Resistant Enterobacteriaceae Bloodstream Infection Treated Successfully With High-Dose Meropenem in a Preterm Neonate. *Front. Pharmacol.*, 11, 566060.

Xiao, H. (2018) Introduction of FDA guideline antibacterial therapies for patients with an unmet medical need for the treatment of serious bacterial diseases. *Drug Evaluation Research*, 41 (1), 41-47.

Yang, T., Mei, H., Wang, J.et al. (2021) Therapeutic Drug Monitoring of Tigecycline in 67 Infected Patients and a Population Pharmacokinetics/Microbiological Evaluation of A. baumannii Study. *Front. Microbiol.*, 12, 678165.

Zahr, N., Urien, S., Llopis, B.et al. (2022) Total and Unbound Pharmacokinetics of Cefiderocol in Critically Ill Patients. *Pharmaceutics*, 14 (12).

Zavrelova, A., Sima, M., Malakova, J.et al. (2023) Superiority of ceftazidime off-label high-dose regimen in PK/PD target attainment during treatment of extensively drug-resistant Pseudomonas aeruginosa infections in cancer patients. *Br. J. Clin. Pharmacol.*, 89 (4), 1452-1461.

Zhang, X.-S., Wang, Y.-Z., Shi, D.-W.et al. (2022) Efficacy and pharmacodynamic target attainment for ceftazidime–avibactam off-label dose regimens in patients with

continuous or intermittent venovenous hemodialysis: two case reports. *Infectious Diseases and Therapy*, 11 (6), 2311-2319.

Zheng, M., Li, F.-H., Liu, J.et al. (2024) Synergistic effects of ceftazidime/avibactam combined with meropenem in a murine model of infection with KPC-producing Klebsiella pneumoniae. *J. Antimicrob. Chemother.*, 79 (5), 1069-1080.

Zhou, Q.T., He, B., Shen, N.et al. (2017) Meropenem Dosing Based on a Population Pharmacokinetic-Pharmacodynamic Model in Elderly Patients with Infection of the Lower Respiratory Tract. *Drugs Aging*, 34 (2), 115-121.

Zhou, Y., Xu, P., Li, H.et al. (2021) Population pharmacokinetics and exposure-response analysis of tigecycline in patients with hospital-acquired pneumonia. *Br. J. Clin. Pharmacol.*, 87 (7), 2838-2846.

Appendices

Appendix 1 Search results for the systematic review

Cochrane: 167 results

- #1: 94324 Trials matching "pharmacokinetic*" in Title Abstract Keyword OR "pharmacodynamic*" in Title Abstract Keyword OR PK PD in Title Abstract Keyword - (Word variations have been searched)
- #2: 34114 Trials matching antibiotics in Title Abstract Keyword OR antibiotic in Title Abstract Keyword - (Word variations have been searched)
- #3: 85830 Trials matching resistance in Title Abstract Keyword OR resistant in Title Abstract Keyword - (Word variations have been searched)

Scopus: 3562 results

201,259 document results

- (TITLE-ABS-KEY (*pharmacokinetic**) OR TITLE-ABS-KEY (*pharmacodynamic**) OR TITLE-ABS-KEY (*pk* AND *pd*)) AND (LIMIT-TO (PUBYEAR , *2022*) OR LIMIT-TO (PUBYEAR , *2021*) OR LIMIT-TO (PUBYEAR , *2020*) OR LIMIT-TO (PUBYEAR , *2019*) OR LIMIT-TO (PUBYEAR , *2018*) OR LIMIT-TO (PUBYEAR , *2017*) OR LIMIT-TO (PUBYEAR , *2016*) OR LIMIT-TO (PUBYEAR , *2015*) OR LIMIT-TO (PUBYEAR , *2014*) OR LIMIT-TO (PUBYEAR , *2013*) OR LIMIT-TO (PUBYEAR , *2012*) OR LIMIT-TO (PUBYEAR , *2011*) OR LIMIT-TO (PUBYEAR , *2010*) OR LIMIT-TO (PUBYEAR , *2009*) OR LIMIT-TO (PUBYEAR , *2008*) OR LIMIT-TO (PUBYEAR , *2007*) OR LIMIT-TO (PUBYEAR , *2006*) OR LIMIT-TO (PUBYEAR , *2005*) OR LIMIT-TO (PUBYEAR , *2004*) OR LIMIT-TO (PUBYEAR , *2003*) OR LIMIT-TO (PUBYEAR , *2002*) OR LIMIT-TO (PUBYEAR , *2001*) OR LIMIT-TO (PUBYEAR , *2000*))

675,038 document results

- (TITLE-ABS-KEY (*antibiotic*) OR TITLE-ABS-KEY (*antibiotics*)) AND (LIMIT-TO (PUBYEAR , *2022*) OR LIMIT-TO (PUBYEAR , *2021*) OR LIMIT-TO (PUBYEAR , *2020*) OR LIMIT-TO (PUBYEAR , *2019*) OR LIMIT-TO (PUBYEAR , *2018*) OR LIMIT-TO (PUBYEAR , *2017*) OR LIMIT-TO (PUBYEAR , *2016*) OR LIMIT-TO (PUBYEAR , *2015*) OR LIMIT-TO (PUBYEAR , *2014*) OR LIMIT-

TO (PUBYEAR , *2013*) OR LIMIT-TO (PUBYEAR , *2012*) OR LIMIT-TO (PUBYEAR , *2011*) OR LIMIT-TO (PUBYEAR , *2010*) OR LIMIT-TO (PUBYEAR , *2009*) OR LIMIT-TO (PUBYEAR , *2008*) OR LIMIT-TO (PUBYEAR , *2007*) OR LIMIT-TO (PUBYEAR , *2006*) OR LIMIT-TO (PUBYEAR , *2005*) OR LIMIT-TO (PUBYEAR , *2004*) OR LIMIT-TO (PUBYEAR , *2003*) OR LIMIT-TO (PUBYEAR , *2002*) OR LIMIT-TO (PUBYEAR , *2001*) OR LIMIT-TO (PUBYEAR , *2000*))

2,176,343 document results

- (TITLE-ABS-KEY (*resistant*) OR TITLE-ABS-KEY (*resistance*)) AND (LIMIT-TO (PUBYEAR , *2022*) OR LIMIT-TO (PUBYEAR , *2021*) OR LIMIT-TO (PUBYEAR , *2020*) OR LIMIT-TO (PUBYEAR , *2019*) OR LIMIT-TO (PUBYEAR , *2018*) OR LIMIT-TO (PUBYEAR , *2017*) OR LIMIT-TO (PUBYEAR , *2016*) OR LIMIT-TO (PUBYEAR , *2015*) OR LIMIT-TO (PUBYEAR , *2014*) OR LIMIT-TO (PUBYEAR , *2013*) OR LIMIT-TO (PUBYEAR , *2012*) OR LIMIT-TO (PUBYEAR , *2011*) OR LIMIT-TO (PUBYEAR , *2010*) OR LIMIT-TO (PUBYEAR , *2009*) OR LIMIT-TO (PUBYEAR , *2008*) OR LIMIT-TO (PUBYEAR , *2007*) OR LIMIT-TO (PUBYEAR , *2006*) OR LIMIT-TO (PUBYEAR , *2005*) OR LIMIT-TO (PUBYEAR , *2004*) OR LIMIT-TO (PUBYEAR , *2003*) OR LIMIT-TO (PUBYEAR , *2002*) OR LIMIT-TO (PUBYEAR , *2001*) OR LIMIT-TO (PUBYEAR , *2000*))

Combine query #1 AND #2 AND #3" 3,562 document results

- ((TITLE-ABS-KEY (*pharmacokinetic**) OR TITLE-ABS-KEY (*pharmacodynamic**) OR TITLE-ABS-KEY (*pk* AND *pd*))) AND ((TITLE-ABS-KEY (*antibiotic*) OR TITLE-ABS-KEY (*antibiotics*))) AND ((TITLE-ABS-KEY (*resistant*) OR TITLE-ABS-KEY (*resistance*))) AND (LIMIT-TO (PUBYEAR , *2022*) OR LIMIT-TO (PUBYEAR , *2021*) OR LIMIT-TO (PUBYEAR , *2020*) OR LIMIT-TO (PUBYEAR , *2019*) OR LIMIT-TO (PUBYEAR , *2018*) OR LIMIT-TO (PUBYEAR , *2017*) OR LIMIT-TO (PUBYEAR , *2016*) OR LIMIT-TO (PUBYEAR , *2015*) OR LIMIT-TO (PUBYEAR , *2014*) OR LIMIT-TO (PUBYEAR , *2013*) OR LIMIT-TO (PUBYEAR , *2012*) OR LIMIT-TO (PUBYEAR , *2011*) OR LIMIT-

TO (PUBYEAR , *2010*) OR LIMIT-TO (PUBYEAR , *2009*) OR LIMIT-TO (PUBYEAR , *2008*) OR LIMIT-TO (PUBYEAR , *2007*) OR LIMIT-TO (PUBYEAR , *2006*) OR LIMIT-TO (PUBYEAR , *2005*) OR LIMIT-TO (PUBYEAR , *2004*) OR LIMIT-TO (PUBYEAR , *2003*) OR LIMIT-TO (PUBYEAR , *2002*) OR LIMIT-TO (PUBYEAR , *2001*) OR LIMIT-TO (PUBYEAR , *2000*)) AND (LIMIT-TO (DOCTYPE , *"ar"*)) AND (LIMIT-TO (LANGUAGE , *"English"*))

Web of science: 2912 results

- Query #1: pharmacokinetic (All Fields) or pharmacodynamic (All Fields) or pk pd (All Fields)
- Query #2: resistance (All Fields) or resistant (All Fields)
- Query #3: antibiotics (All Fields) or antibiotic (All Fields)

advanced search:

- #3 AND #2 AND #1 and 2022 or 2021 or 2020 or 2019 or 2018 or 2017 or 2016 or 2015 or 2014 or 2013 or 2012 or 2011 or 2010 or 2009 or 2007 or 2008 or 2006 or 2005 or 2004 or 2003 or 2002 or 2001 or 2000 (Publication Years) and ArtiCles (Document Types) and English (Languages)

Ovid (Embase): 2083 results

- Query: ((pharmacokinetic or pharmacodynamic or pk pd).af.) AND ((resistance or resistant).af.) AND ((antibiotics or antibiotic).af.)

Year range: 2000 – current, language: English -> 1514 results

Pubmed: 2,695 results

- Query: ((((pharmacokinetic) OR (pharmacodynamic)) OR (pk pd) AND ((fha[Filter]) AND (Clinicaltrial[Filter]) AND (humans[Filter]) AND (english[Filter]))) AND ((antibiotics) OR (antibiotic) AND ((fha[Filter]) AND (Clinicaltrial[Filter]) AND (humans[Filter]) AND (english[Filter])))) AND ((resistance) OR (resistant) AND ((fha[Filter]) AND (Clinicaltrial[Filter]) AND (humans[Filter]) AND (english[Filter])))

Year range: 2000 – 2022

The systematic review was registered in PROSPERO (see attachment for details CRD42022376130)

Appendix 2 Required information for Phase II

Information for extraction	Conversion for prevention of identifier disclose
Length of hospitalisation	
Admission date	
Discharge date	
Age	Converted from date of birth
Ethnicity	If applicable
Gender	
Weight	
eGFR (or CrCl)	If applicable
Serum albumin level	If applicable
Infections	
Isolated bacteria	
Antibiotics	
Route of Administration	
Dosage	
Duration per administration	
Duration (start date-end date)	
MIC	
Patient outcomes	
Results of the last bacterial culture	(Positive or Negative or not available)
Death	(Yes or No)
Admission to ICU	(Yes or No)
Use of following vasopressor drugs such as dopamine, dobutamine, epinephrine, norepinephrine, vasopressin, and phenylephrine.	(Yes or No)
Requirement of mechanical ventilation	(Yes or No)
Recovery	(Yes or No)
Lost of follow-up	(Yes or No)

Appendix 3 Data

This file contains all data used in the thesis project on the PK/PD of antibiotics in critically ill patients. The content is organised across three phases, as follows:

Name of file	Details
Phase 1: A systematic review and meta analysis	
Phase1_Summary	Contains characteristics of 44 studies included in the systematic review.
	Includes study ID, title, type, number of patients, pathogens, resistance profile, antibiotic used, PK/PD targets, outcomes, and whether modification occurred.
Phase1_PRISMA (C)	PRISMA-style summary table of the screening process for the systematic review.
	Shows number of records identified, excluded, and included, consistent with PRISMA 2020.
Phase1_RoB2_RCT	Risk of Bias assessment using the RoB 2 tool for RCTs.
	Each row represents an RCT with judgments across 5 domains (D1–D5) and an overall risk rating.
Phase1_Robins_I_nonRCT	Risk of Bias assessment for non-randomized studies using the ROBINS-I tool.
	Lists study ID, author, and bias ratings across domains such as confounding and missing data.
Phase1_NHLBI_CS	NHLBI quality evaluation for case series studies.
	Includes 9 yes/no questions per study and a final quality rating (Good, Fair, Poor) according to NHLBI tool
Phase1_MA	Data for meta-analysis comparing PK/PD target attainment between modified and unmodified antibiotic regimens.
	Columns: author, number of successes, total, type of modification, and study type.
Phase 2: Clinical cohort from 225 Patients	

Name of file	Details
Phase2_225 patients	Patient-level dataset with demographics, infection details, antibiotic exposure, and outcomes, including variables like age, weight, albumin, creatinine, ICU admission, CrCl, and bacterial species.
Phase2_antibiotic use	Antibiotic-level dataset by course for each patient, including tracks start/end dates, antibiotic name, duration, outcome, and creatinine clearance.
Phase2_lengthofuse	Summarises minimum and maximum dates and total duration of use for each antibiotic per patient.
Phase 3: PK Model Input and Output	
Phase3_meropenem	Dataset used for meropenem population PK model development and simulation.
	Includes dosing history, observed concentrations, covariates (e.g., age, CrCl), and derived parameters (e.g., CL, V).
	Final PK data from the model
Phase3_Cefta.avi	Dataset for ceftazidime/avibactam PK model and simulation.
	Similar structure to meropenem data, includes patient-level PK parameters and model-specific inputs (V, CL).
	Final PK data from the model

See the attachment for details of thesis data (Data of Phase I and Phase II)

This file (PK.PD_simulatedCc_22__Patients.docx) presents the simulated concentration–time profiles and PTA values for the 22 patients included in Phase II. The results were generated using the population PK/PD models described in section 3.3.3.3 (Chapter 3), with dosing regimens were simulated.