

In vitro and *in silico* studies of rational
combination therapies against multidrug-
resistant Gram-negative bacteria

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In the name of Allah, the Most Compassionate, the Most Merciful.

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Hemodialyzer Compared to a Commercial Cartridge

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N. Kroemer, **A. Farooq**, J.W. Decousser, J. Buyck, N. Grégoire, P. Nordmann, S.G. Wicha

Hollow fiber study assessing synergy of ceftazidime/avibactam and fosfomycin in a clinical
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33rd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID),
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III. Abbreviations

ADME	Absorption, Distribution, Metabolism, Excretion
AMR	Antimicrobial resistance
AUC	Area under the curve
C _{max}	Maximum concentration
CFU	Colony-forming units
CL	Clearance
CLSI	Clinical and Laboratory Standards Institute
EC ₅₀	Concentration resulting in half-maximum effect
EMA	European Medicines Agency
E _{max}	Maximum effect
ESBL	Extended-spectrum beta-lactamases
ESBL-E	Extended-spectrum beta-lactamases Enterobacterales
EUCAST	European Committee on Antimicrobial Susceptibility Testing
F	Bioavailability
FDA	Food and Drug Administration
FICI	Fractional inhibitory concentration index
G3P	Glucose-3-phosphate
G6P	Glucose-6-phosphate
GPDI	General Pharmacodynamic Interaction
HFIM	Hollow Fibre Infection Model
IV	Intravenous
KPC	<i>Klebsiella pneumoniae</i> carbapenemase

MIC	Minimal inhibitory concentration
MLE	Maximum likelihood estimation
MOI	Multiplicity of infection
MDR	Multidrug-resistant
NCA	Non-compartmental analysis
NLME	Nonlinear mixed effects
PBP	Penicillin-binding proteins
PD	Pharmacodynamic
PDI	Pharmacodynamic index
PK	Pharmacokinetic
PPB	Plasma protein binding
PTA	Probability of target attainment
Q	Intercompartmental clearance
R	Resistant
S	Susceptible
UTI	Urinary tract infections
V_d	Volume of distribution central compartment
V_p	Volume of distribution peripheral compartment
WHO	World Health Organisation

1 Zusammenfassung

Antimikrobielle Resistenzen (AMR) stellen eine ernsthafte und zunehmende globale Bedrohung dar, die die verfügbaren Optionen zur Behandlung von Infektionen durch multiresistente (MDR) Gram-negative Bakterien, insbesondere solchen die *Carbapenemases* oder *extended-spectrum beta-lactamases* (ESBL) exprimieren, stark einschränken. Folglich erhält das Konzept rationaler Kombinationstherapien als Möglichkeit, die Wirksamkeit bestehender Reserveantibiotika zu maximieren, sowie alternative Ansätze wie die Bakteriophagentherapie, zunehmende Aufmerksamkeit.

Ziel dieser Arbeit war es, die pharmakokinetischen und pharmakodynamischen (PK/PD) Eigenschaften wichtiger Reserve- und neuartiger Antibiotika systematisch und *in vitro* zu charakterisieren. Der Fokus lag hierbei auf synergistischen PD-Interaktionen gegen schwer behandelbare, MDR Isolate von *Klebsiella pneumoniae* und *Escherichia coli*. Durch die Integration von *in vitro*- und *in silico*-Ansätzen, einschließlich verschiedener experimenteller und pharmkometrischer Methoden, wurde die Wirksamkeit und Interaktionsdynamik von Meropenem-Fosfomycin, Ceftazidim/Avibactam-Tigecyclin, und Polymyxin B und dem neuartigen Bakteriophagen pK8 – einzeln und in ausgewählten Kombinationen – quantitativ untersucht.

Die Arbeit umfasste vier grundlegende Studien: **Publikation I** PK/PD-Modellierung von statischen und *hollow-fibre infection model* (HFIM) Experimenten zur Meropenem-Fosfomycin Kombinationstherapie; **Publikation II** Pharmakometrische Beurteilung der Kombinationstherapie von Polymyxin B und Bakteriophage pK8 gegen polymyxinresistente *K. pneumoniae*; **Publikation III** Systematische Untersuchung der PD-Interaktionen zwischen Ceftazidim/Avibactam und Tigecyclin in einer Auswahl klinischer und isogener *E. coli* und *K. pneumoniae*-Isolate; und **Publikation IV** Entwicklung eines pharmakometrischen Plattformmodell zur Definition kombinierter klinischer Breakpoints für die Meropenem-Fosfomycin-Therapie unter Nutzung von Daten aus einer vielfältigen MDR-*K. pneumoniae* Population und unterschiedlichen *in vitro*-Infektionsmodellen.

Die quantitativen Analysen umfassten statische *time-kill* Methoden, *checkerboard* und dynamische *checkerboard* Methoden sowie longitudinale HFIM-Experimente zur Simulation humaner PK-Profile. Darüber hinaus wurden sog. nicht-lineare gemischte Effektmodelle und das *General Pharmacodynamic Interaction Model* angewendet, um neben der Wirksamkeit auch die Art, Richtung und das Ausmaß der Arzneistoffinteraktionen zu charakterisieren. Wo zutreffend,

wurden humane PK-Populationsmodelle eingesetzt, um Simulationen zur Übertragung der *in vitro* Befunde in klinisch relevante Empfehlungen für Dosierung und Erfolgswahrscheinlichkeiten von Therapien zu erstellen.

Die Ergebnisse zeigten eine robuste und reproduzierbare Synergie von Meropenem und Fosfomycin Kombinationstherapien über mehrere MDR *K. pneumoniae*-Stämme hinweg, einschließlich solcher mit *Carbapenemases*. Die PK/PD-Modellierung bestätigte, dass die Synergie hohe kombinierte Effekte erzeugte, die mit Monotherapien nicht erreicht werden konnten. Diese Ergebnisse wurden zusätzlich im *Open Flow Infection Model* und HFIM unter Einsatz simulierter humaner PK validiert, was die Wirksamkeit gegen resistente Isolate und klinische Anwendbarkeit bestätigte. Aufbauend auf diesen Ergebnissen wurden umfassende Simulationen durchgeführt. Bemerkenswert ist, dass die Meropenem-Fosfomycin-Kombination die PD Zielwerte (*Probability of Target Attainment* $\geq 90\%$ für Bakteriostase und 1-log-Reduktion der Bakterien) bei weitaus höheren MIC-Werten als jede Einzelsubstanz (z.B. bis zu 32 mg/L | 512 mg/L für Meropenem | Fosfomycin) zuverlässig erreichte und sowohl die Wiedereinführung eines intravenösen Fosfomycin-Breakpoints im Kombinationskontext als auch optimierte Dosierungsempfehlungen bei schweren MDR *K. pneumoniae*-Infektionen unterstützt.

Eine alternative Strategie untersuchte die Kombination von Polymyxin B mit einem neuartigen, lytischen Bakteriophagen (pK8) gegen ein polymyxinresistentes *K. pneumoniae* Isolat. Die pharmakometrische Modellierung zeigte eine synergistische Interaktion, gekennzeichnet durch Phagen-vermittelte Resensibilisierung gegenüber Polymyxin B und verstärkte bakterizide Effekte bei hoher Phagenzahl pro Bakterien.

Umgekehrt zeigte die systematische Untersuchung von Ceftazidim/Avibactam mit Tigecyclin in klinischen und isogenen *E. coli* und *K. pneumoniae*-Isolaten überwiegend antagonistische PD-Interaktionen. Sowohl *checkerboard*-basierte als auch *time-kill* Untersuchungen, unterstützt durch metabolomische Analysen, belegten, dass der maximal erzielte Effekt in Kombination auf den von Tigecyclin-Monotherapie beschränkt war, was nicht nur fehlenden Nutzen, sondern möglicherweise potenzielle Risiken für dieses Therapie nahelegt.

Diese Arbeit zeigt den Wert und die Notwendigkeit der Integration fortschrittlicher pharmakometrischer Ansätze mit modernen *in vitro*-Methoden, um rationale, quantitative Evidenz für oder gegen spezifische Kombinationsstrategien bei Infektionen ausgelöst durch MDR, Gram-negative Bakterien zu untersuchen.

2 Abstract

Antimicrobial resistance (AMR) poses a critical and escalating global threat, severely limiting the available options for treating infections caused by multidrug-resistant (MDR) Gram-negative bacteria, in particular those expressing carbapenemases or extended-spectrum beta-lactamases (ESBL). Consequently, the concept of rational combination therapies is receiving increasing emphasis as a means to maximize the efficacy of existing last-resort antibiotics, and alternative approaches, such as bacteriophage therapy.

This thesis systematically investigated the pharmacokinetic and pharmacodynamic (PK/PD) properties of key last-resort and novel antimicrobials *in vitro*. The primary focus was on synergistic PD interactions against difficult-to-treat, MDR isolates of *Klebsiella pneumoniae* and *Escherichia coli*. Integrating *in vitro* and *in silico* approaches, like various experimental and pharmacometric methods, this dissertation quantitatively evaluated the efficacy and interaction dynamics of meropenem-fosfomycin, ceftazidime/avibactam-tigecycline, polymyxin B and novel bacteriophage pK8—alone and in selected combinations.

The work encompassed four foundational studies: **Publication I** PK/PD modelling of static and hollow-fibre infection model (HFIM) experiments of meropenem-fosfomycin combination therapy; **Publication II** Pharmacometric assessment of polymyxin B and bacteriophage pK8 combination against polymyxin-resistant *K. pneumoniae*; **Publication III** Systematic investigation of PD interactions between ceftazidime/avibactam and tigecycline in a panel of clinical and isogenic *E. coli* and *K. pneumoniae* isolates; and **Publication IV** The establishment of a pharmacometric framework to define rational combined clinical breakpoints for meropenem-fosfomycin combination therapy, employing data from a diverse MDR *K. pneumoniae* strain panel and multiple *in vitro* infection models.

Quantitative analyses included static time-kill assays, checkerboard and ‘dynamic’ checkerboard methods, and longitudinal HFIM experiments simulating human PK profiles. In addition, nonlinear mixed-effects models and the General Pharmacodynamic Interaction Model were applied to elucidate not only efficacy endpoints but also the nature, directionality, and magnitude of drug interactions. Where appropriate, human population PK models informed simulation studies to translate *in vitro* findings into clinically meaningful guidance on dosing and probability of therapy success.

The results demonstrated robust and reproducible synergy of meropenem-fosfomycin combination therapy across multiple MDR *K. pneumoniae* strains, including those producing KPC carbapenemases. PK/PD modelling confirmed that synergy was mainly driven by meropenem enhancing the potency of fosfomycin, resulting in profound suppression of bacterial regrowth that could not be achieved by monotherapies. These findings were validated in the HFIM using simulated human PK, confirming efficacy against resistant isolates and offering supportive evidence for clinical applicability. Building on these results, comprehensive simulations integrated diverse MDR *K. pneumoniae* strains, low to high dosing regimens, and two distinct dynamic *in vitro* infection models, allowing the proposal of rational, model-based clinical combined breakpoints. Notably, the meropenem-fosfomycin combination reliably achieved pharmacodynamic targets (Probability of Target Attainment $\geq 90\%$ for bacteriostasis and 1-log reduction of bacteria) at substantially higher MICs than either compound alone (e.g., up to 32 mg/L | 512 mg/L for meropenem | fosfomycin), supporting both the reintroduction of an intravenous fosfomycin breakpoint in combination contexts and optimized dosing recommendations in severe MDR *K. pneumoniae* infections.

A distinct strategy combining polymyxin B with a novel lytic bacteriophage (pK8) was explored against a polymyxin-resistant *K. pneumoniae* isolate. Pharmacometric modelling revealed a synergistic interaction characterized by phage-mediated resensitization to polymyxin B.

Conversely, the systematic evaluation of ceftazidime/avibactam with tigecycline in clinical and isogenic *E. coli* and *K. pneumoniae* isolates revealed predominantly antagonistic PD interactions. Both checkerboard-based and time-kill assays, supported by metabolomic analyses, demonstrated that the maximal effect achieved in combination was limited to that of tigecycline monotherapy, suggesting not only lack of benefit but potential risk in this widely considered salvage regimen.

This thesis demonstrates the value and necessity of integrating advanced pharmacometric modelling with state-of-the-art *in vitro* methodology to provide rational, quantitative evidence supporting or refuting specific combination strategies for MDR Gram-negative infections. Furthermore, the proposals for model-based clinical breakpoints, especially for meropenem-fosfomycin, represent a concrete advance toward rationalized, evidence-driven infectious diseases management amid the global AMR crisis. Prospective preclinical and clinical validation are now warranted to translate these findings into improved patient care, thereby maximizing the efficacy and safety of antibiotic therapy against MDR Gram-negative bacteria.

3 Introduction

3.1 Infectious Diseases: Challenges and treatment strategies

Infectious diseases can be defined as illnesses due to pathogens or their toxic product, which arise through transmission from an infected person, an infected animal, or a contaminated inanimate object to a susceptible host¹. Pathogenic microorganism causing infectious diseases are bacteria, viruses, parasites or fungi. Infectious diseases can be grouped in three major categories: diseases which cause high levels of mortality; diseases which place heavy burdens of disability on populations; and diseases which owing to the rapid and unexpected nature of their spread can have serious global repercussions². In brief, these categories often overlap or combine with one another. These diseases are responsible for immense burden on public health systems worldwide, causing approximately 14 million infection-related deaths per year³. Antimicrobials showing antibacterial activity are more specifically referred to as antibiotics. A comprehensive study published in 2022 estimated that, in 2019, infections caused by bacteria—both those susceptible and resistant to antibiotics—were associated with 7.7 million deaths, representing 13.6% of all deaths worldwide³.

3.1.1 *Gram-negative and multidrug-resistant bacteria*

Bacteria can be grouped into two structural subclasses, Gram-positive or Gram-negative, differentiated through the Gram staining method⁴. The bacterial cell wall of Gram-negative bacteria consists of a thin peptidoglycan layer encased by an outer membrane containing lipopolysaccharides, thereby differentiating them from Gram-positive bacteria. The structural difference between these two subclasses results in different penetration of antibiotics into the bacterial cell wall and plays an essential role in the treatment of bacterial infections. Multidrug-resistant (MDR) bacteria, defined as bacteria non-susceptible to one or more antibiotics in at least three categories of antibiotics⁵, pose a major global threat contributing to approximately five million deaths per year⁶. According to the Review on Antimicrobial Resistance, commissioned by the UK Government, Antimicrobial Resistance (AMR) could arguably be the cause of 10 million deaths per year until 2050⁷.

In addition to the intrinsic or natural resistance of some species to selective antibiotics, bacteria are capable of acquiring or developing resistance through various mechanisms. Resistance is generally acquired through horizontal gene transfer or developed through mutations under

selection pressure due to the presence of a drug. Drug resistance mechanisms can be categorized into three main types: drug uptake limitation, drug target modification and drug inactivation⁸. Drug uptake can be limited by reducing the number of porins or altering their selectivity, changing cell wall polarity, as well as through efflux transporters^{9–12}. Examples of drug target modification include altered cell wall properties, ribosomal mutations, and modifications of gene processing enzymes^{13–15}. Finally, enzyme-catalysed drug inactivation is another significant mechanism, where antibiotics are structurally modified, resulting in the loss of their activity^{16–18}.

Contributing to the AMR challenges, many pharmaceutical companies have discontinued their antibiotic drug development programs due to low profit margins. As a result, the outlook of new antibiotics being approved for therapy in the near future is not promising. Global antibiotic shortages are frequent and arise from various causes, predominantly driven by supply chain issues with raw materials, dependence on single-source manufacturers, financial disincentives, and regulatory challenges¹⁹.

Klebsiella pneumoniae (*K. pneumoniae*) and *Escherichia coli* (*E. coli*) are Gram-negative species within the *Enterobacteriaceae* family, which is part of the *Enterobacterales* order. As shown in **Fig. 1**, antibiotic resistant *K. pneumoniae* and *E. coli* represent two of the deadliest pathogens worldwide, associated with more than 1.5 million deaths in the year 2019⁶. The most severe resistance mechanisms identified within *Enterobacterales* spp. are attributed to the expression

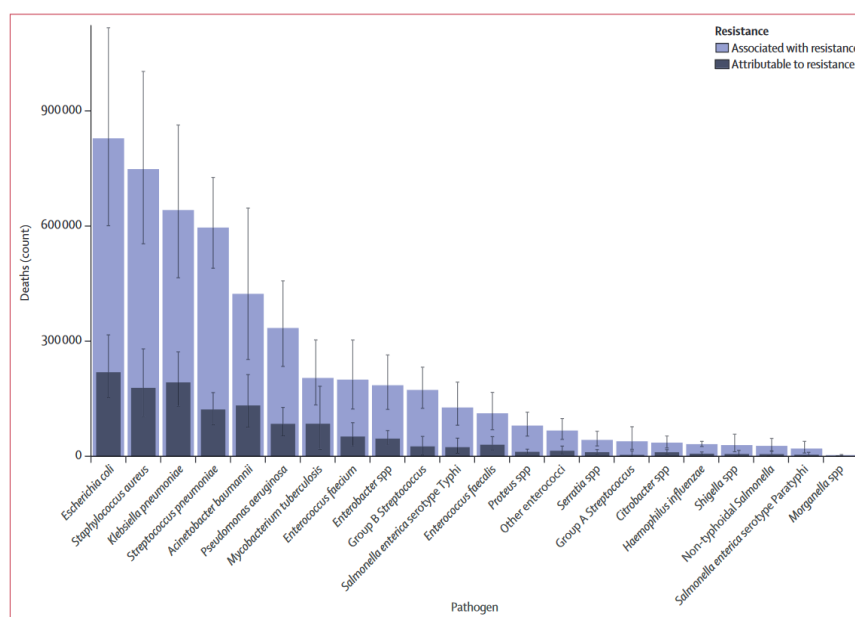


Figure 1 Global deaths (counts) attributable to and associated with bacterial antimicrobial resistance by pathogen, 2019. Estimates were aggregated across drugs, accounting for the co-occurrence of resistance to multiple drugs. Error bars show 95% uncertainty intervals. Reprinted from Murray *et al.* with permission.

of extended spectrum beta-lactamases (ESBLs), which confer resistance to third-generation cephalosporins, and carbapenemases, which lead to resistance against carbapenems. Consequently, ESBL-producing and carbapenem-resistant *Enterobacterales* (ESBL-E and CRE) are also included in the 'WHO

Bacterial Priority List' under the critical group, representing the highest and most challenging category within AMR²⁰.

3.1.2 Antibiotic therapy

Antibiotics, following the direct-acting small molecule paradigm, are used for more than a century in the treatment of bacterial infections. The first substance with antibiotic properties was reported by Bartolomeo Gosio in 1893²¹. In 1928, the discovery of penicillin by Sir Alexander Fleming completely transformed medical practice, and still ranks as the most effective life-saving intervention in medicine, significantly reducing mortality and saving thousands of lives across all age groups²². In the early days of antibiotics, drugs were isolated from fungi that produced them. Today, drug development has progressed to involve structurally modifying drug molecules to bind to targets with high specificity complemented with various other methods in testing their activity. Depending on their mechanism of action, antibiotics inhibit bacterial reproduction (bacteriostatic) or actively kill bacteria (bactericidal). Moreover, antibiotics can be categorized into classes depending on their mechanism of action: Inhibition of cell wall synthesis, inhibition of protein synthesis, depolarization of cell membrane, inhibition of nucleic acid synthesis and inhibition of metabolic pathways⁸.

In microbiology the Minimum Inhibitory Concentration (MIC) refers to the lowest concentration of an antimicrobial agent that inhibits the visible growth of a microorganism. The MIC is a crucial measurement that helps to determine the effectiveness of an antimicrobial agent, such as an antibiotic, against a specific microorganism. Different MIC determination methods were established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) in complete agreement with the recommendations of the International Standards Organisation²³. The EUCAST routinely updates and releases what are known as breakpoint tables for interpreting MIC values. These tables define when pathogens are classified as 'susceptible' (S), 'susceptible, increased exposure' (I), and 'resistant' (R; SIR classification)²⁴. After conducting so called antibiograms of multiple antibiotics against the respective pathogen, clinicians use MIC values and the SIR classification to choose the most effective antibiotic for treating an infection, ensuring that the selected drug can inhibit the growth of the pathogen at achievable concentrations in the patient's body.

However, MIC values do not consider dynamic factors such as drug absorption, distribution, metabolism, and elimination in the body. As a result, MIC-based treatments may not always correlate with clinical outcomes. Harry Eagle was the first to investigate pharmacokinetic/pharmacodynamic (PK/PD) principles for antibiotics during the 1940s and 1950s. Using animal models, they discovered the time-dependent bactericidal activity of penicillin, the concentration-dependent activity of streptomycin and bacitracin, and a mixed pattern for tetracyclines. These insights were applied in clinical settings, revealing that continuous penicillin infusion resulted in the fastest cures while minimizing drug-related toxicities^{25,26}. Based on this groundwork, the concept of the PK/PD indices (PDI) was developed, which have allowed a better understanding of why a particular dosing regimen results in clinical success or failure^{27,28}. By integrating two aspects, the unbound concentration-time profiles (exposure), and the observed antibacterial effect as tested MIC values, these indices help maximising therapeutic outcomes while minimising toxicity. The unbound drug concentration is the portion of the drug in the plasma that is not bound to proteins and is free to exert therapeutic effects, as only unbound drugs can interact with target sites in the body. The most common PK/PD indices include: i. The percentage of a 24-h time period that the unbound or free drug concentration (indicated by ' f ') exceeds the MIC (time-dependent; $fT_{>MIC}$), ii. The maximal unbound drug concentration (concentration-dependent; $\frac{fC_{max}}{MIC}$) and iii. the area under the unbound drug concentration-time curve within 24 h of treatment (time- and concentration-dependent; $\frac{fAUC_{0-24}}{MIC}$). Non-linear least-squares regression analysis is used to identify which PDI correlates best with a specific antibiotic or even class of antibiotics and therefore help to define a target. For example, the effects of beta-lactams are known to be time-dependent and therefore correlate best with $fT_{>MIC}$, which is why these antibiotics are recommended to be administered as prolonged or continuous infusions whenever possible^{29,30}.

Due to the AMR situation worsening over the last few decades, the World Health Organisation (WHO) classified antibiotics for evaluation and monitored use into three groups: 'Access', 'Watch' and 'Reserve' ('AWaRe')³¹. These regularly published 'AWaRe' classifications help healthcare providers to rationalize the use of antibiotics by avoiding the unnecessary use of last-resort antibiotics ('Access' and 'Watch') and ensuring that the most appropriate and effective treatments are used based on the severity and resistance patterns of the infecting pathogen. By adhering to these classifications, healthcare providers can minimize the development and

spread of antimicrobial resistance, preserve the efficacy of existing treatments, and improve patient outcomes.

The antibiotics investigated in this work are introduced in the following sections, with five out of six being marketed last-resort antibiotics that are crucial for combating MDR Gram-negative bacteria. They were investigated in both mono and combination therapies applying *in vitro* and *in silico* methods, aiming to identify rational treatment strategies and enhance our understanding of drug interactions.

3.1.2.1 Meropenem

Meropenem is a bactericidal beta-lactam antibiotic of carbapenem-type which penetrates the outer membrane of the bacterial cell wall and inhibits the peptidoglycan synthesis by covalently binding to penicillin-binding proteins (PBPs) with high affinity. It binds to several PBPs simultaneously with different affinities depending on the species of bacteria³². Meropenem distributes well in interstitial fluids with enhanced cerebrospinal fluid penetration during meningitis, and exhibits variable concentrations across lung, bile, and abdominal tissues³³. According to the mentioned WHO 'AWaRe' classification, meropenem is categorized under 'Watch,' designating it as one of the highest priority agents in human medicine with a significant potential for resistance³¹. Moreover, it serves as the first-line treatment for any infections outside the urinary tract caused by ESBL-E³⁴, and, when combined with the carbapenemase inhibitor vaborbactam, it is also one of the first-line treatments against CRE³⁰. Resistant bacteria harbouring *Klebsiella pneumoniae* carbapenemases (KPCs) can actively hydrolyse meropenem, causing it to lose its effectiveness. It is regularly given intravenously (IV) with 1 or 2 g every 8 h as standard infusion (i. e. over 30 min). Meropenem has a negligible plasma protein binding (PPB) of approx. 2%³⁵. The EUCAST sets the clinical breakpoint for meropenem against Enterobacterales at an MIC of ≤ 2 mg/L for susceptible pathogens.

3.1.2.2 Ceftazidime/avibactam

Ceftazidime is a bactericidal beta-lactam antibiotic of the 3rd generation cephalosporins which penetrates the outer membrane of the bacterial cell wall and inhibits the peptidoglycan synthesis by covalently binding to penicillin-binding proteins with high affinity³⁶. Through expressing ESBL, bacteria achieve the ability to hydrolyse 3rd generation cephalosporins as ceftazidime³⁷. To overcome these resistance mechanisms, a combination therapy was developed using a fixed beta-lactam and beta-lactamase inhibitor — ceftazidime paired with a novel beta-lactamase inhibitor, avibactam. Against *Enterobacterales*, the antibacterial spectrum of

ceftazidime/avibactam is the broadest of the antibiotics available to date³⁸, while resistances against this combination have already been reported³⁹. According to the WHO classification, ceftazidime/avibactam is listed under the last category 'Reserve'³¹, and serves as another first-line treatment for any infections caused by ESBL-E and complicated urinary tract infections (UTIs) caused by CRE. It is given IV as 2 g/0.5 g every 8 h infused over 3 h³⁰. The EUCAST sets the clinical breakpoint for ceftazidime/avibactam against Enterobacterales at an MIC of ≤ 8 mg/L for susceptible pathogens.

3.1.2.3 Fosfomycin

Fosfomycin is an antibiotic with low molecular weight inhibiting the cell wall synthesis of bacteria. In order to exert its bactericidal activity, fosfomycin must reach the bacterial cytoplasm. To enter the cell, fosfomycin uses the active transport proteins GlpT and UhpT by mimicking both glucose-6-P (G6P) and glycerol-3-P (G3P). Thus, fosfomycin can be imported into the bacterial cell via the hexose monophosphate transport system (which is induced by G6P) and via the L-alpha-glycerophosphate transport system (which is induced by G3P). Given as a flat dose of 3 g, it has been used against UTIs for decades^{40–42}. Fosfomycin exhibits minimal binding to plasma proteins⁴³, and is widely distributed across various tissues. Biologically relevant concentrations of fosfomycin have been detected not only in serum but also in the kidneys, bladder, prostate, lungs, bone, cerebrospinal fluid, as well as in inflamed tissues and abscess fluid^{40,44,45}. Fosfomycin IV is a safe and effective option for the treatment of difficult-to-treat infections, categorized by the WHO under 'Reserve' group. Due to its wide distribution across tissues, it showed highest clinical success rates in osteomyelitis, skin and soft tissue infections, intra-abdominal infections and sepsis⁴⁶. Nevertheless, fosfomycin is not recommended to be used alone⁴⁷, in combination with i. e. ceftazidime/avibactam, colistin and other antibiotics, it showed synergy with potentially better clinical outcomes^{46,48–50}. Based on the kidney function of the patients, the resistance of the infecting pathogen and the severity of the infectious disease, fosfomycin IV is dosed with 16 – 24 g divided into 3 – 4 doses. In the past, the clinical breakpoint of fosfomycin against *Enterobacterales* was based on the epidemiological cut-off value, which separated resistant ('R') from susceptible ('S') pathogens with a value of MIC > 32 mg/L. Since the beginning of 2024, EUCAST has withdrawn this clinical breakpoint with the note 'There is currently a lack of clinical evidence to support clinical breakpoints.'

3.1.2.4 Tigecycline

Tigecycline is an antibiotic of the glycylcycline class, which are derivatives of the tetracyclines. In order to exert its bacteriostatic effect, tigecycline binds to the 30S subunit of bacterial ribosomes. The binding of tigecycline prevents the incorporation of amino acid residues into the elongation of peptide chains and results in the loss of peptide formation and bacterial growth. Tigecycline was developed to overcome the main molecular mechanisms of tetracycline resistance, such as tetracycline-specific efflux pump acquisition [e.g. tet(A)] and ribosomal protection [e.g. tet(M)]^{51,52}. After administration of ¹⁴C-labeled tigecycline to rats, tigecycline tissue levels, with the highest concentrations in bone, liver, spleen, and kidney, exceeded those in plasma⁵³. Due to reports of higher mortality rates in tigecycline treatment arms within clinical studies, the US Food and Drug Administration (FDA) issued a black box warning and illustrated that treatment with tigecycline increased the risk of death in FDA-approved and non-approved diseases^{51,54}. Nevertheless, tigecycline is categorized by the WHO under 'Reserve' and represents an alternative treatment option for infections outside the bloodstream and urinary tract caused by CRE, including those with NDM and OXA-48-like producers, especially when beta-lactams are ineffective or not tolerated³⁰. It is dosed with an initial dose of 200 mg IV followed by 100 mg every 12 h, while some reports recommended an 8-hrly dosing for higher effectiveness against CRE⁵⁵. The EUCAST sets the clinical breakpoint for tigecycline against Enterobacterales at an MIC of ≤ 0.5 mg/L for susceptible pathogens.

3.1.2.5 Polymyxin B

Polymyxin B is an antibiotic from the polymyxin class, known for its bactericidal effect against Gram-negative bacteria. It functions by interacting with the bacterial cell membrane, disrupting its integrity, which leads to cell death. Polymyxin B's primary mechanism involves binding to the lipopolysaccharides in the outer membrane of Gram-negative bacteria, which increases the permeability of the membrane and subsequently causes leakage of cellular contents and bacterial cell death⁵⁶. It was developed to combat MDR infections, particularly those resistant to other antibiotic classes. PK studies in animal models have shown that polymyxin B distributes well in tissues, with high concentrations found in organs such as the liver and kidneys⁵⁷. Although effective, the use of polymyxin B is associated with potential nephrotoxicity and neurotoxicity, which limits its use to severe infections where alternatives are not available or effective^{58,59}. Despite these concerns, polymyxin B is categorized by the WHO under the 'Reserve' group, serving as a last-resort treatment for life-threatening infections caused by carbapenem-

resistant *Acinetobacter baumannii* and difficult to treat *Pseudomonas aeruginosa*³⁰. The dosing of polymyxin B varies depending on the severity of the infection, but it is typically administered IV with cautious monitoring of patients' kidney function. The EUCAST only provides a clinical breakpoint as a screening cut-off value for phenotypic resistance, defining *Enterobacterales* with a MIC $\leq$ 2 mg/L as susceptible.

3.1.3 Bacteriophage therapy

Bacteriophages (phages) are viruses capable of binding to surface structures of bacteria, thereby infecting their host and replicating themselves. Lytic phages, also known as virulent phages, rapidly induce the replication and lysis processes after infecting their hosts⁶⁰. They possess a simple structure, typically comprising a protein coat encapsulating their genetic material, which can be either DNA or RNA⁶¹. Due to the mechanism of action of their polysaccharide depolymerase enzymes, phages are typically specific for one bacterial strain and its capsule type⁶².

In the context of antimicrobial therapy, phage therapy offers a promising non-traditional alternative, particularly when effective treatment options are limited, bringing renewed attention to this previously common approach⁶³. Phage therapy has been practiced for almost a century in Eastern Europe⁶⁴, although there are only a few therapies approved relative to traditional antibiotics. The principles of pharmacology are not directly applicable to phages, as phages replicate in the presence of their target while simultaneously acting as an anti-infective agent. Additionally, new phages can be isolated and specifically cultivated against particular pathogens, resulting in a narrow antimicrobial spectrum. This specificity presents a disadvantage, as the success of individual phage therapies cannot be easily translated to real-world scenarios that involve biological variability among infecting pathogens. Furthermore, bacteria typically develop resistance to phages rapidly. Therefore, conducting systematic studies on killing kinetics is essential to understand the relationships among multiplicity of infection (MOI), phage efficacy, and the emergence of resistance⁶⁵. Studies investigating the efficacy and the safety of phage therapy showed that a significant percentage of patients with chronic bacterial infections were able to tolerate phage therapy, yielding good clinical outcomes^{66,67}.

In order to apply phage therapy in a clinical setting, presumptive therapy plays an important role. Presumptive phage therapy, also known as non-personalised phage therapy, is an empirical therapy involving initiating treatment based on a clinical educated guess of the organism

responsible for the infection and its likely susceptibility to phages from a pre-prepared bank, even before laboratory results are available to confirm the exact pathogen and its sensitivities. On the other hand, there is the approach of personalised phage therapy, where phages from a pre-existing library are adapted or trained to the individual pathogen infecting the patient. In this approach phage mutants with an increased range and duration of infectivity are selected *in vitro*⁶⁸. Unfortunately, recent randomized controlled trials using non-personalised phage therapy have failed to demonstrate efficacy⁶⁹. However, personalised phage therapy showed promising results in some cases⁷⁰.

3.1.4 Combination therapy

3.1.4.1 Antimicrobial combination therapy

Antimicrobial combination therapy in principle may offer an opportunity to increase efficacy, protect against the emergence of resistance and in turn potentially even lower infection-related mortality^{71,72}. In empirical therapy, combination treatments can also broaden the antimicrobial spectrum and reduce the risk of inadequate antibiotic coverage⁷². When considering the worsening AMR situation, this underscores the necessity of using already marketed antibiotics with special precaution and as effectively as possible to potentially extend their shelf-lives⁷³. *In vitro* models can be used to identify effective combinations, which can subsequently be validated through animal or clinical studies. When two or more antibiotics are used together, they can demonstrate synergistic effects, meaning their combined effect is greater than the sum of their individual effects⁷⁴. This occurs because the antibiotics might target different mechanisms within the bacteria, leading to a more effective eradication⁷⁵. Also, the suppression of developing resistance is discussed as another potential benefit to combination therapy. One mechanism trying to describe this principle is collateral sensitivity. Evolutionary trade-offs between antimicrobial agents occur when bacteria adapt to different environmental pressures. In the presence of a specific drug, various trade-offs can happen: some mutants may develop resistance to multiple drugs, while others may become more sensitive to a different drug as they gain resistance to the first one^{76,77}.

Another reason supporting antimicrobial combination therapy includes reducing the need for increasing doses of antibiotics that have complex side effects. When exemplifying this with fosfomycin-containing therapies, in addition to potentially achieving high combined effects

through synergy and suppressing resistance, combination therapy could help limit dose escalation, thereby reducing challenging side effects such as hypernatremia.

Antibiotic combination therapy is already well established in some infectious diseases caused by bacteria. Against tuberculosis infections caused by *Mycobacterium tuberculosis*, recommended standard therapies across all age groups and special patient populations are based on combination therapies⁷⁸. In ulcerative infections caused by *Helicobacter pylori*, standard therapy is based on a triple or quadruple combination therapy containing two antibiotics from different classes⁷⁹. Another example is the combination therapy for suspected Gram-negative sepsis and severe infections with *Pseudomonas spp.*, typically including a broad-spectrum beta-lactam and an aminoglycoside or a fluoroquinolone. Moreover, colistin-containing combination therapies started to play an important role as last-resort treatment for MDR bacteria⁷⁴.

In most other infectious diseases, especially those caused by MDR bacteria, combination therapy is not well established and arises out of necessity. Practicing clinicians have limited time in deciding how to deal with difficult to treat infections in patients in severe health condition. Therefore, the choice of which antibiotics to combine is often based on empirical observations rather than a rational approach. Understanding PD interactions between antibiotics is essential for guiding rational combination therapy decisions to maximise efficacy and protect against the emergence of resistance.

3.1.4.2 Phage combination therapy

When using phages as antibacterial agents, one way to overcome their high specificity to a certain bacterial strain and fast resistance development is to combine multiple phages in so called phage cocktails or even combinations of phages and traditional antibiotics⁸⁰. Using phages as a supplement to traditional antibiotics has already shown potential benefits and synergy in phage-antibiotic combination therapy was reported in the literature^{81–83}. A multicentre, multinational, retrospective observational study on personalised phage therapy reported clinical improvement and eradication of the targeted bacteria for 77.2% and 61.3% of infections, respectively⁸⁴. In 100 cases, eradication was 70% less probable when no concomitant antibiotics were used. In one patient a combination of antibiotic re-sensitization to fluoroquinolones and reduced virulence of phage-resistant isolates were reported. Another study investigated IV phage therapy combined with three last-resort antipseudomonal antibiotics in a toddler with post-transplantation sepsis. After further *in vitro* testing with the

infecting pathogen, the study reported synergy between the phage and colistin, gentamicin or aztreonam⁸⁵.

3.2 *In vitro* experimentation and infection models

The 'scientific method', pioneered in the 11th century by Al-Hasan Ibn al-Haytham, also known as Alhazen, in his 'Book of Optics' (transl. '*Kitāb al-Manādhir*')⁸⁶, is a structured process of formulating hypotheses and testing them through repeatable and objective experiments under controlled conditions. Later, the 'scientific method' was further formulated by pioneers like Galileo and Bacon⁸⁷. This method has since then allowed us to explore the physical world, biological systems, and cosmic phenomena through systematic experimentation. *In vitro* (from latin: 'within glass') experimentation allows testing of drugs under highly controlled conditions with precise observations of biological processes without the need of involving animals or humans. In the context of antibiotics, *in vitro* experiments are fundamental for assessing how well a compound inhibits the growth of microorganism under controlled laboratory conditions. These experiments are typically standardised and validated into assays with feasible methods, and they are conducted in every hospital to characterize the pathogens causing infections, consequently influencing clinical treatment decisions.

3.2.1 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing is an *in vitro* microbiological laboratory procedure to determine the effectivity of an antimicrobial against a specific pathogen. In the case of bacteria, the MIC can be determined using different methods according to the Clinical and Laboratory Standards Institute (CLSI) and the EUCAST. The CLSI defines a framework to internationally standardize antimicrobial susceptibility testing across the following methods: Broth Microdilution, Agar Dilution, Disk Diffusion and E-Test⁸⁸. The relevant methods included in this work are Broth Microdilution assay and Agar Dilution assay. Briefly, these assays are performed using either freshly prepared growth media in well-plates, or agar, supplemented with the antibiotic of interest at different 2-fold concentration levels. The prepared well plates or agar plates are spiked with a bacterial inoculum containing a defined number of colony-forming units per mL (CFU/mL), followed by incubation at 35 – 37 °C ambient air for 16–20 h. After assuring sterility and comparing to positive growth controls, the MIC is read visually. For this, each

prepared well is visually inspected for turbidity. The lowest concentration that results in a clear well, indicating that bacterial growth has been inhibited, represents the MIC. In this thesis, MIC testing was conducted for each bacterial strain that was included in the subsequent research activities.

3.2.2 Checkerboard assays

Conventional checkerboard assays are *in vitro* assays testing the combination of two (or more) antibiotics at the same time to identify PD interactions, e. g. synergy, antagonism, indifference. Methodologically they can also be understood as an extension of Broth Microdilution MIC testing with an added dimension with raising concentrations of two antibiotics on two axes instead of one. In this method, varying concentrations of both antibiotics are combined in a grid pattern on a microtiter plate. After adding a distinct amount of bacterial inoculum to each well and incubating overnight, the growth is assessed visually following the visually read turbidity, similar to MIC testing. The following assessment of PD interactions is often based on the calculation of the Fractional Inhibitory Concentration Index (FICI)⁸⁹. The FICI is calculated by dividing the MIC derived in combination (A_{comb} , B_{comb}) by the MIC of the individual agent alone (MIC_A , MIC_B) and is used to reflect type and degree of PD interaction between the drugs:

$$FICI = \frac{A_{comb}}{MIC_A} + \frac{B_{comb}}{MIC_B} = FIC_A + FIC_B \quad (\text{Eq. 1})$$

There are different ways to interpret which of the many combination wells are to be considered in calculating the final FICI. Usually, a FICI value of ≤ 0.5 indicates synergy, a value between 0.5 and 1.0 suggests an additive effect, values from 1.0 to 4.0 imply indifference, and a value > 4.0 denotes antagonism. There are multiple limitations to this method: First of all, turbidity is based on what the naked eye or spectrophotometers ($\lambda = 600 \text{ nm}$) can detect, usually resulting in a lower limit of quantification of $> 10^7 \text{ CFU/mL}$ ⁹⁰. Secondly, checkerboards have a predefined, single end-point, overlooking the dynamics of bacterial growth and antibacterial killing of drugs over time. Consequently, PD interactions derived from FICI values based on conventional checkerboards may potentially lead to an imprecise or incomplete understanding of the synergistic, additive, or antagonistic effects of drug combinations over time.

When investigating the PD interactions of ceftazidime/avibactam and tigecycline in this work, ‘dynamic checkerboard assays’ were performed⁹⁰. These assays were an extension of classical checkerboard assays, but with a total volume of 10 mL, quantification of bacteria at the endpoint

of 24 h, and informative combination concentrations based on the potency of the drugs in the respective strain⁹¹.

3.2.3 Static time-kill assays

Static time-kill assays are *in vitro* assays evaluating the antibacterial effect of drugs by measuring the reduction of the bacterial load over time at constant drug concentrations in growth media. Through sampling the bacterial load at multiple time points they allow a time-dissolved observation of bacterial growth and antibacterial killing effects. This quantitative data can be used to categorize killing effects into non-effective, bacteriostatic, and bactericidal, and to assess the potency (EC_{50}) and maximum effect (E_{max}) of antibiotics against specific bacterial strains. Additionally, when combining different antibiotics in these assays, effect size comparison between antibiotics and assessment on PD interactions of their combination becomes possible. Furthermore, time-kill assays are a cornerstone of the *in vitro* investigation of antimicrobials, often guiding the following *in vivo* animal model experiments. The European Medicines Agency (EMA) mentions in its guideline on the development of antimicrobial medicinal products that time-kill studies should be conducted to obtain preliminary information on the relationship between drug concentrations and antimicrobial effects⁹².

Within the scope of this work, time-kill assays were employed to evaluate single and combination effects of antibiotics and their PD interactions, specifically those of ceftazidime/avibactam-tigecycline, meropenem-fosfomycin and polymyxin B-phage pK8 against MDR *K. pneumoniae* and *E. coli* strains. For each antibiotic, and their combinations multiple concentrations were tested. Additionally, in time-kill assays conducted with meropenem, non-enzymatic and enzyme-induced degradation of meropenem was evaluated for later inclusion in pharmacometric analyses.

3.2.4 Hollow Fibre Infection Model

The Hollow Fibre Infection Model (HFIM) is an *in vitro* infection model used in preclinical studies to evaluate the effect of antimicrobials with defined concentration-time profiles against bacterial pathogens. The HFIM was first described and used by Blaser and Zinner for bacterial testing in the 1980s and by Bilello *et al.* for anti-HIV drugs in the 1990s^{93,94}. Furthermore, the EMA

has organized a workshop in 2015, emphasizing on the HFIM as a method to identify PK/PD indices⁹⁵.

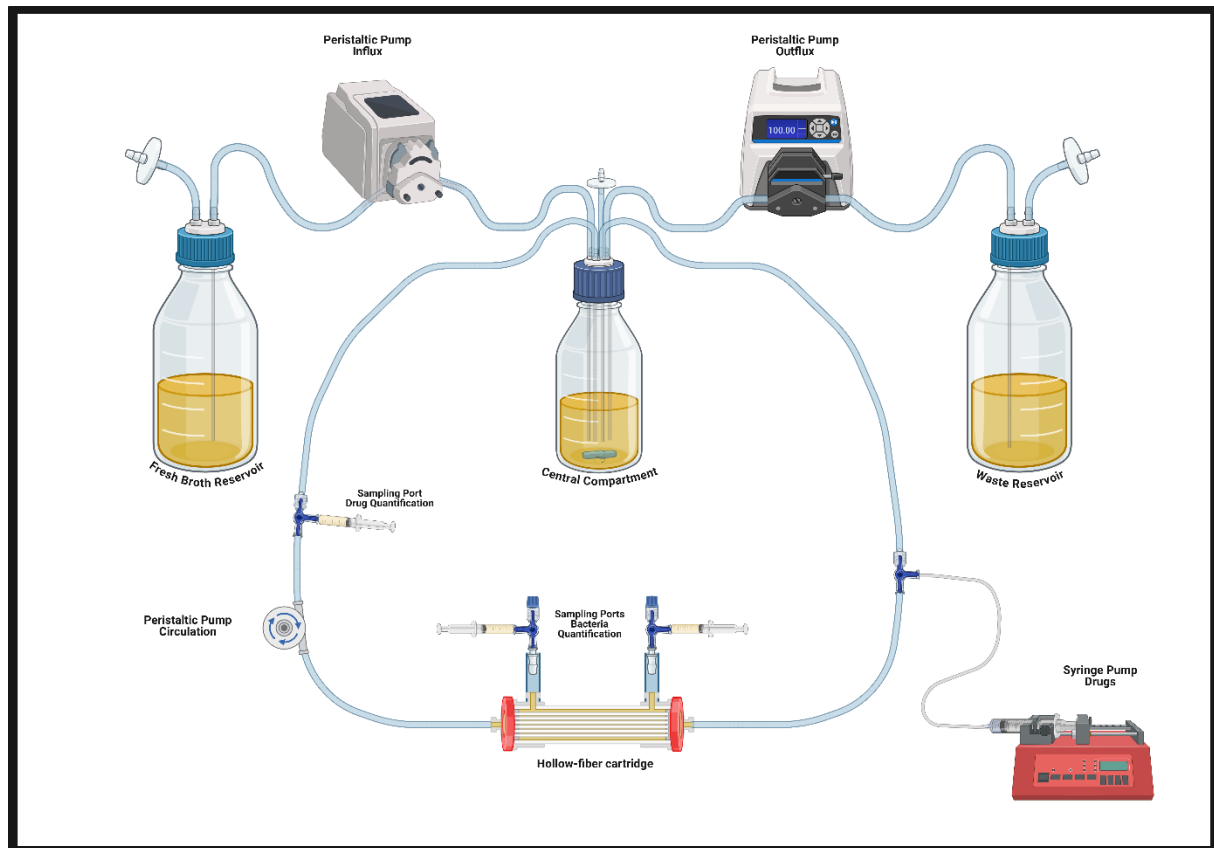


Figure 2 Schematic setup of a Hollow Fibre Infection Model for the *in vitro* testing of single antibiotics or combinations of antibiotics illustrating the key components, including central compartment, broth and waste reservoirs, peristaltic pumps transporting broth, syringe pump for drug infusions, sampling ports, and the hollow fibre cartridge containing bacteria entrapped in the extra capillary space. Constant influx of fresh and drug-free broth enables optimal growth conditions for bacteria over a period of multiple days and the programmable syringe pump allows for mimicking human concentration-time profiles of antibiotics. Created in BioRender. Farooq, A. (2025) <https://BioRender.com/e28m303>.

Typical human PK profiles of one or multiple antimicrobials, usually derived from population PK models, can be mimicked by using programmable peristaltic pumps. These peristaltic pumps allow to simulate drug half-times through precisely calculated dilution pump rates, for both influx and outflux of medium. At the same time, the bacterial pathogen of interest is inoculated within the so-called Hollow Fibre Cartridge. Inside this cartridge there are densely packed semi-permeable hollow fibres with bacteria located in the extracapillary space and broth being pumped through the intrapapillary space. The large surface area of the fibres facilitates the exchange of dissolved nutrients, oxygen, metabolic waste products, and antibiotics. This enables bacteria to grow in an idealized environment for multiple days or even weeks while being exposed to the antibiotic with the underlying PK profile of interest.

Fig. 2 shows the schematic setup of a HFIM with all instruments involved. All components of the HFIM have to be sterilized prior to their setup under an aseptic safety cabinet to avoid microbiological contaminations. The central compartment of the HFIM is filled with a defined amount of constantly stirred, sterile medium and is connected via tubing to two cycles simultaneously. The first being the influx-outflux-cycle (left to right), allowing the influx of fresh and drug-free growth medium with a calculated and set pump rate, and the outflux of used medium. Secondly, the cycle connecting the central compartment to the Hollow Fibre Cartridge using a third peristaltic pump, thereby enabling homogenous distribution of medium containing antibiotic, nutrients and oxygen throughout the whole HFIM (circular). A programmable syringe pump is connected to the cycle via a separate port located before the medium enters the central compartment, making multiple infusions of antibiotics into the system feasible and ensuring system equilibration before being pumped into the bacteria-containing cartridge. Such an automated setup allows to mimic diverse dosing intervals of antibiotics, with dosing events happening outside of usual working hours, overnight for example. Also, the infusion time can be regulated and adjusted to the common clinical situation. Additionally, sampling ports are installed to the bacteria-free system and the extracapillary space of the Hollow Fibre Cartridge for later quantification of antibiotics (PK; exposure) and bacterial burden (PD; response) over time. The HFIM system is usually placed inside a non-sterile incubator with 37 °C ambient air, hence any pressure-regulating ports at the reservoirs and central compartment are sealed with a sterile filter (0.22 µm). Testing of combination therapies of two antibiotics with similar half-lives can simply be performed by simultaneous infusion of both antibiotics with the same syringe and infusion rate at planned dosing times.

Comparing the HFIM with time-kill assays with regard to testing of antibiotics reveals certain key benefits: The HFIM allows for testing of dynamic concentration profiles over time, mimicking typical human PK profiles, PK profiles of special patient populations, or even PK profiles of specific tissues if feasible. This advantage allows to derive PK/PD indices from HFIM studies (i. e. short-infusion vs. long-infusion of the same antibiotic and dose) without the initial need of *in vivo* animal infection models. Further, the HFIM can be used to study antibiotics over days to weeks, closer simulating the real-life situation of infectious diseases. Lastly, a large volume capacity compared to time-kill assays (up to 50- fold) allows for studying emergence of resistance with extended study durations without altering conditions significantly. On the other hand, when comparing the HFIM with *in vivo* animal infection models or clinical studies, limitations

are also revealed: There is no immune system or physiology in the HFIM and you cannot look at distribution processes. Also, due to its *in vitro* nature, the HFIM does not allow observations like those conducted in animal studies, such as toxicological assessments.

This work utilized the HFIM to simulate human PK profiles, derived from population PK models, to demonstrate and confirm synergistic effects of the combinations of meropenem and fosfomycin in killing MDR *K. pneumoniae*. The experiments were conducted following the described setup and with a total duration of 72 h for each experiment.

3.3 Pharmacometric Modelling & Simulations

Pharmacometrics, a term first coined by the editors of the Journal of Pharmacokinetics and Biopharmaceutics in 1982, refers to the formalization of pharmacokinetic–pharmacodynamic modelling into a scientific concept⁹⁶. In its simplest sense, pharmacometrics is the science of quantitative pharmacology⁹⁷. However, it has been more formally defined by Paul Williams and Ene Ette as ‘the science of developing and applying mathematical and statistical methods to characterize, understand, and predict a drug's pharmacokinetic, pharmacodynamic, and biomarker-outcomes behavior.’⁹⁸. Pharmacometrics is positioned at the intersection of PK models, PD models, statistics, stochastic simulation, computer programming and data visualization. As the science of drug development becomes less empirical and more quantitative in nature, the role of pharmacometrics in drug development is becoming increasingly important. Following this, it's essential to note that during the drug development and approval processes, agencies like the FDA and EMA require pharmacometric modelling and simulation analyses, often including *in vitro-in vivo* translation, first-in-human dose predictions, population PK-PD modelling, and clinical trial simulations.

In contrast to non-compartmental analysis (NCA), pharmacometrics uses compartmental models to describe and predict drug behaviour and drug response, providing a more detailed understanding of PK and PD⁹⁹. A pharmacometric model tries to describe a system by employing equations and statistical methods to represent and analyse biological and pharmacological processes of systems. Systems of interest can be *in vitro* laboratory experiments, living organisms as animal models and humans. The process of developing such mathematical models relies on the developers understanding of the system of interest, meaning how the operator thinks the data was created by the system. When conceptually trying to define a mathematical

model, it is helpful to segregate the model into components. Models are built on experimentally derived data, which in its nature is dependent on system inputs, system dynamics, and the instrument used to measure the output of the system⁹⁷. Errors in data collection occur through the measurement error of the instrument but also include process noise that might be part of the systems data output. For differentiating the systemic component of interest from the error, models usually consist of a structural model (systematic component) and a statistical model that describes the variability and error component of the model. The population approach analyses data from groups of individuals or experiments simultaneously and captures the variability component in drug behaviour and response. For animals and humans, this variability is often referred to as inter-individual variability or between-subject variability, whereas in the case of experiments, it is known as inter-experimental variability. It refers to the differences in characteristics and responses observed among individuals within a population, which can result from genetic, physiological, environmental, or lifestyle factors. An example of inter-experimental variability is the differences observed in the resistance rate constant of the same bacterium when tested under drug exposure across different experiments. After accounting for biological variability, there remains a noise or error of the method capturing the data, often referred to as residual variability. This error is often also referred to as residual variability.

Pharmacometric modelling and simulations provides a robust framework for integrating and quantitatively analysing complex data to enhance different aspects of drug development across all phases. Applications of pharmacometric models include *in silico* prediction of chemical properties, evaluation of toxicity, and analysis of structure-activity relationships during the discovery phase. In preclinical development empirical or mechanistic PK/PD models help in predicting the dose-exposure-response relationship, forecasting drug interactions and selecting doses for first-in-human studies. During clinical development, it serves to characterize PK and PD in humans, identify covariates affecting PK/PD, explore differences in effect and safety in tested and simulated dosing regimens, and inform clinical trial design. In the post-marketing phase, it can help to personalise therapy and predict activity in new populations. There are many other applications and only a few are given here.

In this work pharmacometric modelling and simulations was performed to analyse preclinical data from *in vitro* model experiments.

3.3.1 Pharmacokinetics

Edward R. Garrett defined PK as ‘[...] the study of the time courses of absorption, distribution, metabolism, and excretion of drug in the intact total organism’¹⁰⁰. PK in its essence is ‘what the body does to the drug’ after application of the drug using a specific administration route. PK is a discipline trying to describe the absorption, distribution, metabolism and excretion, often referred to as ‘ADME’, of a drug in the body and to reduce a concentration–time profile into a set of parameters that could be used for comparison, evaluation, and prediction⁹⁷. When trying to define a set of parameters describing PK, there are generally two different methods to use: NCA or compartmental analysis (also: regression analysis). The strengths of regression analysis correspond to the weaknesses of the non-compartmental approach, and vice versa. NCA does not necessitate assuming a specific compartmental model for the drug. Instead, it employs the trapezoidal rule to measure the area under the plasma concentration–time curve (**Fig. 3**, AUC). This method, applicable to first-order (linear) models, requires minimal assumptions and can be

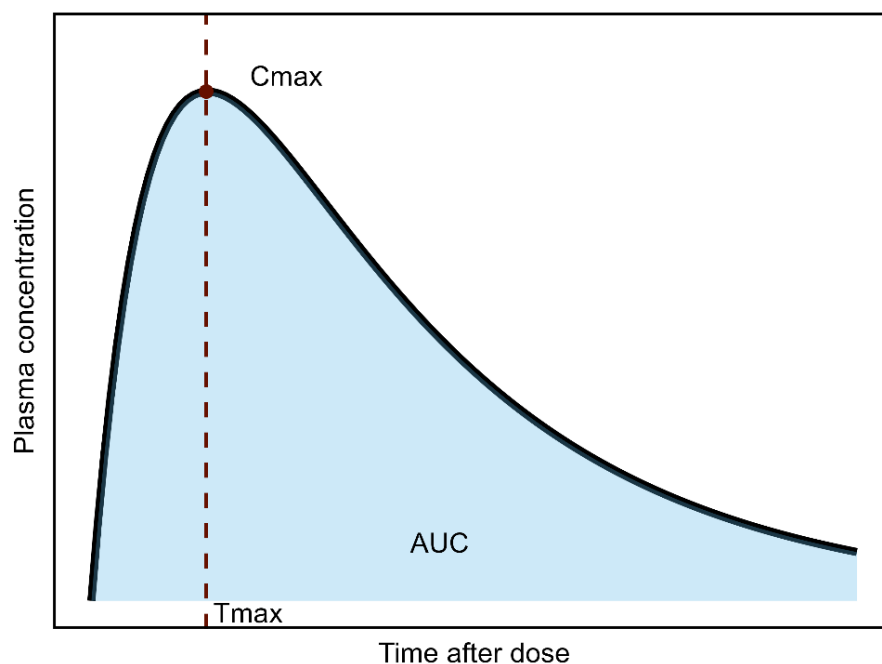


Figure 3 Illustration of a pharmacokinetic (PK) profile after per oral administration of a drug with linear PK. Visualised are secondary parameters which can be derived thereof. Cmax: maximum concentration; Tmax: Time of maximum concentration; AUC: area under the plasma concentration curve.

easily automated. The primary PK parameter of the elimination constant k_e is estimated from the calculation of the slope of the log-transformed concentration-time curve’s terminal phase. Further primary parameters as the clearance (CL) or volume of distribution (V_d) are then calculated using the determined AUC and bioavailability (F) after IV administration^{97,99}:

$$CL = \frac{F \times D}{AUC} \quad (\text{Eq. 2})$$

$$V_d = \frac{D_{IV}}{k_e \times AUC} \quad (\text{Eq. 3})$$

A simplified mathematical representation of the plasma concentration of a drug with linear PK after bolus IV administration can be described as a function of time using a decay equation⁹⁷:

$$c(t) = C_0 \times e^{-k_e \times t} = \frac{D \times F}{V_d} \times e^{-k_e \times t}, \quad (\text{Eq. 4})$$

with $c(t)$ representing the concentration of the drug at time t and the initial concentration C_0 (here also $C_{\max}$). Applying the natural logarithm to this equation reveals its linear character with $\ln(C_0)$ as y-intercept and k_e as slope of the function:

$$\ln[c(t)] = \ln(C_0) - k_e \times t \quad (\text{Eq. 5})$$

Mathematical characterisation of drugs showing more complex behaviour can be performed through utilizing compartmental analysis, such as through the application of non-linear mixed effects models (section 1.3.3). Following the population approach explained in the beginning of this section, population PK models estimate typical PK parameters, such as CL, V_d , volume of second, peripheral compartment (V_p), intercompartmental clearance (Q), etc. Random effects are incorporated to account for different levels of biological variability (inter-individual, within-individual, interoccasion, inter-experimental) observed within the population, and residual variability accounting for the measurement error. Moreover, these models enable the identification of patient-specific covariates (such as bodyweight, renal function, sex) that can explain the variability between individuals, thereby improving our understanding of the PK of a drug⁹⁷. Incorporating random effects into *in vitro* pharmacometric models describing bacterial data enables the characterization of biological variability, including inter-strain, inter-experiment, and potentially other sources of variability.

In vitro experiments with static conditions are usually assumed to have constant concentrations of the tested drug. Processes as non-enzymatic degradation due to thermodynamic effects or enzymatic degradation can significantly decrease drug concentrations in these experiments. Even in such cases PK modelling can be applied. Bioanalytic methods can be coupled with PK modelling to obtain insights on actual drug levels and for the estimation of degradation rate constants. With a more precise characterisation of the experimental conditions any following analyses as on the PD of a drug also become less biased.

This work used population PK models of meropenem and fosfomycin to simulate high-dose regimens in humans. PK of simulated median patients – virtual individuals providing a benchmark for typical drug exposure within the studied population – guided the performed HFIM experiments. Furthermore, in time-kill assays containing meropenem, drug levels were

analysed over time and developed PK/PD models were used to estimate meropenem degradation rate constants.

3.3.2 Pharmacodynamics

The establishment of modern pharmacology as a separate discipline are attributed to Rudolph Buchheim, while the innovation of experimental pharmacology is attributed to Oswald Schmiedeberg, often called as ‘the father of American pharmacology’. In the mid to late 19th century, their work laid the groundwork for pharmacology as a scientific discipline, focusing on the quantifiable effect of drugs on physiological systems¹⁰¹. Later in the 20th century, PK and PD were more clearly separated as distinct, yet connected, areas of study within pharmacology. PD in its essence is ‘what the drug does to the body’ after administration of the drug with a specific dose.

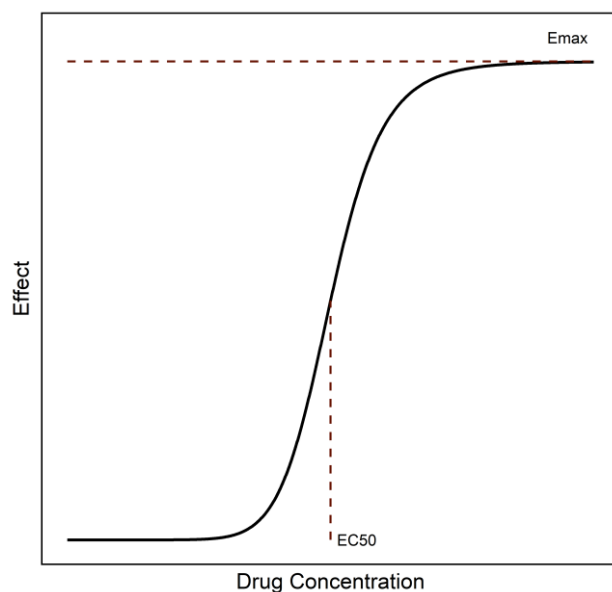


Figure 4 Schematic of sigmoidal concentration-response curve expressed with the sigmoidal E_{max} model.

In the context of antibiotics, PD can refer to different types of observations, also called surrogates, for describing desired effects or undesired toxicity. Some examples for surrogates on effects are the inhibition of bacterial growth or killing effect on bacteria, biomarkers like body temperature in animals or patients, the overall health situation or survival of a population and many more. As described in the earlier sections, *in vitro* experiments with antibiotics usually aim to quantify the PD effect as killing or inhibition of growth of a

bacterial population. This observed PD effect can be characterised as a function of the drug's concentration. Since most drugs follow the fundamental principles of the receptor theory and have saturable targets, effect sizes typically reach an E_{max} when studied over a suitable concentration range¹⁰². Based on the receptor theory and mass balance principles, the E_{max} model, and its extended form, the sigmoidal E_{max} model (**Fig. 4**), are the most commonly used models for describing an observed PD effect $E(c)$:

$$E(c) = \frac{E_{max} \times c^H}{EC_{50}^H + c^H}. \quad (\text{Eq. 6})$$

Here, c is the concentration of the drug, EC_{50} the concentration resulting in a half-maximum effect, and the Hill coefficient H describes the steepness of the concentration-effect relationship.

3.3.3 Nonlinear mixed-effects models

Nonlinear mixed-effects models (NLME) can be used to describe PK, PD, or integrated PK/PD relationships. A model, such as the PK/PD models developed in this work, is considered nonlinear if any of the partial derivatives with respect to its parameters depend on other parameters, or if any of the derivatives are non-existent or discontinuous. One example for a nonlinear model is the aforementioned sigmoidal E_{max} model (Eq. 6), where the concentration c is dependent on the parameters E_{max} and EC_{50} ⁹⁷.

Regression models, in simple words, aim to fit a model to observed data as good as possible. Thereby, an objective function is minimized, which can be as simple as ordinary least squares, or more complex variants of this such as extended least squares¹⁰³.

In the context of data consisting of a population of individuals (experiments, animals, patients), fixed effects are parameters assumed to be constant across all individuals within the given data, representing the structural component of a model, while random effects aim to capture the model's variability components and the error. A given example within this work is the developed PK/PD model describing phage pK8 kinetics and dynamics tested against *K. pneumoniae*. It consists of a number of fixed effects (i. e. bacterial growth rate constant, phage binding kinetics rate constant, maximum bacterial capacity, etc.) and random effects addressing inter-experimental variability (on maximum bacterial capacity), along with an error model that accounts for unexplained residual variability.

Hence, NLME models are regression models that aim to estimate a set of model parameters, both fixed and random, by minimizing the extended least-squares objective function between predicted and observed values using approximation methods. In these models, maximum likelihood estimation (MLE) is utilized because it accommodates model complexity, handles various distributions, provides efficient parameter estimates, and supports robust statistical inference^{104–106}. MLE was first presented by Fisher in the 20th century and represents a cornerstone of today's statistical estimation methods¹⁰⁷. MLE is an estimation method that finds

a set of estimations for model parameters such that the likelihood of actually observing the data is maximal. In complex systems, direct calculation of the likelihood is not practical due to high numbers; therefore, numerical optimization techniques using the log-likelihood are employed to simplify computations and avoid numerical errors. In practice, since optimization routines typically find minima, two times the negative log-likelihood (-2LL) is minimized instead.

By minimizing the differences between observed and predicted values, these models allow to see how accurately different models reflect what's happening in biological and pharmacological systems. Furthermore, through simulations, these models enable the prediction of outcomes under various scenarios, exploration of intervention impacts, and enhanced understanding of complex biological and pharmacological processes, ultimately aiding in model validation and guiding drug development and clinical decision-making.

In the context of this work, NLME models were developed using ordinary differential equation systems and either First-Order Conditional Estimation with interaction in NONMEM® or Stochastic Approximation Expectation-Maximization in Monolix® to conduct -2LL estimations. After developing different PK/PD models, those with the best performance were identified by comparing them on criteria such as biological plausibility, parameter uncertainty, and fit to the observed data, assessed using the Akaike Information Criterion¹⁰⁸.

3.3.4 PK/PD modelling of combination therapies

PK/PD models connect the concentration-time profile (PK) of the drug to its observed effect (PD). This link can be direct or indirect, partially depending on the nature of the PD effect surrogate. Situations where there is a direct link between PK and PD, a decrease of the drug's concentration would result in an immediate reduction of the observed effect⁹⁸. Measuring the PD effect of antibiotics as a decrease of the bacterial population within the infected tissue of patients is usually not feasible, even if some methods are already being investigated^{109,110}. Conversely, for *in vitro* models investigating antibiotic effects against a bacterial population (i. e. time-kill assays) the change of the bacterial burden under antibiotic exposure is precisely captured over time subsequently resulting in a direct link.

When trying to describe the combined effect E_{comb} of two drugs, the most obvious approach is to assume that the effects of the two drugs (E_A , E_B) simply behave additive, resulting in the following equation:

$$E_{comb} = E_A + E_B. \quad (\text{Eq. 7})$$

However, if the effects of drug A and/or B are both near the maximum effect possible, equivalent to eradicating the entire bacterial population in time-kill assays, their combined effect is not expected to be adequately expressed with Eq. 7. Another model intending to describe null-interaction additivity of two drugs with independent mechanisms of action is Bliss Independence¹¹¹. This additivity model assumes that the observed E_{comb} can maximally become the highest E_{max} observed in drug A or B alone:

$$E_{comb} = E_A + E_B - E_A \times E_B \quad (\text{Eq. 8})$$

Since Bliss Independence was derived using a probabilistic approach, E_A and E_B need to be scaled as fractional effects between 0 and 1. After successfully developing two separate PK/PD models for single antibiotics, effects of their combination can be calculated using Eq. 8 and compared to the actually observed E_{comb} . This procedure helps to identify larger differences between Bliss

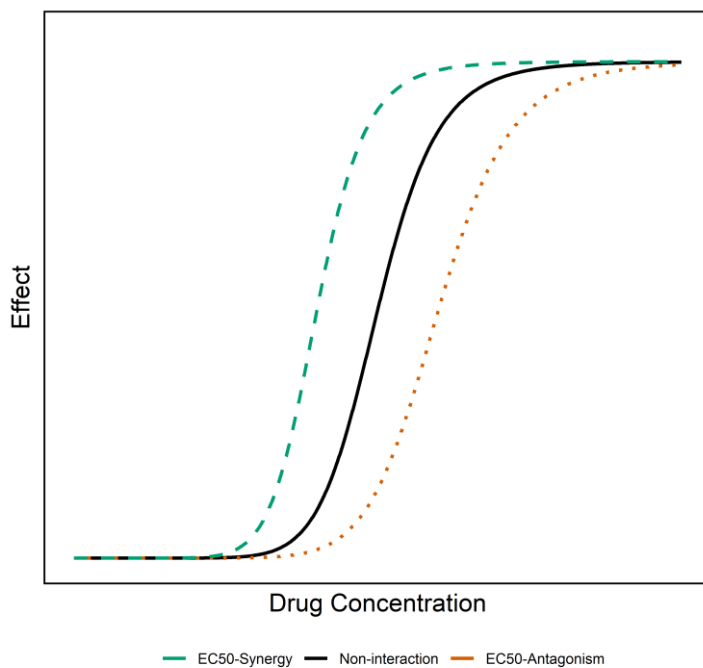


Figure 5 Schematic of sigmoidal concentration-response curve of a drug with its potency (EC_{50}) affected, following the General Pharmacodynamic Interaction term (Eq. 9).

Independence calculated effects and observed effects, indicating a PD interaction between the combined antibiotics. In such cases the PD interaction was characterized using the General Pharmacodynamic Interaction (GPDI) model. The GPDI term is a function of the perpetrator drug concentration, which affects a PD parameter θ (usually either EC_{50} or E_{max}) of the victim drug with INT as maximum possible fractional shift of the PD parameter θ , $EC50_{INT}$ as potency of the perpetrator and H_{INT} representing the sigmoidicity of the interaction (Eq. 9). The fractional shift of the victim's PD parameter caused by the perpetrator drug allows the directional quantification of the PD interaction, i.e., either synergy, antagonism or additivity.

$$\theta \times \left(1 + \frac{INT \times C^{H_{INT}}}{EC50_{INT}^{H_{INT}} + C^{H_{INT}}} \right) \quad (\text{Eq. 9})$$

Fig. 5 visualises how the concentration-effect curve of a victim drug would be influenced in the case of a PD interaction shifting its EC_{50} to higher (antagonism) or lower values (synergy). Understanding the mechanism behind observed PD interactions enhances our ability to design effective combination therapies, potentially extending the utility of existing antibiotics.

In this work longitudinal PK/PD models were developed based on time-kill assay data of meropenem-fosfomycin and polymyxin B-phage pK8 to describe the basal and enzymatic degradation of meropenem, PD killing effects of meropenem, fosfomycin, polymyxin B and phage pK8, and PD interactions between the combination of the mentioned compounds. Additionally, PD interactions between ceftazidime/avibactam and tigecycline were systematically evaluated based on non-longitudinal data from dynamic checkerboard assays. Elucidating PD interactions was based on the principles and methods described in this section and initially involved identifying its type (synergy, antagonism or additivity) and the directionality of interactions, meaning determining a perpetrator-victim relationship. The nature of the interaction was then analysed by comparing models that described the underlying interaction using EC_{50} shifts, E_{max} shifts, or a combination of both. Additionally, in PK/PD models involving phage pK8, the absence of classical PD parameters led to testing shifts in phage kinetics parameters to assess PD interactions. Possible hypotheses were derived, explaining the observed and characterised PD interactions between meropenem-fosfomycin, polymyxin B-phage pK8 and ceftazidime/avibactam-tigecycline.

4 Objectives

The AMR situation is escalating globally, significantly reducing the availability of effective treatment options for infected patients. Antimicrobial combination therapy has the potential to enhance efficacy, guard against resistance, and potentially reduce infection-related mortality. The primary goal of this dissertation was to find rational combination therapies against MDR Gram-negative bacteria. The focus was on identifying PD interactions between last-resort or novel antibiotics using both *in vitro* and *in silico* methods. We explored combinations that included five out of six antibiotics with existing market authorization – meropenem, ceftazidime/avibactam, fosfomycin, tigecycline and polymyxin B - that previously lacked quantitative assessment. Because these antibiotics are already marketed, the tested combinations are believed to be more easily translatable into clinical practice. We conducted novel analyses across three separate combinations resulting in four studies. Each study pursued three main objectives: Firstly, to develop and apply appropriate *in vitro* methods for a comprehensive quantitative evaluation of PD interactions. Secondly, to develop pharmacometric models that accurately capture the combined effects by integrating PD interactions and characterising their directionality, type, and potency. Thirdly, to conduct additional investigations and validate findings, either through further *in vitro* experiments (HFIM, metabolomics) or *in silico* experiments (pharmacometric simulations). These efforts aimed to derive possible mechanisms explaining the observed interactions and highlight their

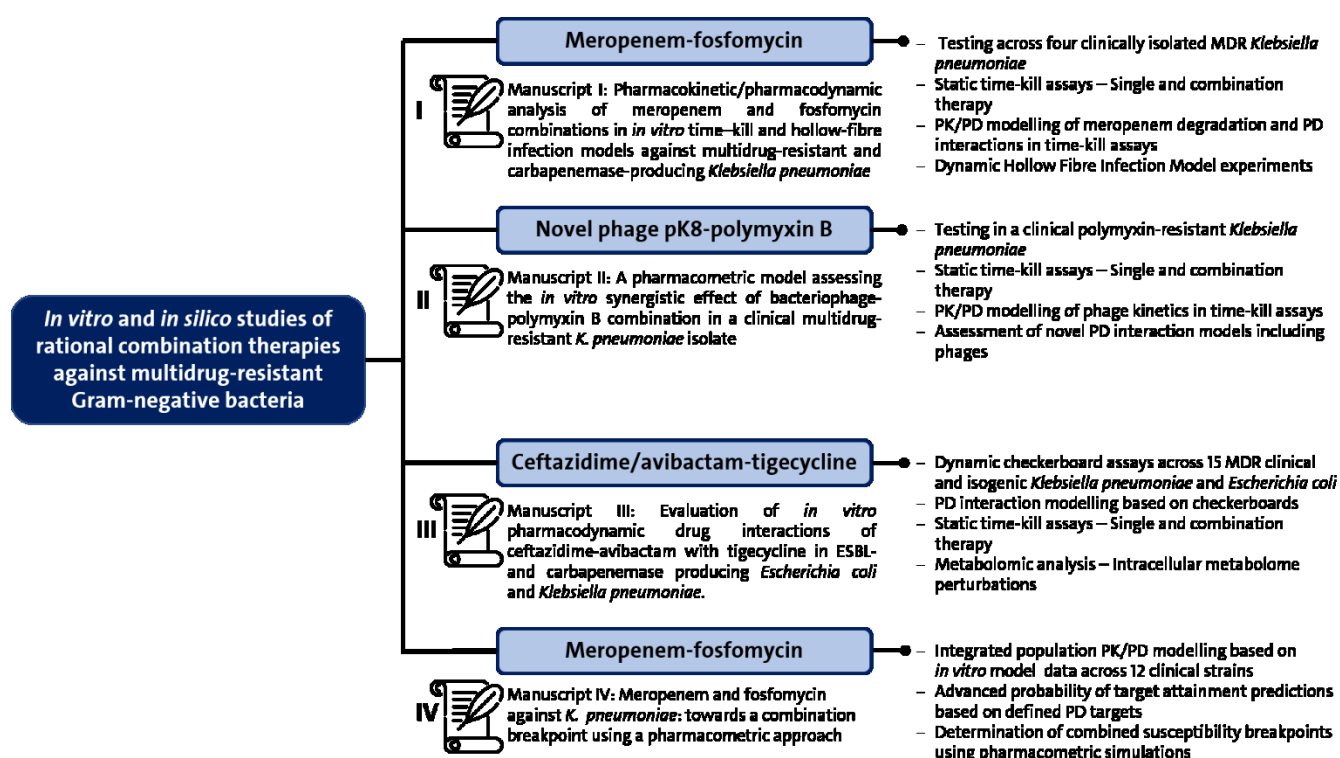


Figure 6 Summary of dissertation: Four main studies and their respective scopes.

potential use in clinical settings. This dissertation is based on four publications, with each addressing the objectives mentioned. The scopes of these publications, which outline the areas of study, methodologies, and contributions to the overall research goals, are summarized in **Fig. 6** and further explained upon in the following part of this section.

Publication I focused on the synergistic potential of combining meropenem and fosfomycin against carbapenem-resistant *K. pneumoniae* strains, which are a significant clinical challenge due to their high resistance levels and few treatment options. Specifically, four clinically isolated MDR *K. pneumoniae* strains were selected. To evaluate the potential synergy between meropenem and fosfomycin, time-kill assays were conducted. These assays were designed to account for the degradation of meropenem over time, ensuring accurate assessment of the drugs' interactions. Further, these interactions were quantitatively assessed using PK/PD modelling, aiming to identify the directionality within the PD interactions. By modelling these interactions, the study aimed not only on quantifying the synergy between the two antibiotics but also to explore underlying mechanisms of action. To validate the synergistic effects observed, HFIM experiments were performed in each strain. HFIM experiments mimicked human PK profiles and therefore provided an insight with higher clinical relevance for their potential use in treating infections caused by carbapenem-resistant *K. pneumoniae*.

Publication II explored phage kinetics and PD interactions between novel phage pK8 and polymyxin B against a polymyxin-resistant, MDR *K. pneumoniae*. Using time-kill assays with time-dissolved sampling for both bacterial and phage count, the dynamics of phage and bacterial populations were monitored. These data were then integrated into pharmacometric models to investigate the phage kinetics, dynamics of phage-resistance, drug effects and PD interactions involved. The study aimed to leverage the unique pharmacology of phages to gain novel insights into the phage-polymyxin interaction, with pharmacometric modelling enhancing the understanding and assessing the clinical potential of this combination as a complementary tool. Various interaction models were assessed to describe the observed interaction between phage pK8 and polymyxin B and subsequently compared to metabolomic findings in this combination.

Publication III systematically investigated the PD interactions between ceftazidime/avibactam and tigecycline across 15 *E. coli* and *K. pneumoniae* strains expressing carbapenemase or ESBL genes. Optimising and standardising laboratory procedures, the study utilized an adaptive and 'dynamic' checkerboard design with single-point sampling after one day of treatment.

Experimental data was included in pharmacometric modelling to quantitatively described the interactions' directionality. In addition, time-kill assays and metabolomic analyses were conducted to provide insights into the mechanisms underlying the drugs' effects. This study also aimed to deepen the usefulness of this combination and its ongoing clinical application.

Publication IV established a comprehensive pharmacometric framework to characterize the antibacterial effects and pharmacodynamic interactions of meropenem and fosfomycin, alone and in combination, against a diverse panel of 12 clinically isolated MDR *Klebsiella pneumoniae* strains. Drawing on dynamic *in vitro* infection model data from two different systems simulating human pharmacokinetics, the study employed nonlinear mixed-effects modelling to describe bacterial kill-kinetics and quantify synergy using advanced interaction models. Simulations with published population PK models were performed across a wide range of dosing regimens and MICs to predict the probability of target attainment (PTA) for clinically relevant efficacy endpoints (bacteriostasis, 1-log, and 2-log killing). Importantly, the study proposes rational clinical breakpoints for the meropenem-fosfomycin combination, providing a framework to support susceptibility testing and optimal dosing recommendations for treating severe infections with MDR *K. pneumoniae*.

5 Cumulative part

The cumulative part of this dissertation presents the key results of four, briefly summarised, peer-reviewed publications.

Three Original Research Articles were published in Journal of Antimicrobial Chemotherapy¹¹², International Journal of Antimicrobial Agents^{113,114}, and Clinical Microbiology and Infection¹¹⁵.

5.1 Publication I

Pharmacokinetic/pharmacodynamic analysis of meropenem and fosfomycin combinations in in vitro time–kill and hollow-fibre infection models against multidrug-resistant and carbapenemase-producing *Klebsiella pneumoniae*

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Journal of Antimicrobial Chemotherapy (2024)
Impact Factor: 3.6 (2024)

Synopsis

One approach against difficult to treat, MDR pathogens is to combine already marketed last-resort antimicrobials in rational combination therapies, thereby extending their shelf times and allowing for higher combined effects against even resistant strains. We quantitatively evaluated the synergistic potential of meropenem and fosfomycin against carbapenem-resistant *K. pneumoniae* strains isolated from clinical cases. Four MDR strains were tested using static time-kill assays accounting for meropenem degradation, followed by pharmacometric PK/PD interaction modelling to characterize the underlying PD interactions. Dynamic HFIM experiments simulating human PK confirmed that combination therapy provided high killing and suppressed regrowth at clinically relevant concentrations, whereas monotherapies failed. The interaction was mainly driven by meropenem enhancing the potency of fosfomycin. These findings support the use of meropenem-fosfomycin combination as a rational strategy against MDR *K. pneumoniae* and highlight the importance of PK/PD-based approaches for optimizing antibiotic combinations.

Pharmacokinetic/pharmacodynamic analysis of meropenem and fosfomycin combinations in *in vitro* time–kill and hollow-fibre infection models against multidrug-resistant and carbapenemase-producing *Klebsiella pneumoniae*

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Background: MDR Gram-negative bacteria, such as ESBL-producing and carbapenemase-producing *Klebsiella pneumoniae*, represent major global health threats. Treatment options are limited due to increasing resistance and slowed development of novel antimicrobials, making it necessary to apply effective combination therapies based on approved antibiotics.

Objectives: To quantitatively evaluate the synergistic potential of meropenem and fosfomycin against carbapenem-resistant *K. pneumoniae* strains isolated from clinics.

Methods: We evaluated four MDR *K. pneumoniae* strains, each expressing KPC-2 or KPC-3, using static time–kill assays that accounted for measured meropenem degradation. This was followed by pharmacokinetic/pharmacodynamic (PK/PD) interaction modelling, which estimated meropenem degradation rate constants and identified perpetrator–victim relationships in PD interactions. Dynamic hollow-fibre infection model (HFIM) experiments were used to confirm synergy.

Results: Static time–kill assays demonstrated high killing effects and suppressed regrowth for the combination of meropenem and fosfomycin, compared with the failure of monotherapy. Meropenem degradation was significantly higher in the presence of bacteria, attributable to carbapenemase activity. Pharmacometric models indicated a synergistic interaction primarily driven by meropenem as the perpetrator, enhancing the potency of fosfomycin. HFIM experiments confirmed *in vitro* synergy, demonstrating continuous bacterial suppression of the combination therapy.

Conclusions: Meropenem and fosfomycin exhibited additive or synergistic potential against carbapenemase-expressing single- or double-resistant *K. pneumoniae* at clinically achievable concentrations. This combination therapy may offer a strategy against MDR infections, possibly improving clinical treatment outcomes. Further *in vivo* research is needed to translate these findings into clinical practice, emphasizing the importance of PK/PD modelling in rationalizing antibiotic use.

Introduction

Infections caused by ESBL-producing and carbapenemase-producing *Klebsiella pneumoniae* are a growing international health problem, gaining increasing attention due to the lack of effective treatment options. *K. pneumoniae* is a Gram-negative bacterium with a propensity for acquiring multidrug resistance,

characterized as a WHO Priority 1 pathogen.^{1,2} MDR *K. pneumoniae*-associated infections present multifaceted difficulties regarding treatment and are associated with high mortality and morbidity. Due to the lengthy process of developing novel antimicrobials, there is an urgency for leveraging already marketed antibiotics. Combination therapy provides an opportunistic approach as it allows for a life extension of existing antibiotics

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through potential synergistic pharmacodynamic (PD) interactions, reducing the need for dose escalations and thus minimized risk of associated side effects. In empirical therapy, combination treatments can help to broaden the antimicrobial spectrum and decrease the risk of no antibiotic coverage.³ Meropenem represents one of the primary treatment options against infections caused by ESBL-producing Enterobacteriaceae;⁴ however, increasing prevalence of carbapenem-resistant Enterobacteriaceae is progressively impairing its efficacy.⁵ IV fosfomycin therapy has shown clinical benefits against infections caused by MDR bacteria, while also being recommended for exclusive use in combination.^{6,7} Although synergy between meropenem and fosfomycin has been shown in *in vitro* studies,^{8,9} the clinical relevance remains unclear. Investigations on the PD interaction are often based on the fractional inhibitory concentration index, limiting the analyses to the imprecise turbidity readout. Also, prior analyses have relied entirely on static chequerboard or static time-kill data, neglecting the dynamic pharmacokinetics (PK) in humans and assuming constant antibiotic concentrations throughout the duration of the experiments. In this study, our objective was to quantitatively elucidate the potential synergy between meropenem and fosfomycin against carbapenem-resistant *K. pneumoniae* strains isolated from clinical patients. To achieve this, static time-kill assays accounting for meropenem degradation were coupled with pharmacometric PK/PD-interaction modelling, enhancing the effective model accuracy by considering enzyme-driven changes. We then conducted dynamic hollow-fibre infection model (HFIM) experiments mimicking typical human PK profiles as verification studies.

Materials and methods

Bacterial isolates and antimicrobials

Four clinical *K. pneumoniae* isolates carrying various resistance genes coding for ESBLs or carbapenemases (KPC-2, KPC-3, OXA-9, OXA-2, CTX-M-2, CTX-M-15, SHV-11, SHV-12, SHV-39, SHV-110, TEM-1) were collected from Medical and Molecular Microbiology, University of Fribourg, Switzerland. Detailed information is given in Text S1 (available as Supplementary data at JAC Online) and Table S1. Meropenem, fosfomycin, glucose-6-phosphate and ceftazidime were obtained from Sigma-Aldrich (USA) and stocks were prepared in sterile 0.9% saline and stored at -80°C until use. In each experiment including fosfomycin, glucose-6-phosphate was added at 25 mg/L according to EUCAST recommendation.¹⁰

Quantification of viable bacteria

For the determination of the bacterial count a diluting and plating procedure was utilized.¹¹ Bacterial samples were serially 10-fold diluted in 48-well plates using 100 μL of sample and 900 μL of sterile 0.9% saline followed by plating on Columbia agar. The agar plates were incubated for 24 h at 37°C ambient air and cfu were counted.

Antimicrobial susceptibility testing

The MICs of meropenem and fosfomycin were determined in accordance with the EUCAST recommendation.¹⁰ Detailed information is given in Text S1.

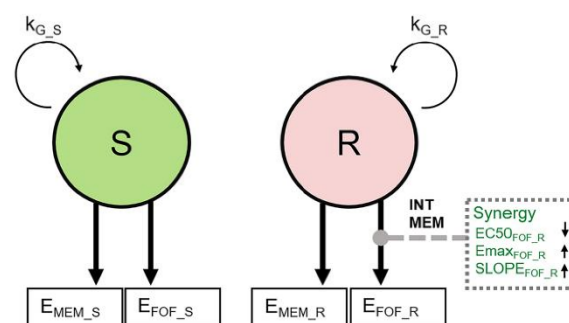


Figure 1. Schematic representation of PD models for static time-kill assays in four tested *K. pneumoniae*. (S), compartment addressing subpopulation of susceptible bacteria; (R), compartment addressing subpopulation of resistant bacteria; $k_{G,S}$, growth rate constant; $k_{G,R}$, growth rate constant; $E_{MEM,S}$, effect of meropenem on susceptible bacteria; $E_{MEM,R}$, effect of meropenem on resistant bacteria; $E_{FOF,S}$, effect of fosfomycin on susceptible bacteria; $E_{FOF,R}$, effect of fosfomycin on resistant bacteria; INT MEM: synergistic PD interaction with perpetrator meropenem affecting fosfomycin effect parameter $EC_{50FOF,R}$ ($\downarrow$) or $E_{maxFOF,R}$ ($\uparrow$) or $SLOPE_{FOF,R}$ ($\uparrow$).

Static time-kill assays

Time-kill assays were conducted with a bacterial inoculum in freshly prepared CAMHB (NutriSelect® Plus, Sigma-Aldrich, USA), incubated at 37°C for 24 h. Meropenem and/or fosfomycin were added in 2-fold dilutions. Bacterial counts were quantified at intervals up to 24 h. Samples were analysed for meropenem degradation and degradation rate constants were later estimated in PK/PD modelling to enhance model accuracy by accounting for enzyme-driven changes in meropenem concentration over time.

Detailed information is given in Text S2.

Pharmacometric modelling

Non-linear mixed-effects modelling was conducted on observed bacterial counts over time in static time-kill assays for all four strains, using NONMEM® 7.5 (Icon Development Solutions, USA). Quantitative analyses were performed using first-order conditional estimation with interaction (FOCE-I) for maximum-likelihood estimations and executed through PsN 5.3.1 (Uppsala University, Uppsala, Sweden). The degradation of meropenem was described using a first-order decay rate estimated from experiments without bacteria, k_{deg} , combined with either an additional constant degradation rate, k_{KPC} , caused by bacteria, or a degradation rate as a function of bacterial concentration. Degradation was implemented as shown in either Equation 1 or Equation 2 with B representing the total bacterial concentration in cfu/mL or transformed as $\ln$ cfu/mL (Equation 7).

$$C_t = C_{t=0} \times e^{-(k_{deg} + k_{KPC}) \times t} \quad (1)$$

$$C_{t,B} = C_{t=0} \times e^{-(k_{deg} + k_{KPC} \times B) \times t} \quad (2)$$

All final pharmacometric models (Figure 1) consisted of two bacterial compartments representing subpopulations of antibiotic-susceptible bacteria (S) and antibiotic-resistant bacteria (R), respectively. Single drug effects of meropenem and fosfomycin were described with separate parameter sets for both subpopulations of bacteria (S and R). The resulting differential equations for growth kinetics of susceptible bacteria B_S

and less susceptible (here termed 'resistant' for clarity) bacteria B_R without drug exposure are:

$$\frac{dB_S}{dt} = k_{g,s} \times \left(1 - \frac{B_S + B_R}{B_{MAX}}\right) \times B_S \quad (3)$$

$$\frac{dB_R}{dt} = k_{g,r} \times \left(1 - \frac{B_S + B_R}{B_{MAX}}\right) \times B_R \quad (4)$$

$$B = B_S + B_R \quad (5)$$

with k_g growth rate constants and B_{MAX} representing maximum bacterial capacity.

Antimicrobial effects of meropenem and fosfomycin were described using slope or maximum effect (E_{max}) models. Detailed information is given in Text S3. Combined effects were quantified using the general pharmacodynamic interaction (GPDI) model.¹² The GPDI model quantifies a potential PD interaction as shifts of the potency (EC_{50}) or efficacy (E_{max}). Single drug effects were quantified as described above while combined effects were described using Bliss independence as null-interaction model with E_A and E_B representing the effect of drug A and B:

$$E_{comb} = E_A + E_B - E_A \times E_B \quad (6)$$

PD interactions were evaluated as follows. In the GPDI model, the GPDI term is a function of the perpetrator drug concentration, which affects a PD parameter θ (either EC_{50} or E_{max}) of the victim drug with INT as maximum possible fractional shift of the PD parameter θ , $EC_{50,INT}$ as potency of the perpetrator and H_{INT} representing the sigmoidicity of the interaction (Equation 7). The GPDI model was then incorporated in Bliss independence (Equation 6). Identified synergy was statistically significant when CIs of INT did not include 0. Detailed information is given in Text S3.

$$\theta \times \left(1 + \frac{INT \times C_{INT}^H}{EC_{50,INT}^H + C_{INT}^H}\right) \quad (7)$$

Therefore, the resulting differential equations for the subpopulations of susceptible bacteria B_S and resistant bacteria B_R under drug exposure are:

$$\frac{dB_S}{dt} = k_{g,s} \times \left(1 - \frac{B_S + B_R}{B_{MAX}}\right) \times B_S - E_{comb,S} \times B_S \quad (8)$$

$$\frac{dB_R}{dt} = k_{g,r} \times \left(1 - \frac{B_S + B_R}{B_{MAX}}\right) \times B_R - E_{comb,R} \times B_R \quad (9)$$

Model selection of competing PK/PD models (i. e. interaction on E_{max} versus EC_{50}) was performed after inspecting biological plausibility of parameters, parameter uncertainty and the Akaike-Information-Criterion for identifying the one representing the observed data best.¹³

HFIM

PK

Population PK simulations were performed to obtain typical human concentration-time profiles for meropenem and fosfomycin. Published population PK models of meropenem and fosfomycin developed upon clinically measured samples were recoded using NONMEM® 7.5.^{14,15} Simulations of 1000 virtual patients were performed for a high-dose regimen of 2 g meropenem three times daily (2 g q8h) and 8 g fosfomycin three times daily (8 g q8h). PK of simulated median patients—virtual individuals providing a benchmark for typical drug exposure within the

studied population—guided the following HFIM experiments. In order to streamline the experimental procedure, both antibiotics were administered as bolus infusions while maintaining the clinically expected minimum (C_{min}) and maximum (C_{max}) concentrations. Detailed information is given in Text S2, Table S2 and Figure S1.

Pharmacodynamics

Dynamic time-kill assays simulating human concentration-time profiles of meropenem and fosfomycin were performed over 72 h at 37°C in ambient air in all four *K. pneumoniae* strains using the HFIM. Meropenem and fosfomycin high-dose treatments were mimicked with 2 g q8h and 8 g q8h, respectively. Killing kinetics and actual drug concentrations were monitored through dense sampling followed by quantification. Detailed information on HFIM equipment and set-up is given in Text S2.

Bioanalysis

Samples collected from static time-kill assays and the central compartment of HFIM experiments were analysed using LC-MS/MS in accordance with the EMA guideline on validation of bioanalytical procedures.¹⁶ Detailed information is given in Text S4 and Tables S3–S6.

Results

Antimicrobial susceptibility testing

The MIC values for all tested strains with identified resistance genes can be found in Table 1. All tested strains were classified as resistant to meropenem according to EUCAST,¹⁰ with varying susceptibility to fosfomycin. *K. pneumoniae* inherently carries fosfomycin resistance genes, potentially leading to high-level resistance through elevated expression or mutations in structural proteins.^{17,18}

Static time-kill assays

Meropenem concentrations and degradation

Meropenem concentrations in CAMHB without bacteria decreased from 125.5 mg/L at 0 h to 70.9 mg/L at 24 h (56.5% of initial value). Meropenem degradation at 24 h was significantly more pronounced in all tested scenarios with the presence of KPC-2- or KPC-3-expressing bacteria (Figure 2, Text S5 and Figure S2), ranging between 45.4% and 4.4% in *K. pneumoniae* 2977_{KPC-2}, 23.6%–18.8% in *K. pneumoniae* N2859_{KPC-2}, 24.7%–4.7% in *K. pneumoniae* N864_{KPC-3} and 31.0%–4.9% in *K. pneumoniae* R307_{KPC-2}, compared with the initial value. Mono and combination scenarios of meropenem with higher effects and lower bacterial concentrations at 24 h showed a trend of resulting in lower total degradation of meropenem compared with scenarios with low effects and high bacterial burden.

PD

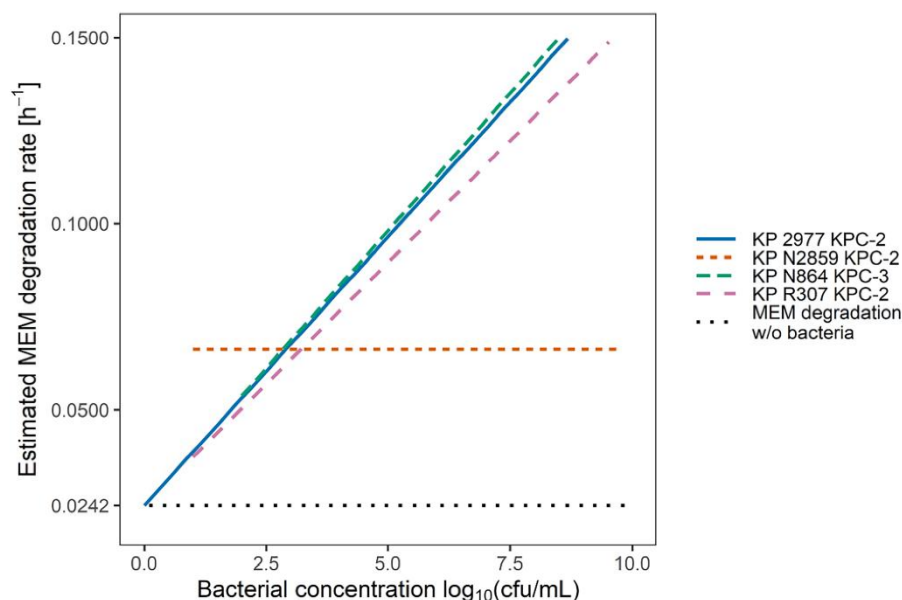
In static time-kill assays, the tested *K. pneumoniae* strains showed non-susceptibility or resistance to both meropenem alone and fosfomycin alone as regrowth was observed within 24 h at 64–128 mg/L and 128–512 mg/L, respectively. Concentrations above what is clinically realizable (≥ 64 mg/L meropenem and ≥ 256 mg/L fosfomycin) were required to suppress regrowth. Notably, when combining meropenem and fosfomycin, substantial reduction followed by suppression of

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Table 1. Tested strains with identified resistance genes and determined MIC values

Strain	Resistance genes	MIC _{MEM} (mg/L)	MIC _{FOF} (mg/L)
<i>K. pneumoniae</i> 2977 _{KPC-2}	KPC-2, CTX-M-2, SHV-12, OXA-9, TEM-1, SHV-110	32	32
<i>K. pneumoniae</i> N2859 _{KPC-2}	KPC-2, SHV-1	64	4
<i>K. pneumoniae</i> N864 _{KPC-3}	KPC-3, OXA-9, TEM-1, SHV-11	32	16
<i>K. pneumoniae</i> R307 _{KPC-2}	KPC-2, CTX-M-2, OXA-2, OXA-9, SHV-39, TEM-1	128	64

MEM, meropenem; FOF, fosfomycin.

**Figure 2.** Estimated total meropenem degradation rate versus bacterial concentration in log₁₀ cfu/mL. MEM, meropenem; KP, *K. pneumoniae*; w/o, without.

regrowth were observed for clinically achievable concentrations in three of four strains.

For *K. pneumoniae* 2977_{KPC-2} (Figure 3) ≤128 mg/L meropenem alone and ≤256 mg/L fosfomycin alone exhibited up to 4.5 log₁₀ reduction followed by regrowth. When combining both antibiotics at 32 mg/L, up to 4 log₁₀ reduction was achieved and regrowth was suppressed in all replicates.

For *K. pneumoniae* N2859_{KPC-2} (Figure 4) ≤128 mg/L meropenem alone and ≤64 mg/L fosfomycin alone exhibited up to 4 log₁₀ reduction followed by regrowth. When combining both antibiotics at only 8 mg/L (meropenem) and 32 mg/L (fosfomycin), up to 4 log₁₀ reduction was achieved and regrowth was suppressed in all replicates.

For *K. pneumoniae* N864_{KPC-3} (Figure 5) ≤64 mg/L meropenem alone and ≤128 mg/L fosfomycin alone exhibited up to 3 log₁₀ reduction followed by regrowth. When combining both antibiotics at 32 mg/L (meropenem) and 256 mg/L (fosfomycin), up to

6.5 log₁₀ reduction was achieved at 24 h and regrowth was suppressed in all replicates.

Lastly, for *K. pneumoniae* R307_{KPC-2} (Figure 6) ≤64 mg/L meropenem alone and ≤2048 mg/L fosfomycin alone exhibited up to 4 and 6 log₁₀ reduction, respectively, followed by regrowth. When combining both antibiotics at 32 or 64 mg/L (meropenem) and 512 mg/L (fosfomycin), up to 6 log₁₀ reduction was achieved and regrowth was suppressed in all replicates, with full eradication of bacteria at 24 h.

Pharmacometric modelling

Table 2 shows the final parameter estimates of all pharmacometric models. Initial reduction of bacteria was mainly described through effects on subpopulation (S) while observed regrowth under drug exposure was mainly driven through subpopulation (R). The developed pharmacometric models adequately

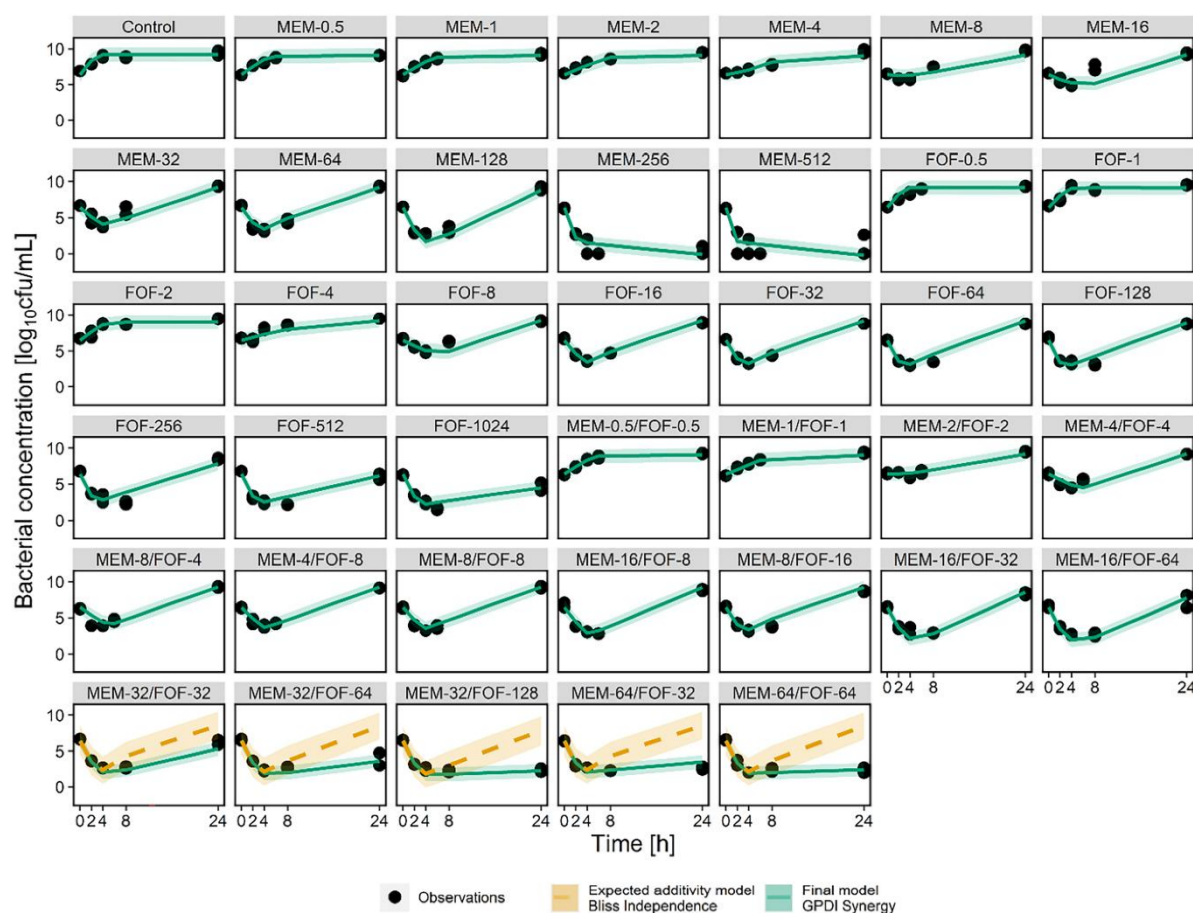


Figure 3. Stratified visual predictive check of final PD model characterizing the bacterial load in $\log_{10}$ cfu/mL for meropenem and fosfomycin against clinical isolate *K. pneumoniae* 2977_{KPC-2} ($\text{MIC}_{\text{MEM}} = 32$ mg/L; $\text{MIC}_{\text{FOF}} = 32$ mg/L). Dots, observations; solid green line, median prediction of final model incorporating synergy; shaded green area, 90% prediction intervals of final model; dashed line, median prediction of calculated additivity by Bliss independence; shaded orange area, 90% prediction intervals of calculated additivity. MEM, meropenem; FOF, fosfomycin.

described meropenem degradation in the presence of bacteria (Figure S2). The estimated degradation rate constants (k_{KPC}) in the case where bacteria-induced degradation was implemented as a function of the underlying bacterial count $\log_{10}$ cfu/mL (Eq. 3), three of the four strains showed comparable rate constants (Figure 2 and Table 2), while the remaining strain (*K. pneumoniae* N2859_{KPC-2}) maintained a degradation rate constant independent of bacterial load. Bacterial growth and killing kinetics in both the mono and combination meropenem and fosfomycin time-kill assays from low to high concentrations were accurately characterized, while also identifying the interaction type (EC_{50} or E_{max}) and its directionality (Figures 3–6). When trying to quantify combined effects as null-interaction additivity calculated with Bliss independence (Equation 1), they were substantially underpredicted in three of four strains (Figures 3, 4 and 6).

A monodirectional PD interaction model with meropenem as perpetrator drug significantly lowering the EC_{50} of fosfomycin

on (R) was identified in two of four strains and outperformed the opposite directionality model with fosfomycin being the perpetrator and meropenem the victim drug (Figures 3 and 4). For *K. pneumoniae* 2977_{KPC-2} and *K. pneumoniae* N2859_{KPC-2}, meropenem lowered the EC_{50} of fosfomycin on (R) by 98.0% (95% CI 97.4%–98.5%) from 529 to 10.6 mg/L and by 82.1% (95% CI 70.5%–92.9%) from 36.3 to 6.50 mg/L, respectively. For *K. pneumoniae* N864_{KPC-3}, the null-interaction additivity calculated with Bliss independence correctly predicted bacterial concentration in combination scenarios (Figure 5). For *K. pneumoniae* R307_{KPC-2}, showing the highest MIC values for all tested strains ($\text{MIC}_{\text{MEM}} = 128$ mg/L; $\text{MIC}_{\text{FOF}} = 64$ mg/L), a monodirectional PD interaction model with meropenem as perpetrator drug raising the slope of fosfomycin on (R) by 6.6-fold (95% CI 5.5-fold to 8.4-fold) was identified (Figure 6). This resulted in only high concentrations of both meropenem, as driver of the interaction, and fosfomycin, restoring its effect, showing synergy in suppression of regrowth.

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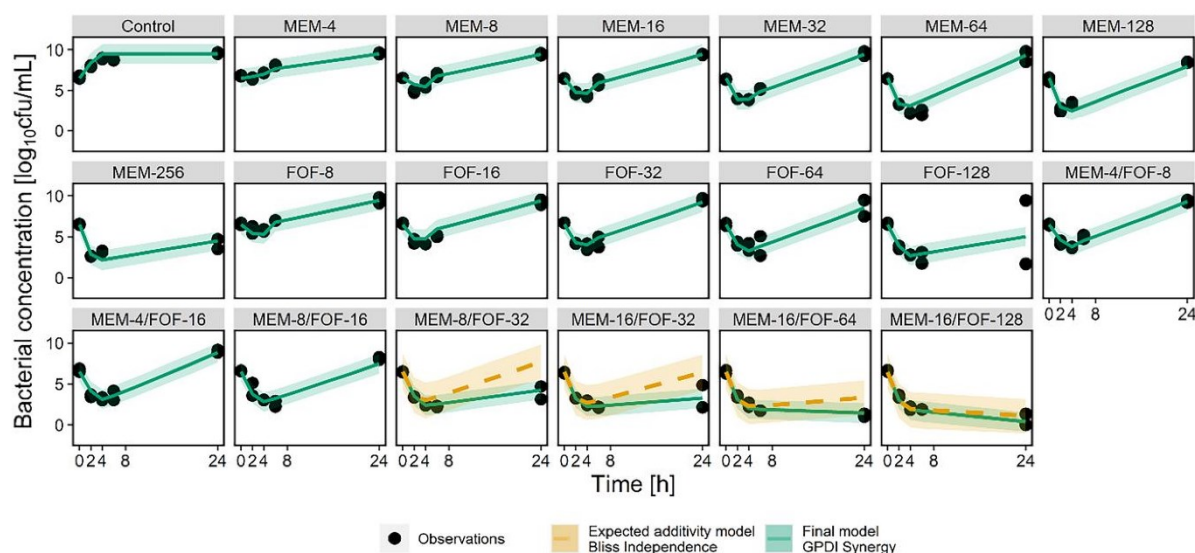


Figure 4. Stratified visual predictive check of final PD model characterizing the bacterial load in $\log_{10}$ cfu/mL for meropenem and fosfomycin against clinical isolate *K. pneumoniae* N2859_{KPC-2} ($MIC_{MEM}=64$ mg/L; $MIC_{FOF}=4$ mg/L). Dots, observations; solid green line, median prediction of final model incorporating synergy; shaded green area, 90% prediction intervals of final model; dashed line, median prediction of calculated additivity by Bliss independence; shaded orange area, 90% prediction intervals of calculated additivity. MEM, meropenem; FOF, fosfomycin.

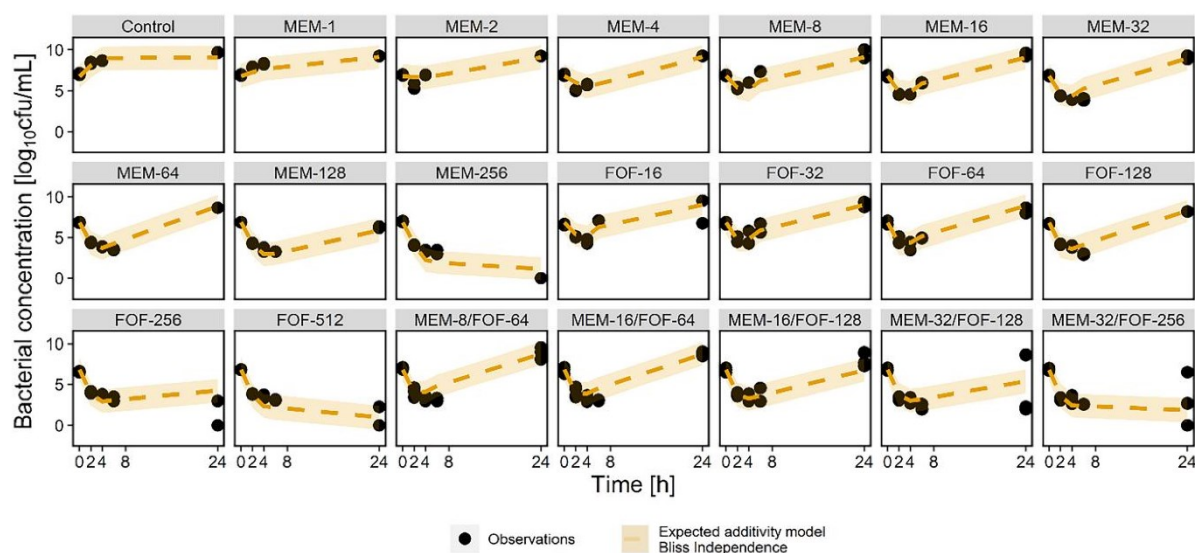


Figure 5. Stratified visual predictive check of final PD model characterizing the bacterial load in $\log_{10}$ cfu/mL for meropenem and fosfomycin against clinical isolate *K. pneumoniae* N864_{KPC-3} ($MIC_{MEM}=32$ mg/L; $MIC_{FOF}=16$ mg/L). Dots, observations; dashed line, median prediction of final model applying additivity by Bliss independence; shaded orange area, 90% prediction intervals of final model. MEM, meropenem; FOF, fosfomycin.

HFIM

PK

Target PK profiles of meropenem and fosfomycin and measured concentrations from collected samples are shown in Figure 7. The

planned target profiles of fosfomycin were accurately achieved in all experiments. Meropenem concentrations in monotherapy experiments (Figure 7a) were in line with the planned target profile following one or two dosing events. Thereafter, degradation of meropenem occurred even shortly after dosing. In these

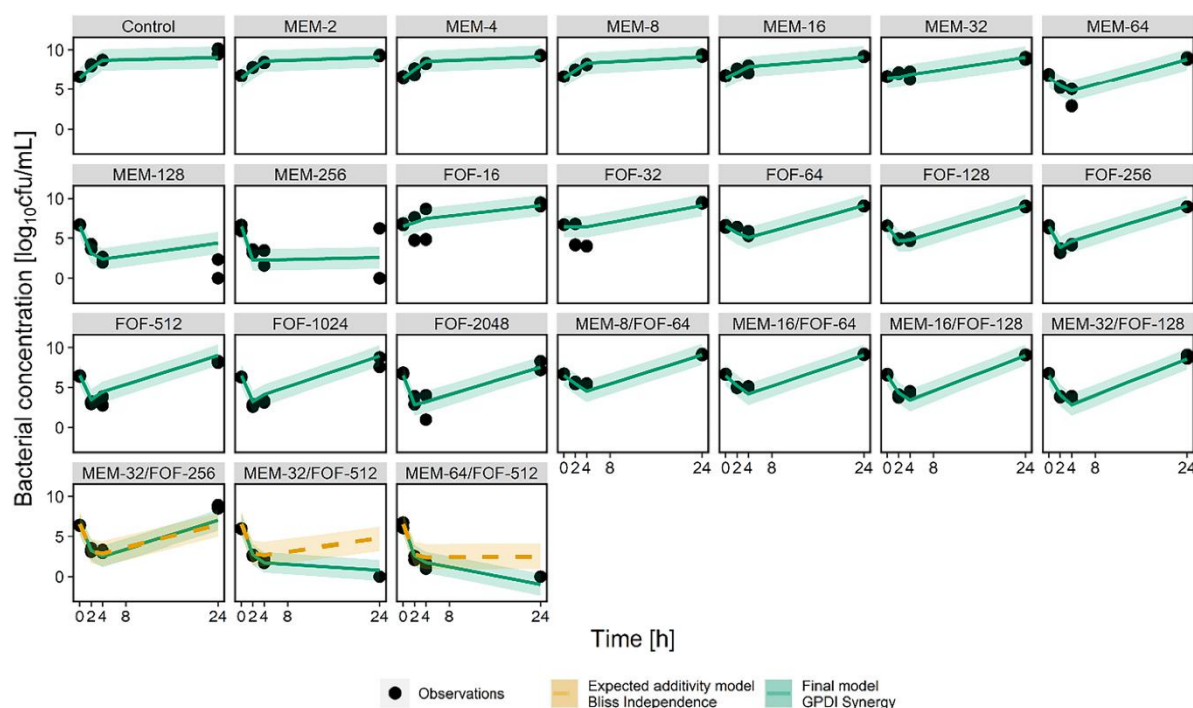


Figure 6. Stratified visual predictive check of final PD model characterizing the bacterial load in $\log_{10}$ cfu/mL for meropenem and fosfomycin against clinical isolate *K. pneumoniae* R307_{KPC-2} ($MIC_{MEM} = 128$ mg/L; $MIC_{FOF} = 64$ mg/L). Dots, observations; solid green line, median prediction of final model incorporating synergy; shaded green area, 90% prediction intervals of final model; dashed line, median prediction of calculated additivity by Bliss independence; shaded orange area, 90% prediction intervals of calculated additivity. MEM, meropenem; FOF, fosfomycin.

monotherapy experiments, bacteria regrew quickly and reached stationary phase (compare with Figure 8). Meropenem concentrations in combination therapy experiments (Figure 7c) were initially in line with the planned target profile but showed degradation over time, though it was less pronounced than in monotherapy experiments.

PD

Killing kinetics and natural growth of performed HFIM experiments in four tested strains with their respective MIC values are shown in Figure 8. Control scenarios showed exponential growth approaching the stationary phase at 10^9 – 10^{11} cfu/mL depending on the tested strain.

For *K. pneumoniae* 2977_{KPC-2} ($MIC_{MEM} = 32$ mg/L; $MIC_{FOF} = 32$ mg/L), high-dose meropenem with 2 g q8h resulted in 2 $\log_{10}$ reduction within the first 4 h, followed by rapid regrowth and the bacterial concentration reaching the stationary phase at 24 h. High-dose fosfomycin with 8 g q8h resulted in 4 $\log_{10}$ reduction at 4 h, followed by slow and steady regrowth of bacteria, reaching stationary phase at 48 h. Combination therapy of high-dose meropenem plus high-dose fosfomycin resulted in 4 $\log_{10}$ reduction followed by no regrowth of bacteria until the end of the experiment.

K. pneumoniae N864_{KPC-3} ($MIC_{MEM} = 32$ mg/L; $MIC_{FOF} = 16$ mg/L), being similarly susceptible or resistant to both antibiotics, showed a comparable response where both mono therapies resulted in initial

reduction and regrowth of bacteria, while combination therapy showed substantial killing without regrowth.

For *K. pneumoniae* N2859_{KPC-2} ($MIC_{MEM} = 64$ mg/L; $MIC_{FOF} = 4$ mg/L), representing the lowest MIC_{FOF} of all tested strains, high-dose fosfomycin therapy and meropenem plus fosfomycin combination therapy did not differ in their response; both scenarios resulted in substantial killing without regrowth.

For *K. pneumoniae* R307_{KPC-2} ($MIC_{MEM} = 128$ mg/L; $MIC_{FOF} = 64$ mg/L), representing the strain with the highest MIC_{MEM} and MIC_{FOF} values, meaning being least susceptible to both antibiotics, mono and combination scenarios resulted in initial reduction of bacteria, followed by regrowth, approaching the stationary phase at 24 h.

Discussion

This study quantitatively elucidated the synergy between meropenem and fosfomycin against carbapenem-resistant *K. pneumoniae* strains. By integrating static time-kill assays and pharmacometric modelling, we demonstrated that the combination of these antibiotics exhibits significant synergistic PD interactions, particularly in strains expressing high levels of resistance against meropenem and partial resistance to fosfomycin. Previous studies often relied on static *in vitro* models, lacking dynamic human PK; our study, by utilizing HFIM experiments, simulated typical human PK profiles.

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Table 2. Parameter estimates for final PK/PD models of meropenem and fosfomycin against four tested *K. pneumoniae*

Parameter	Final estimate (95% CI)			
	<i>K. pneumoniae</i>			
	2977 _{KPC-3}	N2859 _{KPC-3}	N864 _{KPC-2}	R307 _{KPC-2}
Bacterial system				
Growth rate constant susceptible bacteria (S) (h^{-1})	2.43 (1.97–3.04)	2.23 ^b (1.85–2.57)	1.51 ^b (1.18–1.87)	1.43 ^b (1.22–1.68)
Growth rate constant resistant bacteria (R) (h^{-1})	0.91 (0.81–1.02)	2.23 ^b (1.85–2.57)	1.51 ^b (1.18–1.87)	1.43 ^b (1.22–1.68)
Inoculum susceptible bacteria (S) ($\log_{10}$ cfu/mL)	6.42 (6.29–6.54)	6.51 (6.29–6.72)	6.83 (6.60–7.06)	6.48 (6.28–6.68)
Inoculum resistant bacteria (R) ($\log_{10}$ cfu/mL)	1.80 (1.61–1.99)	2.17 (1.90–2.44)	2.93 (2.61–3.23)	2.34 (1.94–2.72)
Maximum bacterial capacity ($\log_{10}$ cfu/mL)	9.22 (9.12–9.33)	9.53 (9.33–9.74)	9.02 (8.72–9.33)	9.13 (8.86–9.40)
Meropenem degradation				
MEM degradation rate constant without bacteria (h^{-1})	0.0242 ^a	0.0242 ^a	0.0242 ^a	0.0242 ^a
Bacteria-induced MEM degradation rate constant (h^{-1})	—	0.042 (0.039–0.045)	—	—
Bacteria-induced MEM degradation rate constant as function of bacterial concentration ($h^{-1} \times \log_{10}$ mL/cfu)	0.0027 (0.0020–0.0039)	—	0.0028 (0.0026–0.0030)	0.0025 (0.0021–0.0030)
Mono drug effect parameter				
Slope of MEM on (S) (L/mg $\times$ h^{-1})	1.42 (1.05–1.92)	—	—	0.045 (0.037–0.054)
E_{max} of MEM on (S) (h^{-1})	—	6.84 (6.06–7.82)	4.72 (3.54–5.81)	—
EC ₅₀ of MEM on (S) (mg/L)	—	8.84 (5.03–16.42)	3.04 (1.57–5.91)	—
Hill coefficient of MEM on (S)	0.30 (0.25–0.36)	1 ^a	1 ^a	1 ^a
Slope of MEM on (R) (L/mg $\times$ h^{-1})	—	—	—	—
E_{max} of MEM on (R) (h^{-1})	1.10 (1.01–1.24)	2.55 (2.13–2.97)	2.30 (1.90–2.70)	1.58 (1.36–1.84)
EC ₅₀ of MEM on (R) (mg/L)	91.7 (80.3–101)	29.3 (17.4–50.4)	45.7 (27.1–84.6)	15.8 (10.5–24.3)
Hill coefficient of MEM on (R)	20 ^a	1 ^a	1 ^a	1 ^a
Slope of FOF on (S) (L/mg $\times$ h^{-1})	—	—	—	—
E_{max} of FOF on (S) (h^{-1})	6.06 (5.47–6.77)	5.52 (4.52–6.69)	5.26 (3.81–6.07)	6.78 (5.52–9.98)
EC ₅₀ of FOF on (S) (mg/L)	7.28 (5.94–9.67)	4.77 (1.18–10.4)	9.88 ^a	114 (50.7–240)
Hill coefficient of FOF on (S)	1.29 (0.97–1.67)	1 ^a	1 ^a	1 ^a
Slope of FOF on (R) (L/mg $\times$ h^{-1})	—	—	—	0.00045 (0.00033–0.00058)
E_{max} of FOF on (R) (h^{-1})	0.99 (0.90–1.07)	2.51 (2.13–2.92)	2.10 (1.51–2.69)	—
EC ₅₀ of FOF on (R) (mg/L)	529 (369–764)	36.3 (26.4–49.9)	145 (106–257)	—
Hill coefficient of FOF on (R)	1 ^a	1 ^a	1.15 (0.79–1.90)	1 ^a
GPDl model parameters				
Interaction type	Synergy	Synergy	Null interaction	Synergy

Continued

Table 2. Continued

Parameter	Final estimate (95% CI)			
	<i>K. pneumoniae</i>			
	2977 _{KPC-3}	N2859 _{KPC-3}	N864 _{KPC-2}	R307 _{KPC-2}
Maximum interaction MEM affecting EC ₅₀ of FOF on (R) (%)	−98.0 (−98.5 to −97.4)	−82.1 (−92.9 to −74.0)	—	—
EC ₅₀ of interaction MEM affecting EC ₅₀ of FOF on (R) (mg/L)	8.70 (5.93–10.1)	0.29 (0.02–0.84)	—	—
Maximum interaction MEM affecting slope of FOF on (R) (%)	—	—	—	6.58 (5.50–8.42)
EC ₅₀ of interaction MEM affecting slope of FOF on (R) (mg/L)	—	—	—	14.1 (11.8–16.1)
Hill coefficient of interaction	20 ^a	1 ^a	—	20 ^a
Residual variability model				
Additive residual variability PD (log ₁₀ cfu/mL)	0.58 (0.54–0.62)	0.72 (0.66–0.79)	0.86 (0.79–0.94)	0.80 (0.73–0.89)
Additive residual variability PK (mg/L)	5.49 (2.15–11.1)	0.07 (0.05–0.12)	—	3.43 (2.32–5.51)
Proportional residual variability PK (%)	13.7 (3.72–33.4)	—	19.0 (13.7–29.0)	—

95% CIs were calculated using log-likelihood profiling. (S), bacteria of susceptible compartment; (R), bacteria of resistant compartment; MEM, meropenem; FOF, fosfomycin.

^aFixed parameter.

^b(S) and (R) same growth rate constant.

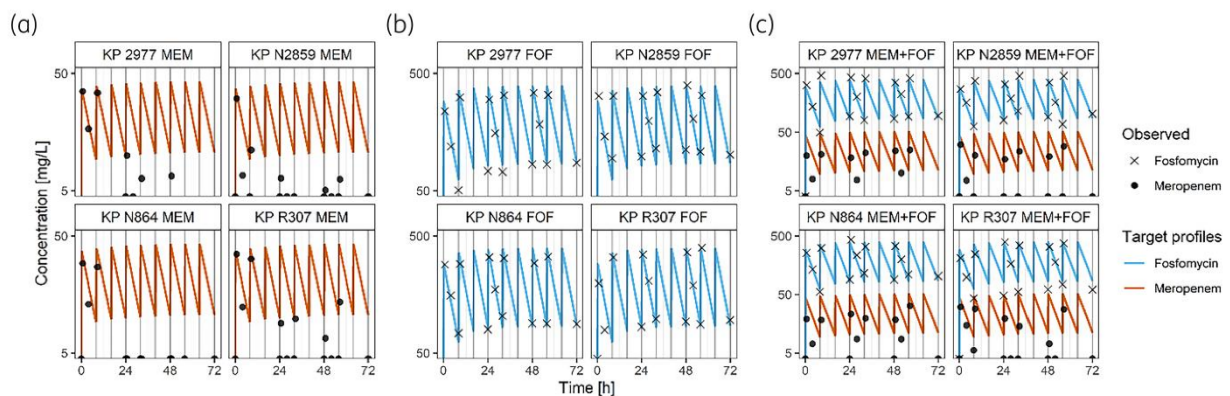


Figure 7. Target and measured concentration–time profiles for high-dose meropenem (2 g q8h) and high-dose fosfomycin (8 g q8h) dosing regimen mimicked in dynamic HFIM experiments stratified by four tested strains and scenarios: mono therapy meropenem (a), mono therapy fosfomycin (b), combination therapy (c). Dots, observations meropenem; crosses, observations fosfomycin; solid lines, target profiles; MEM, meropenem; FOF, fosfomycin; KP, *K. pneumoniae*.

Our findings reveal several key insights. First, the combination therapy of meropenem and fosfomycin significantly suppressed bacterial regrowth at clinically achievable concentrations, even when monotherapy of meropenem and fosfomycin failed to do so. This finding suggests that leveraging combination therapy could enhance treatment outcomes in clinical settings where monotherapy might be expected to be insufficient. MIC values could be a primary decision factor, as the only combination therapy that failed in the HFIM was the

one associated with the highest MIC values. Second, we could successfully categorize PD interactions into additive and synergistic, while gaining knowledge regarding the directionality of identified synergy by applying the GPDI model. In two of four strains (*K. pneumoniae* 2977_{KPC-2} and *K. pneumoniae* N2859_{KPC-2}), synergy was best described with a substantial reduction of the EC₅₀ of fosfomycin on subpopulation *B_R*, driven by the concentration of meropenem as perpetrator of the interaction, while in *K. pneumoniae* N864_{KPC-3}, additivity was

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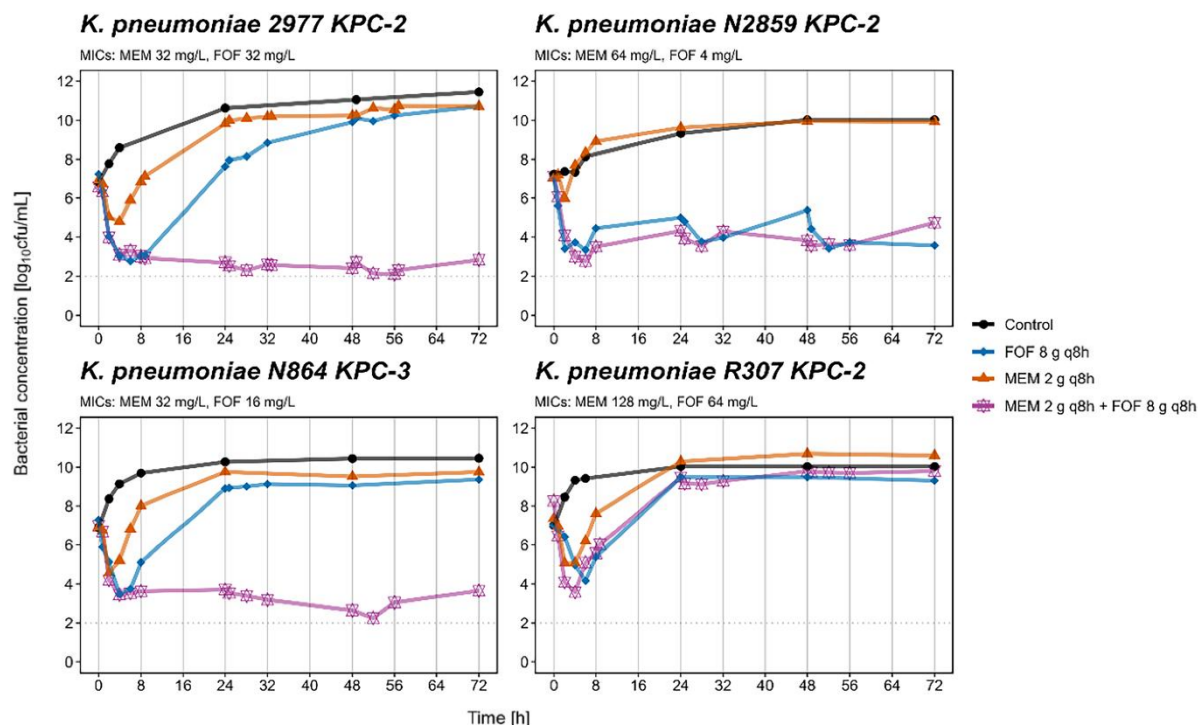


Figure 8. Observed killing kinetics in HFIM experiments for four clinical carbapenemase-producing, meropenem-resistant *K. pneumoniae* strains. Each plot shows the bacterial count in $\log_{10}$ cfu/mL over the experimental time of 72 h for control scenarios, high-dose meropenem 2 g q8h, high-dose fosfomycin 8 g q8h and combination therapy. Shapes, observations; dotted lines, lower limit of quantification; vertical lines, dosing events; MEM, meropenem; FOF, fosfomycin.

identified. Additionally, in dynamic HFIM experiments over 3 days, which better reflect the clinical situation, there was a successful reduction of bacterial burden and suppression of re-growth in combination therapy compared with both monotherapies for *K. pneumoniae* 2977_{KPC-2} and *K. pneumoniae* N864_{KPC-3}. In contrast, for *K. pneumoniae* N2859_{KPC-2}, both fosfomycin monotherapy and combination therapy were equally successful in reducing bacterial burden over 72 h, consistent with the estimated EC_{50} on the B_R subpopulation and its expected efficacy alone. Thirdly, the presented study is, to our knowledge, the first to integrate actual drug levels from static time–kill assays into the development of PK/PD models, consequently addressing the degradation of unstable antibiotics such as meropenem, bacteria-induced degradation and the time-dependent effect sizes. The estimated degradation rate constant of meropenem in CAMHB was estimated to be 0.0242 h^{-1} ($t_{1/2} = 28.6\text{ h}$), which is in line with previously published data.¹⁹ Additionally, bacteria-induced degradation rate constants were successfully estimated to exceed total meropenem degradation to more than three times the size of the basal meropenem degradation without bacteria. This additional degradation can be explained through high expression of KPC-2 or KPC-3 in bacteria, enabling the active hydrolysis of meropenem through a well-described enzymatic reaction.^{20,21} As expected, there was an observed decline of meropenem concentrations over time in the HFIM experiments for both mono and

combination therapy (Figure 7), attributed to the bacteria-driven degradation of the antibiotic. However, in meropenem monotherapy experiments, the rate of the degradation following 24 h was notably greater than when administered in combination. This discrepancy may be due to the accumulation of KPC-2 or KPC-3 in the HFIM cartridge.

The mechanism underlying the observed synergy between carbapenems has been discussed by Samonis et al.,²² in which β -lactams are suggested to inhibit later stages of cell-wall synthesis. As meropenem was identified as the perpetrator in three of the four PD interactions within our study, its potential role in augmenting bacterial cell-wall vulnerability may facilitate the penetration of fosfomycin. This assumes meropenem can bind PBPs despite carbapenemase expression, based on initial killing effects seen in static time–kill assays and HFIM experiments. Furthermore, this would also explain the potentiation of the drug effect through a shift in the $EC_{50\text{FOF}}$ on B_R , as indicated by the identified interaction types. Kroemer et al.^{23,24} provided a similar explanation to describe the synergy between ceftazidime/avibactam and fosfomycin.

Although the HFIM serves as an *in vitro* model that more accurately simulates biological response compared with static assays, its primary shortcoming is the lack of an immune response, thus providing an optimal environment for bacterial growth. Therefore, the contribution of the immune system in an animal or human model may result in an even higher

likelihood of successfully reducing bacterial burden. A limitation of the present study's *in vitro* nature is thus the absence of the full complexity of human infections and human–host interactions. Additionally, the selection of a limited number of four carbapenemase-expressing *K. pneumoniae* strains does not capture the panel of diversity in severely infected patients. Our set *in vitro* conditions allowed bacteria to reach an early phase of exponential growth and a starting inoculum of approximately 10^6 cfu/mL. In contrast, patients typically seek clinical care a few days after infection onset, and information regarding the actual bacterial burden at infection sites is very limited.

Despite this, our results offer evidence for the clinical potential of meropenem and fosfomycin combination therapy against MDR, carbapenemase-expressing *K. pneumoniae*. These findings support the inclusion of meropenem and fosfomycin combination therapy for treating infections caused by carbapenem-resistant Enterobacteriaceae in *in vivo* animal model studies and clinical pilot studies, as already conducted for other fosfomycin-containing combination therapies of interest.²⁵ The combination of meropenem and fosfomycin might serve as an alternative to the recommended treatments for infections caused by KPC-producers, such as meropenem/vaborbactam or ceftazidime/avibactam.⁴ Furthermore, high-dose fosfomycin can cause adverse effects, which may limit the use of IV fosfomycin, depending on their severity.^{26,27} Here the rationale could be combining fosfomycin with meropenem, subsequently reducing the need of fosfomycin dose escalation and its side effects. Moreover, the cost of treatment with off-patent drugs like meropenem and fosfomycin would be significantly lower, making it more accessible for a wider population of patients. Future research should focus on clinical trials to validate these *in vitro* findings and optimize dosing strategies to maximize therapeutic efficacy and minimize resistance development.

Acknowledgements

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Transparency declarations

None to declare.

Supplementary data

Text S1–S5, Figures S1 and S2 and Tables S1 and S2 are available as Supplementary data at JAC Online.

References

- 1 WHO. WHO bacterial priority pathogens list, 2024: Bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. 2024. <https://www.who.int/publications/i/item/9789240093461>.
- 2 Tyers M, Wright GD. Drug combinations: a strategy to extend the life of antibiotics in the 21st century. *Nat Rev Microbiol* 2019; **17**: 141–55. <https://doi.org/10.1038/s41579-018-0141-x>
- 3 Tamma PD, Cosgrove SE, Maragakis LL. Combination therapy for treatment of infections with Gram-negative bacteria. *Clin Microbiol Rev* 2012; **25**: 450–70. <https://doi.org/10.1128/CMR.05041-11>
- 4 Tamma PD, Aitken SL, Bonomo RA et al. Infectious Diseases Society of America 2023 guidance on the treatment of antimicrobial resistant Gram-negative infections. *Clin Infect Dis* 2023; ciad428: <https://doi.org/10.1093/cid/ciad428>
- 5 Pascale R, Giannella M, Bartoletti M et al. Use of meropenem in treating carbapenem-resistant Enterobacteriaceae infections. *Expert Rev Anti Infect Ther* 2019; **17**: 819–27. <https://doi.org/10.1080/14787210.2019.1673731>
- 6 Anastasia A, Bonura S, Rubino R et al. The use of intravenous fosfomycin in clinical practice: a 5-year retrospective study in a tertiary hospital in Italy. *Antibiotics* 2023; **12**: 971. <https://doi.org/10.3390/antibiotics12060971>
- 7 Bassetti M, Graziano E, Berruti M et al. The role of fosfomycin for multidrug-resistant gram-negative infections. *Curr Opin Infect Dis* 2019; **32**: 617. <https://doi.org/10.1097/QCO.0000000000000597>
- 8 AL-Quraini M, Rizvi M, AL-Jabri Z et al. Assessment of in-vitro synergy of fosfomycin with meropenem, amikacin and tigecycline in whole genome sequenced extended and pan drug resistant *Klebsiella pneumoniae*: exploring a colistin sparing protocol. *Antibiotics* 2022; **11**: 153. <https://doi.org/10.3390/antibiotics11020153>
- 9 Bakthavatchalam YD, Shankar A, Muthurandhi Sethuvel DP et al. Synergistic activity of fosfomycin–meropenem and fosfomycin–colistin against carbapenem resistant *Klebsiella pneumoniae*: an in vitro evidence. *Future Sci OA* 2020; **6**: FSO461. <https://doi.org/10.2144/fsoa-2019-0074>
- 10 EUCAST. Breakpoint tables for interpretation of MICs and zone diameters, version 14.0. 2024. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_14.0_Breakpoint_Tables.pdf.
- 11 Eliopoulos GM, Moellering RC Jr. Antimicrobial combinations. In: Lorian V, ed. *Antibiotics in Laboratory Medicine*. Williams & Wilkins Co., 1996; 330–96.
- 12 Wicha SG, Chen C, Clewe O et al. A general pharmacodynamic interaction model identifies perpetrators and victims in drug interactions. *Nat Commun* 2017; **8**: 2129. <https://doi.org/10.1038/s41467-017-01929-y>
- 13 Akaike H. A new look at the statistical model identification. *IEEE Trans Autom Control* 1974; **19**: 716–23. <https://doi.org/10.1109/TAC.1974.1100705>
- 14 Trang M, Griffith DC, Bhavnani SM et al. Population pharmacokinetics of meropenem and vaborbactam based on data from noninfected subjects and infected patients. *Antimicrob Agents Chemother* 2021; **65**: e0260620. <https://doi.org/10.1128/AAC.02606-20>
- 15 Pfausler B, Spiss H, Dittrich P et al. Concentrations of fosfomycin in the cerebrospinal fluid of neurointensive care patients with ventriculostomy-

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- associated ventriculitis. *J Antimicrob Chemother* 2004; **53**: 848–52. <https://doi.org/10.1093/jac/dkh158>
- 16** EMA. ICH M10 on bioanalytical method validation – scientific guideline, Step 5. 2023. <https://www.ema.europa.eu/en/ich-m10-bioanalytical-method-validation-scientific-guideline>.
- 17** Ito R, Mustapha MM, Tomich AD et al. Widespread fosfomycin resistance in Gram-negative bacteria attributable to the chromosomal *fosA* gene. *mBio* 2017; **8**: e00749-17. <https://doi.org/10.1128/mbio.00749-17>
- 18** Wang Y-P, Chen Y-H, Hung I-C et al. Transporter genes and *fosA* associated with fosfomycin resistance in carbapenem-resistant *Klebsiella pneumoniae*. *Front Microbiol* 2022; **13**: 816806. <https://doi.org/10.3389/fmicb.2022.816806>
- 19** Wicha SG, Kloft C. Simultaneous determination and stability studies of linezolid, meropenem and vancomycin in bacterial growth medium by high-performance liquid chromatography. *J Chromatogr B* 2016; **1028**: 242–8. <https://doi.org/10.1016/j.jchromb.2016.06.033>
- 20** Rasmussen BA, Bush K. Carbapenem-hydrolyzing beta-lactamases. *Antimicrob Agents Chemother* 1997; **41**: 223–32. <https://doi.org/10.1128/AAC.41.2.223>
- 21** Calderaro A, Buttrini M, Piergianni M et al. Evaluation of a modified meropenem hydrolysis assay on a large cohort of KPC and VIM carbapenemase-producing Enterobacteriaceae. *PLoS One* 2017; **12**: e0174908. <https://doi.org/10.1371/journal.pone.0174908>
- 22** Samonis G, Maraki S, Karageorgopoulos DE et al. Synergy of fosfomycin with carbapenems, colistin, netilmicin, and tigecycline against multidrug-resistant *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* clinical isolates. *Eur J Clin Microbiol Infect Dis* 2012; **31**: 695–701. <https://doi.org/10.1007/s10096-011-1360-5>
- 23** Kroemer N, Martens M, Decusser J-W et al. Evaluation of *in vitro* pharmacodynamic drug interactions of ceftazidime/avibactam and fosfomycin in *Escherichia coli*. *J Antimicrob Chemother* 2023; **78**: 2524–34. <https://doi.org/10.1093/jac/dkad264>
- 24** Kroemer N, Amann LF, Farooq A et al. Pharmacokinetic/pharmacodynamic analysis of ceftazidime/avibactam and fosfomycin combinations in an *in vitro* hollow fiber infection model against multidrug-resistant *Escherichia coli*. *Microbiol Spectr* 2024; **12**: e0331823. <https://doi.org/10.1128/spectrum.03318-23>
- 25** Fois M, De Vito A, Cherchi F et al. Efficacy and safety of ceftazidime-avibactam alone versus ceftazidime-avibactam plus fosfomycin for the treatment of hospital-acquired pneumonia and ventilator-associated pneumonia: a multicentric retrospective study from the SUSANA cohort. *Antibiotics* 2024; **13**: 616. <https://doi.org/10.3390/antibiotics13070616>
- 26** Wenzler E, Ellis-Grosse EJ, Rodvold KA. Pharmacokinetics, safety, and tolerability of single-dose intravenous (ZTI-01) and oral fosfomycin in healthy volunteers. *Antimicrob Agents Chemother* 2017; **61**: e00775-17. <https://doi.org/10.1128/aac.00775-17>
- 27** Abdallah TAK, Elajez R, Ibrahim TB et al. Efficacy and safety of intravenous fosfomycin for the treatment of difficult-to-treat Gram-negative bacterial infections. *J Infect Public Health* 2021; **14**: 1620–2. <https://doi.org/10.1016/j.jiph.2021.09.025>

5.2 Publication II

A pharmacometric model assessing the *in vitro* synergistic effect of a bacteriophage-polymyxin B combination in a clinical multidrug-resistant *K. pneumoniae* isolate

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Synopsis

Another approach in finding rational combination therapies against MDR pathogens is to combine conventional small molecule antibiotics with novel bacteriophages. Even if bacteriophages offer a promising alternative, their clinical application comes with challenges as high specificity resulting in fast resistance development. Our idea in this study was to overcome this limitation of bacteriophage monotherapy by combining them with polymyxin B, as one of the clinically used last-resort antibiotics against *K. pneumoniae* infections. For this we investigated phage kinetics and pharmacodynamic interactions between novel phage pK8 and polymyxin B against a clinically isolated *K. pneumoniae* strain tested to be polymyxin B resistant. Time-kill assay data with frequent sampling of phages and bacteria were combined with pharmacometric modelling to describe not only phage-bacteria kinetics, but also killing dynamics of mono- and combination therapy. The findings showed that the combination treatments associated with high phage doses allowed for higher killing rates and suppression of regrowth of bacteria, while monotherapies failed. The developed pharmacometric model describes observed synergy as a resensitization of polymyxin B when combined with phage pK8 and proves the concept and paves the way for further *in vivo* investigations validating bacteriophage-based combination therapies to fight MDR infections.



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A pharmacometric model assessing the *in vitro* synergistic effect of a bacteriophage-polymyxin B combination in a clinical multidrug-resistant *K. pneumoniae* isolate

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ABSTRACT

Background: *Klebsiella pneumoniae* represents one of the most critical pathogens globally and bacteriophage (phage) therapy offers a promising alternative for treatment. Phage therapy poses challenges due to its high specificity and fast resistance development in bacteria. One promising option to overcome these challenges is combining phages with conventional antibiotics.

Objective: This study investigates phage kinetics and pharmacodynamic interactions between novel phage pK8 and polymyxin B against a clinical multidrug-resistant *K. pneumoniae*.

Methods: Time-kill assays with time-dissolved sampling of phages and bacteria were coupled with pharmacometric modelling to describe phage-bacteria kinetics and killing dynamics. Additionally, different interaction models were investigated to assess the observed synergy between phage pK8 and polymyxin B.

Results: Key findings reveal that while polymyxin B alone showed no effect and phage pK8 alone was not efficacious enough to prevent the regrowth of *K. pneumoniae* II-503, their combined application resulted in notable bactericidal effects up to complete eradication. This was particularly notable in scenarios with higher phage doses. The developed pharmacokinetic/pharmacodynamic model describes synergy as bacterial resensitisation to polymyxin B when combined with phage pK8.

Conclusions: The study shows that phage pK8 and polymyxin B combination effectively combats a clinical polymyxin-resistant *K. pneumoniae* strain. These promising results pave the way for further *in vivo* studies to validate and refine treatment strategies for tackling multidrug-resistant infections.

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1. Introduction

Multidrug-resistant Gram-negative bacteria have become an international health problem, gaining major attention due to the lack of effective and safe treatment options, and the low pace in the development of novel antimicrobial agents [1,2]. *Klebsiella pneumoniae* represents one of the most critical pathogens, causing severe infections worldwide, associated with high morbidity and mortality [3,4]. Bacteriophage (phage) therapy offers a promising alternative, particularly when effective treatment options are limited,

bringing renewed attention to this previously abandoned approach [5]. Phage therapy has been practiced for almost a century in Eastern Europe [6], although there are only a few therapies approved relative to traditional antibiotics. The principles of pharmacology cannot be applied to phages, as phages replicate in the presence of their target while simultaneously functioning as an anti-infective agent against it. Moreover, phages are isolated and actively cultivated against a specific pathogen, consequently displaying a narrow spectrum of antimicrobial activity [7]. This specificity has the disadvantage that the effectiveness of an individual phage therapy cannot be directly transferred to real-world scenarios with a range of biological variability of infecting pathogens. In addition, resistance development of a bacterium against phages usually occurs rapidly [8]. Hence, systematic killing kinetics studies are needed to understand the relationships between the multiplicity of infection (MOI), phage killing and emergence of resistance

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[9]. One potential solution to the aforementioned challenges is the use of combination therapy involving phages and antibiotics [10–12]. Han et al. have mechanistically investigated the combination of polymyxin B with the novel phage pK8 against a polymyxin-resistant *mcr-1*-carrying clinical isolate *K. pneumoniae* II-503 using *in vitro* time-kill assays [13]. The metabolomic analyses provided mechanistic insights to bacterial killing by phage-polymyxin B combination therapy. Polymyxins exert their antimicrobial action by permeabilising the bacterial outer membrane through interaction with the lipid A component of lipopolysaccharides [14], while phages use receptor-binding proteins to recognise and infect host bacteria, propagate within them, and ultimately cause cell lysis [15]. The current study involves the exposure of *K. pneumoniae* II-503 to various MOIs combined with different concentrations of polymyxin B. We further extended the *in vitro* investigation of phage pK8 and polymyxin B by assessing pharmacodynamic (PD) interactions through pharmacometric modelling with the aim to better understand the interaction from a quantitative perspective and to corroborate the mechanism of interaction. Therefore, we evaluated various structural models to describe the infection-replication-lysis cycle between phages and bacteria. Finally, we investigated different PD interaction models by testing different possible mechanisms of interaction and compared the *in silico* findings to the mechanistic insight by Han et al. [13].

2. Material and methods

2.1. Bacterial isolate and antibiotics

K. pneumoniae II-503 is an *mcr-1*-carrying clinical isolate, demonstrating resistance to polymyxins with a minimum inhibitory concentration of 8 mg/L [16]. The MIC was determined with a broth microdilution method using cation-adjusted Mueller-Hinton Broth (CAMHB) [17]. Sterile solutions of polymyxin B (sulphate; Beta Pharma, Shanghai, China) were prepared by filter sterilisation through 0.22 µm syringe filters (Sartorius, Germany).

2.2. Bacteriophage isolation and amplification

The phage pK8 was isolated with *K. pneumoniae* II-503 as the host strain [13]. The amplification of pK8 was conducted using a plate lysate method as described in the Phage on Tap protocol [18]. Plaque assay was conducted by serially diluting phage stock in SM buffer (50 mM Tris-HCl pH 7.4, 100 mM NaCl, 8 mM MgSO₄) and spotting (10 µL) on a double-layer agar seeded with *K. pneumoniae* II-503.

2.3. Static time-kill assays

The time-kill kinetics were conducted with *K. pneumoniae* II-503 at starting inocula of 10⁶ and 10⁸ cfu/mL. Briefly, a single colony was inoculated in 10 mL CAMHB and incubated over 16 h at 37 °C. The overnight culture was diluted in 1:100 with fresh CAMHB and incubated for ~2 h to achieve an OD_{600nm} of 0.5 (equivalent to 10⁸ cfu/mL). For groups with a starting inoculum of 10⁶ cfu/mL, the culture of OD_{600nm} of 0.5 was further diluted at 1:100 with fresh CAMHB. Treatment groups include polymyxin B ranging from 2 to 16 mg/L and phage pK8 ranging from MOI 0.000001 to 50. At 0, 1, 2, 4, 6 and 24 h, 200 µL of the culture was collected and centrifuged at 10,000 × g for 10 min at 4 °C. The bacterial pellet was resuspended in an equal volume of 0.9% NaCl solution and plated on nutrient agar. The supernatant of groups containing phage pK8 was plated on double-layer agar with *K. pneumoniae* II-503. The agar plates were incubated for ~16 h at 37 °C, and bacterial colonies and phage plaques were enumerated.

2.4. Pharmacometric modelling

Nonlinear-mixed-effects modelling was conducted on observed bacterial and phage counts over time in static time-kill assays, using Monolix® 2023R1 (Lixsoft, France). Quantitative analyses were performed using the Stochastic Approximation Expectation Maximisation (SAEM) algorithm for maximum-likelihood estimations. Below limit of quantification data was handled using the M3 method, as implemented in Monolix, where such data is treated as censored in the likelihood calculation [19,20]. The model (Fig. 1) was patterned after a previously published model with further modifications [21]. It consisted of three bacterial compartments representing phage-susceptible (B_S), phage-resistant (B_R), and phage-infected-bacteria (B_I), as well as one phage compartment addressing free phage particles (V). The developed model describes the binding of free phage particles to phage-susceptible bacteria (B_S), followed by the lysis of bacteria and the release of a certain number of phage particles per bacterium. Simultaneously, phage-susceptible bacteria (B_S) can develop resistance against phage-binding following a specific mutation rate constant. Antimicrobial effects of polymyxin B were assessed using slope or maximum effect (Emax) models, where further information can be found in Supplementary Text S1.

When bacteria were exposed to phages, the implementation of the aforementioned infection-replication-lysis cycle results in the following differential equations with bacterial growth rate constant k_g , maximum bacterial capacity B_{max} , burst size N_{burst} , infected bacteria lysis rate constant k_{lysis} , phage to host binding rate constant k_{bind} , thermodynamic phage decay rate constant k_{decay} and bacterial resistance rate constant k_{mut} :

$$\frac{dV}{dt} = N_{burst} \times k_{lysis} \times B_I - k_{bind} \times V \times B_S - k_{decay} \times V \quad (1)$$

$$\frac{dB_S}{dt} = k_g \times \left(1 - \frac{B_S + B_R + B_I}{B_{max}}\right) \times B_S - k_{bind} \times V \times B_S - k_{mut} \times B_S \quad (2)$$

$$\frac{dB_R}{dt} = k_g \times \left(1 - \frac{B_S + B_R + B_I}{B_{max}}\right) \times B_R + k_{mut} \times B_S \quad (3)$$

$$\frac{dB_I}{dt} = k_{bind} \times V \times B_S - k_{lysis} \times B_I \quad (4)$$

Different PD interaction models were implemented following four principle in biologically plausible assumptions on this particular combination therapy: synergistic PD interaction addressing (i) a resensitisation of polymyxin B effect in combination with phages, (ii) a suppression of resistance to phages, (iii) an increase of k_{bind} due to polymyxin B interacting with the outer membrane and (iv) a reduction of k_g . The modelling effects of (i) was performed as an additional effect only occurring with combined polymyxin B and phage exposure. Effect size calculation was done similarly to modelling effects of a traditional drug with a power model but as a function of both the MOI_{t=0} (ln(pfu/mL)/ln(cfu/mL)) and the concentration of polymyxin B C_{PMB} with slope parameter of the resensitisation. Slope_{PMB} and Hill coefficient H_{PMB} and H_{INT} :

$$E_{PMB}(C_{PMB}, MOI_{t=0}) = Slope_{PMB} \times (MOI_{t=0})^{H_{INT}} \times C_{PMB}^{H_{PMB}} \quad (5)$$

In the case of (ii), (iii) and (iv) the General Pharmacodynamic Interaction (GPDI) model [22] was applied. In the GPDI model, the GPDI term is a function of the perpetrator drug concentration, which affects a PD parameter θ (typically EC50 or Emax, here: k_{mut} , k_{bind} , k_g) of the victim drug with INT as maximum possible fractional shift of the PD parameter θ , $EC50_{INT}$ as potency of the

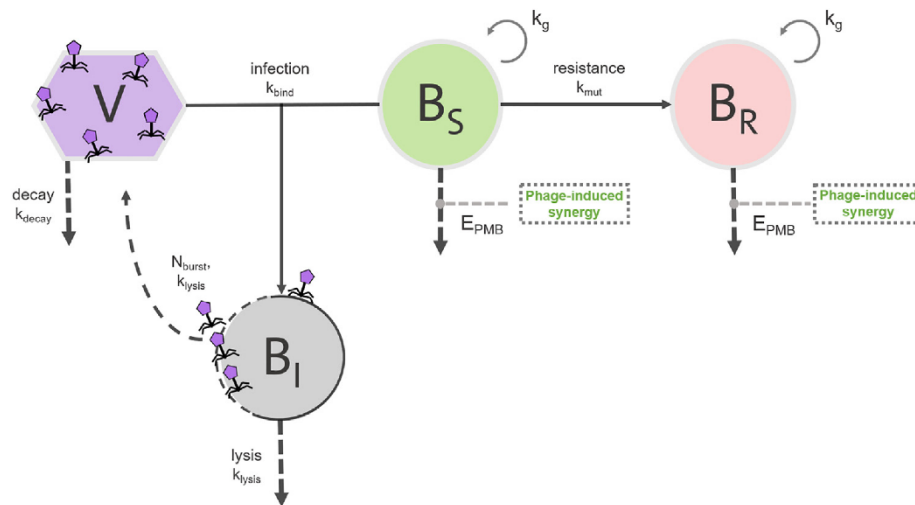


Fig. 1. Schematic representation of final pharmacometric model for novel phage pK8 and polymyxin B (PMB) against *K. pneumoniae* II-503 in static time-kill assays. B_S : compartment addressing phage-susceptible bacteria; B_R : compartment addressing phage-resistant bacteria; B_I : compartment addressing phage-infected bacteria; V : free phage particles; k_g : growth rate constant; N_{burst} : burst size; k_{lysis} : infected bacteria lysis rate constant; k_{bind} : phage to host binding rate constant; k_{decay} : thermodynamic phage decay rate constant; k_{mut} : bacterial resistance rate constant; E_{PMB} : effect of polymyxin B on bacteria with synergy when combined with phage pK8.

perpetrator and H_{INT} representing the sigmoidicity of the interaction (Eq. (6)). Further information can be found in Supplementary Text S2.

$$\theta \times \left(1 + \frac{INT \times C_{INT}^{H_{INT}}}{EC50_{INT}^{H_{INT}} + C_{INT}^{H_{INT}}} \right) \text{ for } \theta \in \{k_{mut}, k_{bind}, k_g\} \quad (6)$$

Exemplified for modelling polymyxin B as perpetrator of the PD interaction reducing the k_{mut} of bacteria, the following equation results:

$$k_{mut, INT} = k_{mut} \times \left(1 - \frac{INT \times C_{PMB}^{H_{INT}}}{EC50_{INT}^{H_{INT}} + C_{PMB}^{H_{INT}}} \right) \quad (7)$$

Model selection of competing pharmacokinetic/pharmacodynamic (PK/PD) models (e.g., different PD interaction) was performed after inspecting biological plausibility of parameters, parameter uncertainty and the Akaike-Information-Criterion for identifying the one representing the observed data best [23].

3. Results

3.1. Static time-kill assays

3.1.1. Phage kinetics

Observed free phage particles are visualised as triangles in Fig. 2. Phage concentration increased and reached a plateau of approximately 10^7 – 10^9 pfu/mL in all experiments, with a slow decline of the phage concentration towards 24 h. In some scenarios, starting at a bacterial inoculum $t = 0$ below approximately 10^5 cfu/mL (e.g., plots R, S, X, Y), phage proliferation occurred slower in the early phase of the experiment. Also, this was observed in certain instances of very low initial phage concentrations (e.g., plots G and K). Conversely, in other cases (e.g., plot H), this trend was not observed.

3.1.2. Bacterial kinetics

Observed bacterial counts are visualised as dots in Fig. 2. In control scenarios (plot A) bacteria showed growth until reaching a maximum bacterial capacity of $10^{7.5}$ – $10^{8.5}$ cfu/mL. Single drug

exposure of polymyxin B of up to 16 mg/L resulted in no observable killing effect, thus bacterial growth was comparable to control scenarios (plots B–F). Exposure to phage pK8 alone resulted in killing or bacteriostasis in the early period of all tested scenarios. Phage pK8 at MOIs ranging from 0.00001 to 46, demonstrated substantial initial killing in the early phase with the bacterial count falling below the limit of quantification at 1 h, followed by regrowth of bacteria in the late phase. In some cases, bacterial concentrations even dropped below the limit of quantification (plots H, N, O, Q, T). In scenarios involving combined exposure to polymyxin B (2–16 mg/L) and phage pK8 (plots AA–AJ), similar observations were made for experiments with initial MOIs below one: strong initial killing of bacteria in the early phase followed by subsequent regrowth. Scenario plot AA, which represents the combination of initial $MOI_{t=0} = 0.91$ with polymyxin B at 2 mg/L, demonstrated bacterial regrowth that did not exceed the initial inoculum $t = 0$. One notable scenario with $MOI_{t=0} = 0.91$, combined with polymyxin B 4 mg/L (plot AF) exhibited rapid initial bacterial killing, reducing the bacterial concentration below the limit of quantification by $t = 1$ h and maintaining this level until the final sampling at 24 h. Furthermore, in this scenario no bacteria could be detected at any sampling points later than 0 h indicating total eradication of bacteria. The synergistic activity of phage pK8 and polymyxin B was not as prominent with the higher starting bacterial inoculum of 10^8 cfu/mL.

3.2. Pharmacometric modelling

The quantitative assessment of the PD interaction of phage pK8 and polymyxin B is detailed in Fig. 1, Table 1 and Fig. S1, providing an overview of the parameter estimates and their implications. Table 1 shows the final parameter estimates of the pharmacometric model with their confidence intervals. Single drug effect parameters of polymyxin B approached zero, since no killing effect of polymyxin B alone was observed. These parameters were consequently removed from the model. Maximum bacterial capacity B_{max} was estimated to be 8.03 (7.62–8.50) $\log_{10}$ cfu/mL and inter-experimental variability was successfully described with a random effect of 13.0% (9.75–17.5%). Binding kinetics rate constant, k_{bind} describing phages (V) infecting susceptible bacteria (B_S) was fixed to $10^{-6.9}$ h $^{-1}$ from previously published literature [21]. Lysis rate

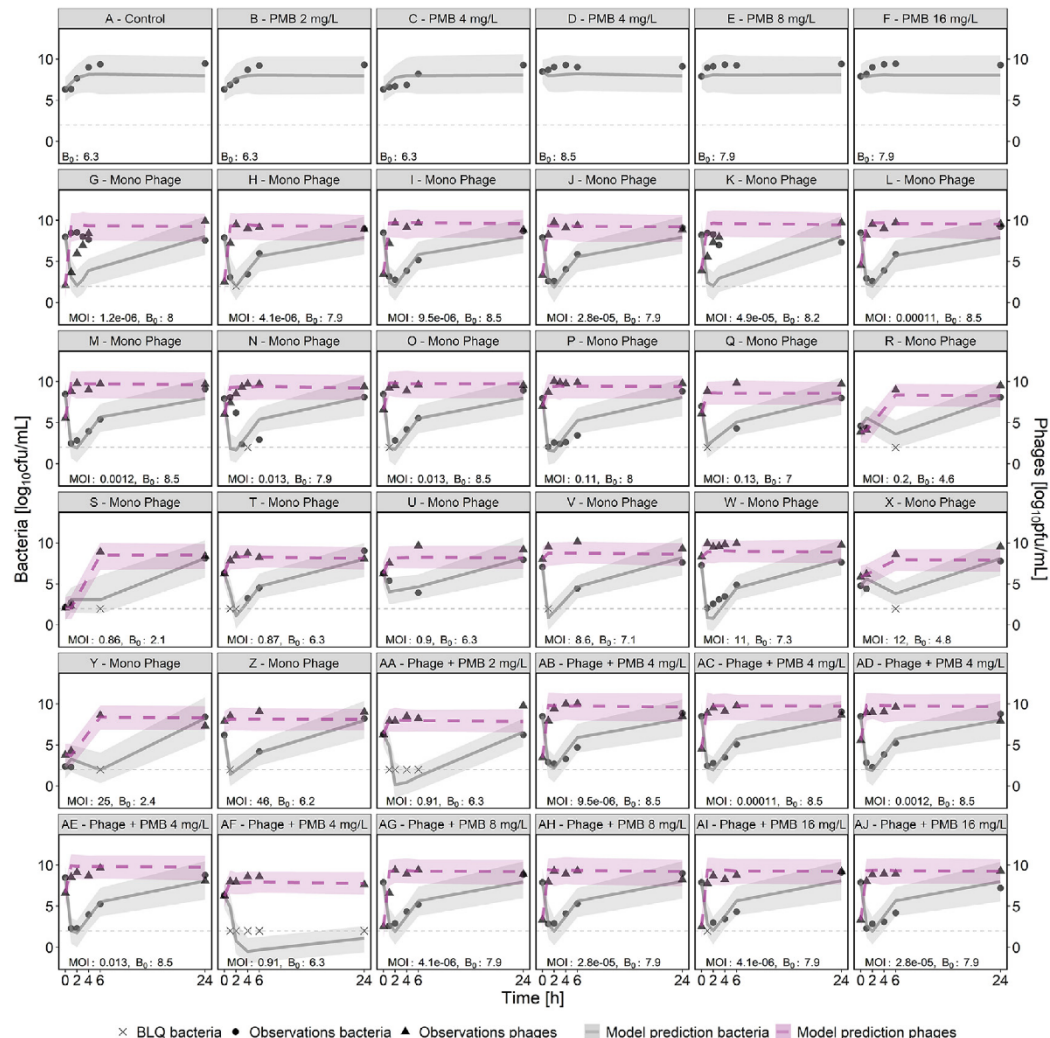


Fig. 2. Stratified visual predictive checks of final pharmacokinetic/pharmacodynamic model characterising the bacterial concentration and phage load. Dots: observations bacteria; triangles: observations phages; solid grey line: median bacteria prediction of model incorporating synergy; shaded grey area: 90% prediction intervals of bacteria; solid purple line: median phage prediction of model incorporating synergy, shaded purple area: 90% prediction intervals of phages; MOI: multiplicity of infection; B_0 : inoculum_{t=0h} bacteria.

constant of infected bacteria (B_i) was estimated to 15.5 (14.2–17.5) h^{-1} which translates to an infection-to-lysis half-time of 2.68 min, and burst size N_{burst} estimated to a value of 36.1 (29.6–44.1). The phage decay rate constant k_{decay} , describing reduction of phage particles in absence of bacteria due to thermodynamic and other processes, was fixed to a literature value [24]. Synergy, modelled as a resensitisation of the effect of polymyxin B when combined with phage pK8 (AIC = 1781), outperformed the competing models describing synergy as a suppression of resistance development to phages (AIC = 1815), an increase of k_{bind} due to polymyxin B interacting with the outer membrane (non-convergence) or a reduction of k_g (AIC = 1847). The resensitisation of polymyxin B effect only occurring in combination with phage pK8 was estimated as a $Slope_{PMB} = 1.88$ (1.68–2.14) $\ln(cfu)/\ln(pfu) \times L/mg \times h^{-1}$. With H_{INT} set to 20, the synergy was modelled as an on-off effect depending on the initial $MOI_{t=0}$. The visual predictive checks (Fig. 2) show an overall high quality of the model prediction when compared with observed data. The developed model described both the observed bacterial and phage count in mono and combination scenarios from low to high MOIs well, while also identifying the interaction-type of the combination. Misspecifications occurred

in only two scenarios (plots G and K), where the predicted early proliferation and the effect of phage pK8 were overestimated, although the late phase was still accurately predicted.

4. Discussion

The present study demonstrates the successful development and application of a pharmacometric model to quantitatively describe phage killing kinetics and the PD interaction in time-kill assays when combined with polymyxin B against a polymyxin-resistant *mcr-1*-carrying *K. pneumoniae* isolate. Key findings reveal that while polymyxin B alone showed no effect, its combined application with phage pK8 resulted in notable bactericidal effects and decreased bacterial burden, particularly in scenarios with higher initial MOIs. Phage pK8 alone was also able to significantly reduce bacterial concentrations, yet in none of the tested scenarios was it efficacious enough to prevent the regrowth of *K. pneumoniae* II-503.

Our data build upon previous work by Han et al., which elucidated the synergistic killing of phage pK8 and polymyxin B combination using metabolomics [13]. Han et al. revealed that

Table 1

Parameter estimates for final pharmacokinetic/pharmacodynamic model of polymyxin B and novel phage pK8 against tested *K. pneumoniae* strain. Confidence intervals (95% CI) were calculated using log-likelihood profiling.

Parameter	Final estimate (95% CI)
Bacterial system	
Growth rate constant bacteria k_g (h^{-1})	2.13 (2.02–2.35)
Resistance to phages rate constant k_{mut} (h^{-1})	$10^{-6.77}$ ($10^{-6.31}$ – $10^{-7.22}$)
Maximum bacterial capacity B_{max} ($\log_{10}$ cfu/mL)	8.03 (7.62–8.50)
Phage infection-replication-lysis cycle	
Binding kinetics rate constant of phages to susceptible bacteria (B_s) k_{bind} (h^{-1})	$10^{-6.9}$ (FIX) ^a
Phage-induced lysis rate constant of infected bacteria (B_i) k_{lysis} (h^{-1})	15.5 (14.2–17.5)
Burst size of new phage particles after bacterial lysis N_{burst} (pfu $\times$ cfu ⁻¹)	36.1 (29.6–44.1)
Thermodynamic phage decay rate constant k_{decay} (h^{-1})	0.011 (FIX) ^b
Interaction-model parameters	
Resensitisation of polymyxin B in presence of phages	
Slope polymyxin B effect $Slope_{PMB}$ ($\ln(\text{cfu/mL})/\ln(\text{pfu/mL}) \times \text{mL/g} \times h^{-1}$)	1.88 (1.68–2.14)
Hill coefficient of interaction H_{PMB} (–)	0.468 (0.463–0.473)
Hill coefficient of interaction H_{INT} (–)	20 (FIX)
Inter-experimental variability	
Random effect maximum bacterial capacity B_{max} (%CV) ^c	13.0 (9.75–17.6)
Residual variability model	
Additive residual variability bacteria ($\log_{10}$ cfu/mL)	0.93 (0.83–1.06)
Additive residual variability phages ($\log_{10}$ pfu/mL)	0.84 (0.72–0.97)

^a parameter fixed to literature value; Delattre et al. [21].

^b parameter fixed to literature value; Cairns et al. [24].

^c %CV was calculated as follows: $\%CV = \sqrt{\exp(\omega^2)} - 1 \times 100\%$.

although pK8 is a highly virulent phage, *K. pneumoniae* II-503 could rapidly developed resistance against it. Our pharmacometric model, structured to consist of phage-susceptible bacteria B_s transition to phage-resistant bacteria B_R following resistance rate constant k_{mut} , aligns with their findings. In contrast to Delattre et al. model, which used multiple infected compartments and included a pharmacokinetic (PK) component for murine *in vivo* studies, we employed a simplified structure with a single infected bacterial population, reflecting our focus on *in vitro* dynamics [21]. It is important to note that in the study by Han et al., distinct differences in cell surface morphology and membrane potential of *K. pneumoniae* II-503 isolate were observed when treated with polymyxin B, phage pK8 and the combination of both. Polymyxin B alone caused outer membrane disorganisation even without showing killing effects while combination treatment showed a mixture of lysed cells and a disorganised outer membrane. Metabolomic analysis revealed that phage pK8 and polymyxin B induced similar metabolic changes to phage alone at 4 h, but only the combination treatment caused a significant metabolic change at 24 h, indicating that the synergy was driven by the initial killing activity from the phage and the suppression of resistance was attributed by the combination of phage and polymyxin B. Following the evaluation of four biologically plausible mechanisms to describe the PD interaction and a comparison of our *in silico* findings with the significant mechanistic discoveries made by Han et al., we can affirm agreement. The pharmacometric model demonstrating synergy as resensitisation of polymyxin B when combined with phage pK8 showed the best performance in describing the observed data support the idea of collateral sensitivity regarding polymyxin B efficacy [25,26].

Our approach enhances the understanding of PD interactions between conventional and alternative antimicrobials by quantitatively describing their combined effect sizes. It also highlights the potential for these methodologies to be applied to other combinations, whether *in vitro* or in more distinct contexts such as *in vivo* animal model studies, while considering their inherent differences. Coupling *in vitro* studies with modelling techniques serves as important tools for elucidating phage PK/PD. These approaches have the potential to inform the rationale for combination thera-

pies, the role of the MOI in treatment success, and provide insights into bacterial killing and resistance dynamics. In this work, key PD and biological parameters such as the burst size, lysis rate, bacterial growth rate, resistance emergence rate, and maximum bacterial capacity, were estimated directly from our experimental data. Other variables, including the phage adsorption rate (k_{bind}) and phage decay rate (k_{decay}), were fixed based on established literature values. It should be noted that all parameter estimates remain specific to the tested phage-bacterium pair, and our model did not explicitly account for evolutionary trade-offs or strain-specific adsorption rates. In addition, limited late time-point sampling may affect predictions of regrowth and resistance. Broader data from multiple antibiotics, bacterial strains, and extended time courses will be essential to improve the robustness and generalizability of future models.

This study employs time-dissolved sampling of both bacteria and phages, rather than relying on checkerboard assays with insensitive turbidity criteria, to better understand the underlying kinetics and interaction dynamics. This methodology, as detailed in previous literature [9,21], provides a more accurate evaluation of how these interactions might influence therapeutic outcomes. Our PD interaction modelling results suggest that phage pK8 may enhance the effectiveness of polymyxin B by modulating the bacterial outer membrane. Whether comparable synergistic effects would be achieved with other bacterial membrane-disrupting antibiotics, remains to be investigated in future studies, as differences in cell surface interactions or bacterial susceptibility could influence the interaction dynamics.

As our analysis is based on *in vitro* data from a single phage-bacterium pair, these findings cannot be directly translated to clinical trials or patient care. While our model predictions closely matched the observed dynamics for this specific combination and offer valuable mechanistic insights, key infection parameters may differ with other strains or in different environments. Further *in vivo* studies and validation in diverse clinical isolates are needed to inform phage selection and optimise dosing for real-world application. The potential interactions with the host immune system can be quantitatively assessed through *in vivo* animal experiments and subsequent strengthen the pharmacometric model. Incorporat-

ing coevolution processes with multiple phage and bacterial strains in future models would also help enhance the translatability of phage-antibiotic studies.

Due to the high specificity of phages and limited time in deciding which therapy to apply, more data is needed to determine in which species or strains of bacteria the presumptive application of a specific phage or phage-antibiotic combination therapy is appropriate. Clinical evidence supporting specific combination therapies should be advanced, such as through case studies. Such studies have been conducted for adaptive and presumptive phage-cocktail treatments against urinary tract infections [27,28]. Notably, ongoing clinical efforts such as a randomised, placebo-controlled phase III trial of phage therapy combined with antibiotics for infective endocarditis (ClinicalTrials.gov Identifier: NCT06870409) – including patients infected by *K. pneumoniae* – underscore the increasing interest in bringing phage-antibiotic combinations into clinical practice [29]. For the first time, this study has successfully built a pharmacometric model describing the PK and PD of phage and polymyxin B against *K. pneumoniae*. Pharmacometric models play an essential role in clinical practice as they inform the design of effective phage-antibiotic combination therapies, improving treatment of multidrug-resistant infections and patient outcomes.

Ethical approval: Not required.

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Authors contributions: SCN, AF, JL and SGW designed the study. SCN planned experiments. SCN, HHY and HW performed experiments. AF conducted modelling analyses. MLH and YWL provided methodological guidance and contributed to the research. AF wrote the original draft, all authors read, edited and approved the final version of the manuscript.

Data availability: All original data generated during the study are included in the article in the form of figures and tables. For any further inquiries, please contact the corresponding author.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2025.107628.

References

- [1] Dutescu IA, Hillier SA. Encouraging the development of new antibiotics: are financial incentives the right way forward? A systematic review and case study. *Infect Drug Resist* 2021;14:415–34. doi:10.2147/IDR.S287792.
- [2] Lack of new antibiotics threatens global efforts to contain drug-resistant infections. <https://www.who.int/news/item/17-01-2020-lack-of-new-antibiotics-threatens-global-efforts-to-contain-drug-resistant-infections> [accessed 12.25.2024].
- [3] World Health Organization. WHO bacterial priority pathogens list, 2024: bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. <https://www.who.int/publications/i/item/9789240093461> [accessed 07.26.2024].
- [4] Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, Gray A, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet* 2022;399:629–55. doi:10.1016/S0140-6736(21)02724-0.
- [5] Lin DM, Koskella B, Lin HC. Phage therapy: an alternative to antibiotics in the age of multi-drug resistance. *World J Gastrointest Pharmacol Ther* 2017;8:162. doi:10.4292/wjgpt.v8.i3.162.
- [6] Diallo K, Dublanchet A. A century of clinical use of phages: a literature review. *Antibiotics* 2023;12:751. doi:10.3390/antibiotics12040751.
- [7] Pirisi A. Phage therapy—advantages over antibiotics? *Lancet* 2000;356:1418. doi:10.1016/S0140-6736(05)74059-9.
- [8] Kortright KE, Chan BK, Koff JL, Turner PE. Phage therapy: a renewed approach to combat antibiotic-resistant bacteria. *Cell Host Microbe* 2019;25:219–32. doi:10.1016/j.chom.2019.01.014.
- [9] Nang SC, Lin YW, Petrovic Fabijan A, Chang RYK, Rao GG, Iredell J, et al. Pharmacokinetics/pharmacodynamics of phage therapy: a major hurdle to clinical translation. *Clin Microbiol Infect* 2023;29:702–9. doi:10.1016/j.cmi.2023.01.021.
- [10] Ryan EM, Alkawareek MY, Donnelly RF, Gilmore BF. Synergistic phage-antibiotic combinations for the control of *Escherichia coli* biofilms *in vitro*. *FEMS Immunol Med Microbiol* 2012;65:395–8. doi:10.1111/j.1574-695X.2012.00977.x.
- [11] Knezevic P, Curcin S, Aleksic V, Petrusic M, Vlaski L. Phage-antibiotic synergism: a possible approach to combatting *Pseudomonas aeruginosa*. *Res Microbiol* 2013;164:55–60. doi:10.1016/j.resmic.2012.08.008.
- [12] Oechslin F, Piccardi P, Mancini S, Gabard J, Moreillon P, Entenza JM, et al. Synergistic interaction between phage therapy and antibiotics clears *Pseudomonas aeruginosa* infection in endocarditis and reduces virulence. *J Infect Dis* 2017;215:703–12. doi:10.1093/infdis/jiw632.
- [13] Han ML, Nang SC, Lin YW, Zhu Y, Yu HH, Wickremasinghe H, et al. Comparative metabolomics revealed key pathways associated with the synergistic killing of multidrug-resistant *Klebsiella pneumoniae* by a bacteriophage-polymyxin combination. *Comput Struct Biotechnol J* 2022;20:485–95. doi:10.1016/j.csbj.2021.12.039.
- [14] Velkov T, Thompson PE, Nation RL, Li J. Structure–activity relationships of polymyxin antibiotics. *J Med Chem* 2010;53:1898–916. doi:10.1021/jm900999h.
- [15] Nogueira CL, Pires DP, Monteiro R, Santos SB, Carvalho CM. Exploitation of a *Klebsiella* bacteriophage receptor-binding protein as a superior biorecognition molecule. *ACS Infect Dis* 2021;7:3077–87. doi:10.1021/acscinfecdis.1c00366.
- [16] Nang SC, Han ML, Yu HH, Wang J, Torres VVL, Dai C, et al. Polymyxin resistance in *Klebsiella pneumoniae*: multifaceted mechanisms utilized in the presence and absence of the plasmid-encoded phosphoethanolamine transferase gene *mcr-1*. *J Antimicrob Chemother* 2019;74:3190–8. doi:10.1093/jac/dkz314.
- [17] Kahlmeter G, Turnidge J. Wild-type distributions of minimum inhibitory concentrations and epidemiological cut-off values—Laboratory and clinical utility. *Clin Microbiol Rev* 2023;36:e00100–22. doi:10.1128/cmr.00100-22.
- [18] Bonilla N, Rojas MI, Netto Flores Cruz G, Hung S-H, Rohwer F, Barr JJ. Phage on tap—a quick and efficient protocol for the preparation of bacteriophage laboratory stocks. *PeerJ* 2016;4:e2261. doi:10.7717/peerj.2261.
- [19] Beal SL. Ways to fit a PK model with some data below the quantification limit. *J Pharmacokinet Pharmacodyn* 2001;28:481–504. doi:10.1023/a:1012299115260.
- [20] Bergstrand M, Karlsson MO. Handling data below the limit of quantification in mixed effect models. *AAPS J* 2009;11:371–80. doi:10.1208/s12248-009-9112-5.
- [21] Delattre R, Seurat J, Haddad F, Nguyen TT, Gaborieau B, Kane R, et al. Combination of *in vivo* phage therapy data with *in silico* model highlights key parameters for pneumonia treatment efficacy. *Cell Rep* 2022;39. doi:10.1016/j.celrep.2022.110825.
- [22] Wicha SG, Chen C, Clewe O, Simonsson USH. A general pharmacodynamic interaction model identifies perpetrators and victims in drug interactions. *Nat Commun* 2017;8:1–11. doi:10.1038/s41467-017-01929-y.
- [23] Akaike H. A new look at the statistical model identification. *IEEE Trans Autom Control* 1974;19:716–23. doi:10.1109/TAC.1974.1100705.
- [24] Cairns BJ, Timms AR, Jansen VAA, Connerton IF, Payne RJH. Quantitative models of *in vitro* bacteriophage-host dynamics and their application to phage therapy. *PLoS Pathog* 2009;5. doi:10.1371/journal.ppat.1000253.
- [25] Roemhild R, Andersson DI. Mechanisms and therapeutic potential of collateral sensitivity to antibiotics. *PLoS Pathog* 2021;17:e1009172. doi:10.1371/journal.ppat.1009172.
- [26] Yekani M, Azargun R, Sharifi S, Nabizadeh E, Nahand JS, Ansari NK, et al. Collateral sensitivity: an evolutionary trade-off between antibiotic resistance mechanisms, attractive for dealing with drug-resistance crisis. *Health Sci Rep* 2023;6:e1418. doi:10.1002/hsr2.1418.
- [27] Ujmajuridze A, Chanishvili N, Goderdzishvili M, Leitner L, Mehnert U, Chkhotua A, et al. Adapted bacteriophages for treating urinary tract infections. *Front Microbiol* 2018;9:1832. doi:10.3389/fmicb.2018.01832.

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- [28] Leitner L, Ujmajuridze A, Chanishvili N, Goderdzishvili M, Chkonia I, Riggava S, et al. Intravesical bacteriophages for treating urinary tract infections in patients undergoing transurethral resection of the prostate: a randomised, placebo-controlled, double-blind clinical trial. *Lancet Infect Dis* 2021;21:427–36. doi:[10.1016/S1473-3099\(20\)30330-3](https://doi.org/10.1016/S1473-3099(20)30330-3).
- [29] ClinicalTrials.gov Identifier: NCT06870409. Bacteriophages in addition to antibiotics for the treatment of patients with infective endocarditis. <https://clinicaltrials.gov/study/NCT06870409> [accessed 09.10.2025].

5.3 Publication III

Evaluation of *in vitro* pharmacodynamic drug interactions of ceftazidime-avibactam with tigecycline in ESBL- and carbapenemase producing *Escherichia coli* and *Klebsiella pneumoniae*

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Synopsis

Despite the fact that tigecycline is becoming less relevant as last-resort antibiotic against MDR pathogens due to safety concerns, it is reported to be used in combination therapy against difficult to treat infections. Since no quantitative assessment of the last-resort combination of ceftazidime/avibactam and tigecycline was available, we conducted a study to systematically evaluate their pharmacodynamic interactions in fifteen clinical or isogenic *E. coli* and *K. pneumoniae* isolates. For this we performed checkerboard assays with an adaptive experimental design, time-kill assays, and metabolomic analyses. The results revealed antagonistic interactions between ceftazidime/avibactam and tigecycline in majority of tested strains, limiting maximum effect sizes of the combination therapy to those of tigecycline monotherapy. Additionally, metabolomic findings corroborated the identified antagonism mechanistically. Our study concludes that the clinical benefit of ceftazidime/avibactam and tigecycline combination therapy would expect to have limited clinical benefit.



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Evaluation of *in vitro* pharmacodynamic drug interactions of ceftazidime-avibactam with tigecycline in ESBL- and carbapenemase producing *Escherichia coli* and *Klebsiella pneumoniae*



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ABSTRACT

Background: Combination therapy offers a promising option to enhance efficacy and prevent resistance. A comprehensive and quantitative assessment of the last-resort combination of ceftazidime/avibactam and tigecycline is not available.

Objective: This study systematically investigated the pharmacodynamic interaction between ceftazidime/avibactam and tigecycline in clinical and isogenic *Escherichia coli* and *Klebsiella pneumoniae* strains harbouring genes that encode various carbapenemases or ESBLs.

Methods: An adaptive *in vitro* 'dynamic' checkerboard design and pharmacometric modelling were employed for the evaluation of pharmacodynamic interactions in fifteen bacterial isolates. Additionally, time-kill assays and metabolomic analyses were used to provide mechanistic insights.

Results: Antagonistic drug interactions between ceftazidime/avibactam and tigecycline were identified in the majority of tested strains. Time-kill assays confirmed antagonistic interactions, with tigecycline limiting ceftazidime/avibactam total killing. Metabolomic analyses of mono and combined drug exposure to bacteria revealed matching metabolomes in tigecycline alone and the combination with ceftazidime/avibactam, corroborating the identified antagonism between these drugs.

Conclusions: Our study reveals that the antagonistic interaction between ceftazidime/avibactam and tigecycline can undermine ceftazidime/avibactam's efficacy, suggesting limited clinical benefit in combining these antibiotics. Therefore, further research is encouraged to explore this and alternative combinations or approaches that may offer better clinical outcomes.

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1. Introduction

Infections caused by multidrug-resistant *Enterobacteriaceae* such as ESBL-producing *Escherichia coli* (*E. coli*) and carbapenemase-producing *Klebsiella pneumoniae* (*K. pneumoniae*), representing WHO priority-1 pathogens [1], have become an international health problem and gained major attention due to the lack of

effective and safe treatment options and the low pace in the development of novel antimicrobial agents. Novel beta-lactam and beta-lactamase-inhibitor combinations, such as ceftazidime-avibactam have become available as promising options against those strains [2], but resistance against these antibiotics has already been reported [3,4]. Ceftazidime/avibactam is effective against KPC and OXA-48-producing *Enterobacteriaceae*, which drive resistance against ceftazidime alone. Tigecycline represents an alternative treatment option for infections caused by carbapenem-resistant *Enterobacteriaceae*, including those with NDM and OXA-48-like producers, especially when beta-lactams are ineffective or not tolerated [5]. Antimicrobial combination therapy in princi-

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Table 1
Determined MIC, EC₅₀ and E_{max} of ceftazidime/avibactam and tigecycline in tested strains.

Strain	Type	Resistance Genes	CZA			TGC		
			MIC [mg/L]	EC ₅₀ [mg/L]	E _{max} log ₁₀ [CFU/mL]	MIC [mg/L]	EC ₅₀ [mg/L]	E _{max} log ₁₀ [CFU/mL]
<i>E. coli</i> WT	Isogenic	empty plasmid	0.125	0.11	9.10	0.125	0.13	5.60
<i>E. coli</i> OXA-181	Isogenic	OXA-181	0.125	0.18	8.90	0.125	0.16	5.04
<i>E. coli</i> OXA-48	Isogenic	OXA-48	0.125	0.15	9.32	0.125	0.10	6.32
<i>E. coli</i> KPC-2	Isogenic	KPC-2	0.125	0.10	8.89	0.125	0.10	6.69
<i>E. coli</i> KPC-3	Isogenic	KPC-3	1	2.37	9.44	0.125	0.07	5.35
<i>E. coli</i> YAL_AMA	Clinical	CTX-M-15, OXA-244	0.125	0.10	9.09	0.25	0.11	4.88
<i>E. coli</i> JUM_JEA	Clinical	CTX-M-15, OXA-48	0.06	0.04	7.75	0.25	0.22	6.23
<i>E. coli</i> OLA_HAM	Clinical	OXA-244, CTX-M-15	0.5	0.21	9.22	0.25	0.17	4.69
<i>K. pneumoniae</i> WT	Isogenic	-	0.06	0.07	8.81	0.06	0.09	8.81
<i>K. pneumoniae</i> OXA-48	Isogenic	OXA-48	0.06	0.10	9.28	0.25	0.12	9.28
<i>K. pneumoniae</i> KPC-2	Isogenic	KPC-2	0.125	0.07	9.00	0.25	0.12	9.00
<i>K. pneumoniae</i> KPC-3	Isogenic	KPC-3	0.25	0.08	8.57	0.25	0.10	8.94
<i>K. pneumoniae</i> 2977	Clinical	KPC-2, CTX-M-2, OXA-9, TEM-1	1	0.67	9.39	0.125	0.15	2.94
<i>K. pneumoniae</i> N864	Clinical	KPC-3	2	2.04	9.75	0.5	0.34	3.51
<i>K. pneumoniae</i> R307	Clinical	KPC-2	2	0.56	9.36	0.25	1.15	7.65

Abbreviations: CZA: ceftazidime/avibactam; TGC: tigecycline.
Avibactam fixed to 4 mg/L.

ple may offer an opportunity to increase efficacy, protect against the emergence of resistance and in turn potentially even lower infection-related mortality. *In vitro* studies examining the pharmacodynamic interactions between ceftazidime/avibactam and tigecycline, particularly using the Fractional Inhibitory Concentration Index (FICI) in checkerboard assays, predominantly showed indifferent results, with approximately 65% of strains in one study and 18 out of 19 strains in another demonstrating this outcome [6,7]. However, these studies primarily used a turbidity read-out, which does not allow differentiation between effect sizes below the turbidity threshold, leaving details and mechanistic insights into this combination unexplored. Combination therapies that include tigecycline are still in clinical use, and previous studies have explored their potential benefits. Some studies indicate that including tigecycline in combination treatments can lead to lower clinical failure and all-cause mortality rates, though other research has not found a marked difference in mortality between those receiving combination therapy and those on monotherapy [8–10].

The aim of this study was to investigate on the ceftazidime/avibactam-tigecycline combination from a pharmacological and quantitative perspective. We performed an *in vitro* screening based on an optimized checkerboard design [11], and utilized a quantitative, model-based PD-interaction assessment using the General Pharmacodynamic Interaction (GPDI) model [12]. The GPDI model approach provides quantitative insights into the PD interaction by identifying the nature (antagonism, additivity, synergy), affected PD parameter (e.g., potency EC₅₀ or maximum effect E_{max}) as well as effect size as shift of the PD parameters. Moreover, metabolomics studies were conducted and compared to the PD interactions to provide mechanistic insight.

2. Materials and methods

2.1. Bacterial isolates

A total of 8 *E. coli* (5 isogenic and 3 clinical) and 7 *K. pneumoniae* (4 isogenic and 3 clinical) isolates carrying various resistance genes coding for ESBLs or carbapenemases were collected from University of Poitiers, France, Université Paris-Est-Créteil Val-De-Marne, France and Medical and Molecular Microbiology, University of Fribourg, Switzerland. Isogenic *E. coli* CFT073 and isogenic *K. pneumoniae* CIP53153 strains carried the cloning vector pACYC184 with chloramphenicol and/or tetracycline resistance gene for the selection of recombinant bacteria from non-recombinant bacte-

ria using Columbia agar (Carl Roth, Germany) containing 25 mg/L chloramphenicol or 10 mg/L tetracycline. Isogenic strains of *E. coli* and *K. pneumoniae* were derived from their respective wild-type (WT) strains, with plasmids modified to include specific resistance genes listed in Table 1.

2.2. Chemicals

Tigecycline was obtained from USP (USA, LOT: R09410), ceftazidime, chloramphenicol from Sigma-Aldrich (USA, LOT: LRAC5653, 0000082469), avibactam sodium from Ambeed Inc. (USA, LOT: A169351), tetracycline hydrochloride from Chemodex (Switzerland).

2.3. Antimicrobial susceptibility testing and quantification of viable bacteria

Minimal inhibitory concentrations (MICs) of ceftazidime/avibactam and tigecycline were determined as triplicates using the broth microdilution method in accordance to the European Committee of Antimicrobial Susceptibility Testing (EUCAST) [13]. Detailed information on antimicrobial susceptibility testing and quantification of viable bacteria is given in Supplementary Text S1.

2.4. EC₅₀ and E_{max} determination

The half-effective concentration EC₅₀ and the maximal effect E_{max} were estimated from triplicate data of experiments, by inoculating and pre-incubating the bacterial isolates (approx. 1 × 10⁶ CFU/mL) in ca-MHB at 37°C in ambient air for 2 h using 48-well-plates followed by adding a series of 2-fold dilutions of each antibiotic. The bacterial count was determined after incubating for 24 h as described above.

Drug effect was defined as the reduction in bacterial population using the sigmoidal maximum effect model (Eq. [1]) describing the drug effect *E* as function of its concentration *C* and the model parameters E_{max}, EC₅₀ and *H* (Hill factor).

$$E(C) = \frac{E_{\max} \times C^H}{EC_{50}^H + C^H} \quad (1)$$

For each antibiotic, the EC₅₀ and E_{max} were obtained through analysing the concentration-effect-relationship using a sigmoidal maximal effect model and non-linear fitting methods. Detailed information is given in Supplementary Text S2 and Table S1.

2.5. PD interaction screening

An optimized 'dynamic' checkerboard design was chosen to efficiently screen for PD-interactions [11]. It combined ceftazidime/avibactam and tigecycline at different effective concentrations (EC_{08} , EC_{44} , EC_{82}) obtained from the EC_{50} and E_{max} determination. The experiments were conducted by inoculating bacterial isolates (approx. 1×10^6 CFU/mL) in ca-MHB. The inoculated wells were pre-incubated without drug at 37°C in ambient air for 2 h. Thereafter, the antibiotics were added and the well plates were incubated for 48 h at 37°C. The bacterial count was determined at 0, 24 and 48 h and subpopulations being phenotypically resistant to 5 x MIC were quantified at 0 and 48 h using Columbia agar plates containing each antibiotic at 5 x MIC. In the case of the tigecycline containing agar, pyruvate (2%) and ascorbic acid (0.3%) were added as stabilizing agents before pouring the agar [14,15]. Mutation frequencies were determined by dividing the count of resistant subpopulations by the total bacterial population count.

Each experiment was performed as $n \geq 2$. Four different combination scenarios containing ceftazidime/avibactam-tigecycline at EC_{08} - EC_{44} , EC_{44} - EC_{08} , EC_{44} - EC_{82} , EC_{82} - EC_{44} , one mono scenario for each antibiotic at EC_{44} and one growth control scenario were evaluated [11]. Detailed information on nominal concentrations tested in experiments is given in Table S2.

2.6. Assessment of antimicrobial drug interactions using the GPDI model

The experimental endpoint data at 24 h from the checkerboard assays was modelled using the GPDI model [16]. The GPDI model quantifies a potential PD interaction as shifts of the EC_{50} or E_{max} . Single drug effects were quantified as described above using Eq. (1). Bliss Independence was used as null-interaction model with E_A and E_B representing the effect of drug A and B (Eq. (2)). Ceftazidime/avibactam were treated as a single drug, with avibactam set at a fixed concentration of 4 mg/L in all experiments.

$$E = E_A + E_B - E_A \times E_B \quad (2)$$

PD interactions were evaluated as follows: The GPDI term is a function of the perpetrator drug concentration, which affects a PD parameter θ (either EC_{50} or E_{max}) of the victim drug with INT as maximum possible fractional shift of the PD parameter θ , EC_{50INT} as potency of the perpetrator and H_{INT} (Eq. [3]). Whenever $-1 < INT < 0$ applies to E_{max} based PD interactions, antagonism is described, and whenever $-1 < INT < 0$ applies to EC_{50} based PD interactions, synergy is described (vice versa for $0 < INT$). In our study, INT was consistently estimated as a single parameter, leading to a mutual relationship. Detailed information is given in Supplementary Text S3.

$$\theta \times \left(1 + \frac{INT \times C^{H_{INT}}}{EC_{50}^{H_{INT}} + C^{H_{INT}}} \right) \quad (3)$$

An interaction was statistically significant if the confidence intervals of INT did not overlap with zero ($P \leq 0.05$). Model selection of competing models (i.e., interaction on E_{max} vs. EC_{50}) was performed after inspecting biological plausibility of parameters, parameter uncertainty and the Akaike-Information-Criterion for identifying the one representing the observed data best [17].

2.7. Time-kill assays

Two *E. coli* and two *K. pneumoniae* isolates were tested, with one isogenic and one clinical strain of each species. These

strains expressed various resistance genes and were susceptible to ceftazidime/avibactam and tigecycline (Table 1). The selection criteria also included previously identified interactions, with three strains showing antagonism and one showing additivity in prior screenings. Time-kill assays were performed by inoculating and pre-incubating the bacterial isolate (approx. 1×10^6 CFU/mL) in ca-MHB for 2 h at 37°C ambient air before adding ceftazidime/avibactam and/or tigecycline. Tigecycline and ceftazidime/avibactam concentrations of 1 x MIC, 2 x MIC, 4 x MIC as well as combinations of both antibiotics at 1 x MIC_{CZA} – 1 x MIC_{TGC} , 2 x MIC_{CZA} – 2 x MIC_{TGC} , 4 x MIC_{CZA} – 4 x MIC_{TGC} and growth controls were studied over 30 h with samples collected at 0, 2, 4, 7, 24 and 30 h. Each experiment was performed as duplicate with a final volume of 10 mL and a lower limit of detection of 10 CFU/mL. Furthermore, PD interaction modelling was conducted on growth control, 4 x MIC_{CZA} , 4 x MIC_{TGC} and 4 x MIC_{CZA} – 4 x MIC_{TGC} data using non-linear mixed effects modelling in NON-MEM® version 7.5.0 and PsNB® version 5.4.0. Detailed information is given in Supplementary Text S4.

2.8. Metabolomic analysis

Mechanistical investigation of the PD interaction between ceftazidime/avibactam-tigecycline was studied in a selected clinical isolate of *E. coli* (strain JUM_JEA, Table 1) using metabolomic analyses in mono- and combination treatment scenarios. Time-kill assays were conducted for ceftazidime/avibactam and tigecycline concentrations of 4 x MIC as well as combinations of both antibiotics at 4 x MIC_{CZA} – 4 x MIC_{TGC} and growth controls as four replicates over 4 h with samples at 0, 2 and 4 h. Further information on sample preparation and metabolomic analysis can be found in Supplementary Text S6.

3. Results

3.1. MIC, EC_{50} and E_{max} determination

All tested strains with identified resistance genes, determined MIC, EC_{50} and E_{max} values can be found in Table 1. All tested strains were susceptible to ceftazidime/avibactam and tigecycline according to EUCAST [13].

Ceftazidime/avibactam showed bactericidal effects resulting in high E_{max} values ($> 7 \log_{10}$ CFU/mL) with full eradication of bacteria while tigecycline showed mainly bacteriostatic effects with low E_{max} values in most of the tested strains. However, tigecycline showed high E_{max} values against four isogenic *K. pneumoniae* strains (Fig. 1).

3.2. Assessment of antimicrobial drug interactions using the GPDI model

Table 2 shows the results of the *in vitro* interaction screening after the GPDI model evaluation for the combination of ceftazidime/avibactam-tigecycline. The GPDI model identified antagonism in form of a reduced E_{max} of ceftazidime/avibactam and tigecycline in seven of eight *E. coli* and in three of seven *K. pneumoniae* strains. The effect sizes of the interaction parameter were high ranging from 3.41 to 7.43 $\log_{10}$ CFU/mL reduction of the E_{max} , i.e., the effect of ceftazidime/avibactam (7.75–9.75 $\log_{10}$ CFU/mL) was fully antagonized and the weaker effect of tigecycline (2.94–6.69 $\log_{10}$ CFU/mL) remained. Four isogenic *K. pneumoniae* strains where tigecycline showed a relatively high E_{max} (6.32–9.23 $\log_{10}$ CFU/mL) and one isogenic *E. coli* strain showed no significant interaction thus the data was best described by the null-interaction model. Heatmaps visualizing the combined effects

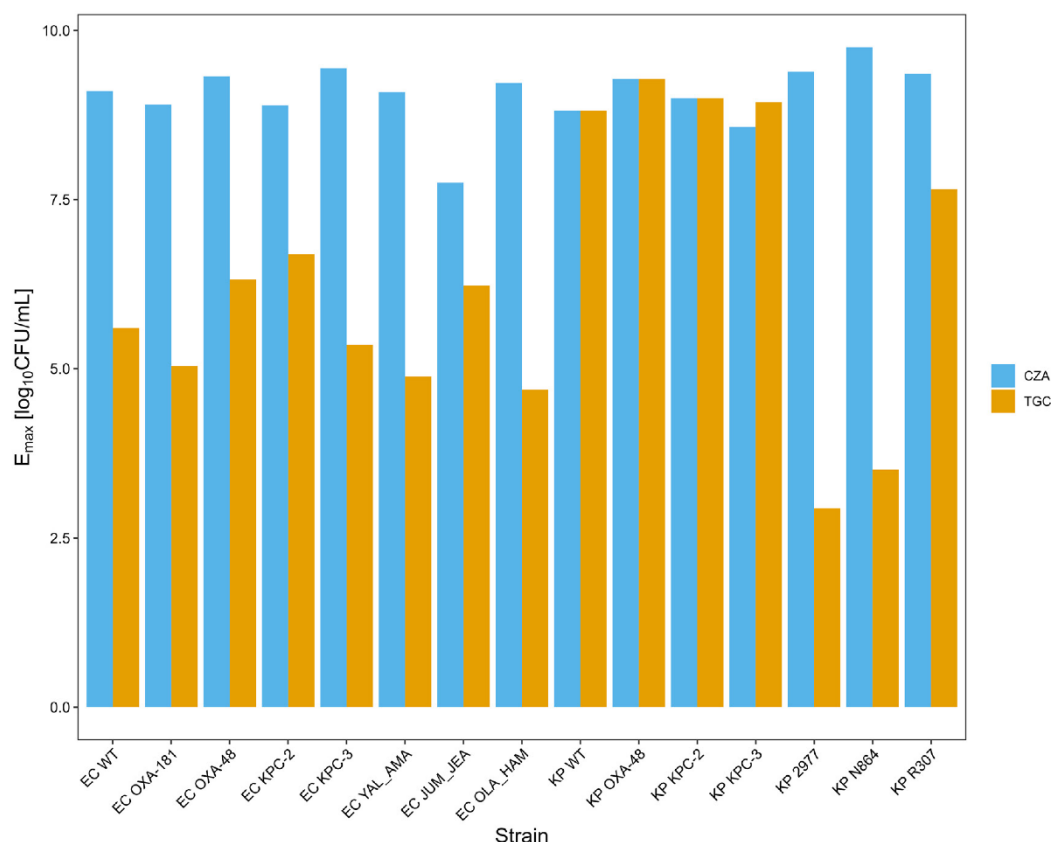


Fig. 1. Comparison of the maximum effect ($E_{\max}$) of ceftazidime/avibactam (CZA) and tigecycline (TGC) across tested bacterial strains. The bars represent the $E_{\max}$ values measured as maximum $\log_{10}$ CFU/mL reduction of bacteria for each strain.

Table 2

Predicted interactions between ceftazidime/avibactam-tigecycline after evaluation of performed checkerboard experiments.

Strain	Affected PD-parameter	Interaction-type	INT (CI 95%)	CZA $E_{\max}$ $\log_{10}$ [CFU/mL]	TGC $E_{\max}$ $\log_{10}$ [CFU/mL]
<i>E. coli</i> WT	$E_{\max}$	Antagonism	-0.40 (-0.63 to -0.18)	9.10	5.60
<i>E. coli</i> OXA-181	$E_{\max}$	Antagonism	-0.50 (-0.70 to -0.30)	8.90	5.04
<i>E. coli</i> OXA-48	-	Null-interaction	-0.05 (-0.23 to 0.12)	9.32	6.32
<i>E. coli</i> KPC-2	$E_{\max}$	Antagonism	-0.27 (-0.51 to -0.04)	8.89	6.69
<i>E. coli</i> KPC-3	$E_{\max}$	Antagonism	-0.31 (-0.53 to -0.09)	9.44	5.35
<i>E. coli</i> YAL_AMA	$E_{\max}$	Antagonism	-0.37 (-0.61 to -0.12)	9.09	4.88
<i>E. coli</i> JUM_JEA	$E_{\max}$	Antagonism	-0.53 (-0.70 to -0.36)	7.75	6.23
<i>E. coli</i> OLA_HAM	$E_{\max}$	Antagonism	-0.44 (-0.70 to -0.19)	9.22	4.69
<i>K. pneumoniae</i> WT	-	Null-interaction	-0.03 (-0.10 to 0.15)	8.81	8.81
<i>K. pneumoniae</i> OXA-48	-	Null-interaction	-0.10 (-0.35 to 0.14)	9.28	9.28
<i>K. pneumoniae</i> KPC-2	-	Null-interaction	0 (-0.26 to 0.25)	9.00	9.00
<i>K. pneumoniae</i> KPC-3	-	Null-interaction	-0.08 (-0.32 to 0.16)	8.57	8.94
<i>K. pneumoniae</i> 2977	$E_{\max}$	Antagonism	-0.52 (-0.79 to -0.24)	9.39	2.94
<i>K. pneumoniae</i> N864	$E_{\max}$	Antagonism	-0.48 (-0.74 to -0.22)	9.75	3.51
<i>K. pneumoniae</i> R307	$E_{\max}$	Antagonism	-0.20 (-0.33 to -0.07)	9.36	7.65

Abbreviations: CZA: ceftazidime/avibactam; TGC: tigecycline.

Avibactam fixed to 4 mg/L.

The table shows the predicted interactions by the GPDI model between combined antibiotics, PD parameters which were affected in found interactions, the type of interaction (i.e., antagonism, synergy, null-interaction additivity).

calculated by the final models can be found in Supplementary Fig. S3.

Table 3 presents the emergence of 5 x MIC resistant subpopulations and the calculated mutation frequencies observed in the checkerboard experiments after 48 h. In general, phenotypically resistant subpopulations were found more frequently during mono-exposure to ceftazidime/avibactam EC₄₄ or tigecycline EC₄₄ compared to combined exposure ceftazidime/avibactam EC₄₄ + tigecycline EC₀₈ or tigecycline EC₄₄ + ceftazidime/avibactam EC₀₈.

3.3. Time-kill assays

Scenarios of ceftazidime/avibactam alone at 1 x MIC, 2 x MIC and 4 x MIC exhibited bactericidal activity in the first hours followed by regrowth in the case of 1 x MIC and full eradication of bacteria for 2 x MIC and 4 x MIC at 30 h exposure (Fig. 2, Plots CZA). Tigecycline exhibited bacteriostatic activity over 30 h exposure for *E. coli* JUM_JEA and *K. pneumoniae* 2977 at concentrations of 1 x MIC, 2 x MIC and 4 x MIC (Fig. 2, Plots TGC) with no notable

Table 3

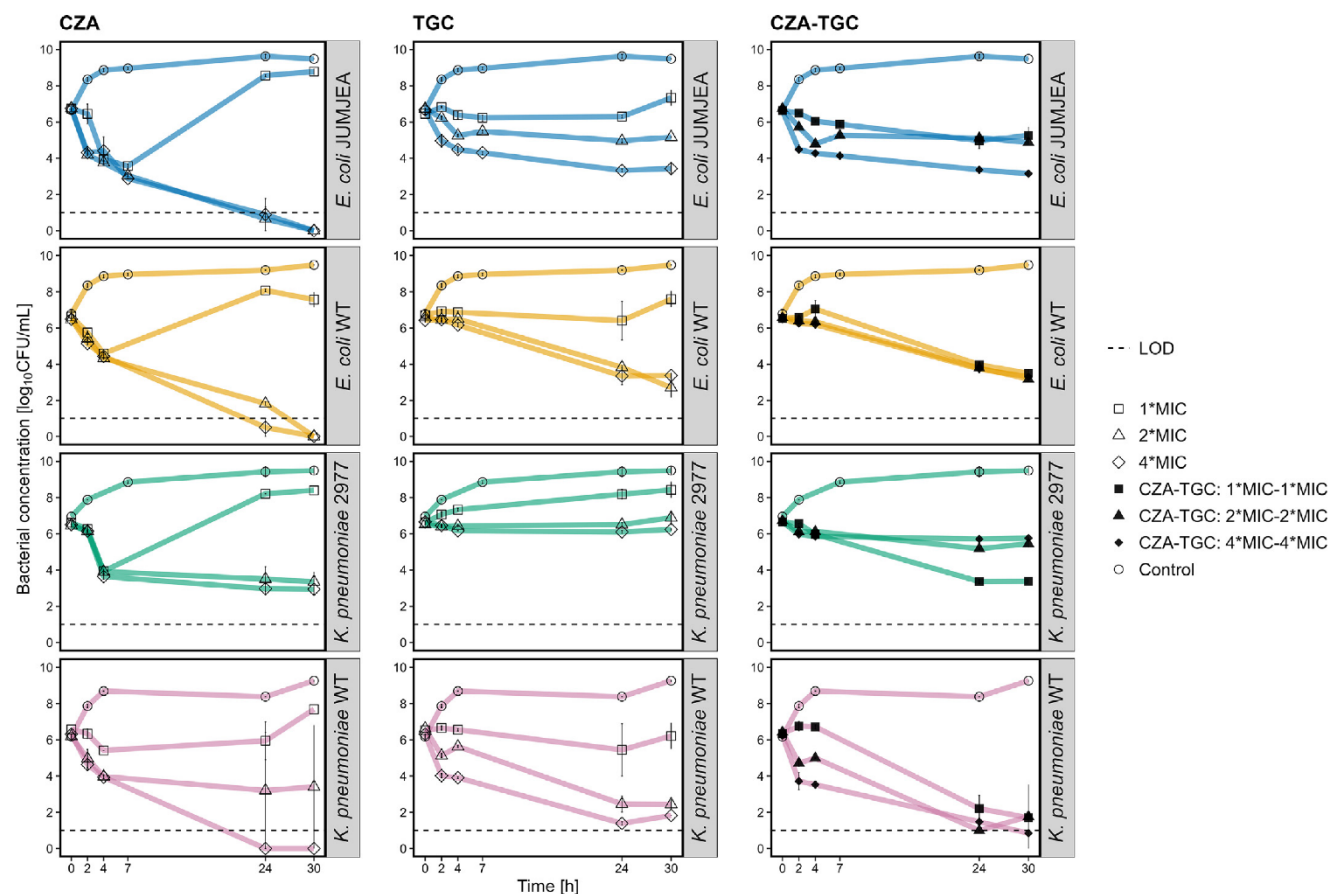
Overview on the emergence of resistant subpopulations and calculated mutation frequencies in the checkerboard experiments after 48 h.

Strain	Emergence of resistant subpopulations against 5x MIC CZA		Emergence of resistant subpopulations against 5x MIC TGC	
	Mono exposure EC ₄₄ CZA	Combined exposure EC ₄₄ CZA + EC ₀₈ TGC	Mono exposure EC ₄₄ TGC	Combined exposure EC ₄₄ TGC + EC ₀₈ CZA
<i>E. coli</i> WT	3.47×10^{-06} 0	0 0	0 2.63×10^{-08}	0 0
<i>E. coli</i> OXA-181	2.90×10^{-07} 1.78×10^{-07}	2.04×10^{-07} 0	0 1.18×10^{-08}	1.01×10^{-04} 2.22×10^{-06}
<i>E. coli</i> OXA-48	0 0	0 0	0 0	0 0
<i>E. coli</i> KPC-2	1.62×10^{-06} 0	0 0	1.82×10^{-07} 0	0 0
<i>E. coli</i> KPC-3	0 3.84×10^{-03}	6.18×10^{-02} 0	0 0	0 0
<i>E. coli</i> YAL_AMA	0 7.54×10^{-06}	0 0	0 1.08×10^{-06}	0 0
<i>E. coli</i> JUM_JEA	0 0	0 0	0 1.32×10^{-07}	0 0
<i>E. coli</i> OLA_HAM	0 0	0 0	0 4.19×10^{-07}	0 0
<i>K. pneumoniae</i> WT	1×10^{-04} 4.48×10^{-05}	7.81×10^{-06} 3.79×10^{-06}	4.71×10^{-05} 0	0 0
<i>K. pneumoniae</i> OXA-48	0 0	0 0	0 0	0 0
<i>K. pneumoniae</i> KPC-2	1.62×10^{-08} 0	0 0	1.82×10^{-07} 0	0 0
<i>K. pneumoniae</i> KPC-3	0 3.84×10^{-03}	6.18×10^{-02} 0	0 0	0 0
<i>K. pneumoniae</i> 2977	2.30×10^{-03} 0	0 0	7.55×10^{-05} 0	6.67×10^{-03} 5.43×10^{-02}
<i>K. pneumoniae</i> N864	1.31×10^{-06} 3.85×10^{-08}	0 0	1.4×10^{-05} 0	0 0
<i>K. pneumoniae</i> R307	0 0	0 0	2.80×10^{-02} 6.73×10^{-01}	8.70×10^{-03} 0

Abbreviations: CZA: ceftazidime/avibactam; TGC: tigecycline.

Avibactam fixed to 4 mg/L.

Resistances are reported as two mutation frequencies (separated by |), accounting for variable emergence of resistance in the two checkerboard duplicates. Mutation frequencies were determined by dividing the count of resistant subpopulations by the total bacterial population count.

**Fig. 2.** Results of time-kill assays with bacterial concentrations during 30-h exposure of mono ceftazidime/avibactam (CZA), mono tigecycline (TGC) and combination (CZA-TGC) scenarios at MIC-fold drug concentrations against two *E. coli* and two *K. pneumoniae* strains (all susceptible to CZA and TGC). Presented control scenarios were individually evaluated in respective strains. LOD: Limit of detection.

killing or growth. In *E. coli* WT and *K. pneumoniae* WT, 2 x MIC and 4 x MIC. Compared to the growth control scenarios, raising the ceftazidime/avibactam concentration resulted in higher activity in all tested strains, while raising tigecycline concentration did not increase its effect beyond bacteriostasis in two of four strains. When both antibiotics were combined the combined effect was limited to that of tigecycline alone indicating that the maximum effect of ceftazidime/avibactam is antagonized (Fig. 2, Plots CZA-TGC). In *K. pneumoniae* 2977 the killing effect of combination scenario 4 x MIC – 4 x MIC even decreased when compared to 1 x MIC – 1 x MIC.

The models where tigecycline antagonistically impacts the killing rate constant of ceftazidime/avibactam on the (R) population outperformed the opposite directionality models across all strains (Fig. S1, S2 and Table S3). The final parameter estimates, detailed in Table S4, show significant reductions in killing rate constant of ceftazidime/avibactam on the (R) population, varying from notable in *K. pneumoniae* WT to almost complete reduction in *E. coli* WT.

3.4. Metabolomic analysis

Principal Component Analysis (PCA) was employed to visualize the distinct metabolomic profiles of bacterial cell pellets under varying treatments at timepoints 2 and 4 h (Fig. 3). According to the PCA score plot of our metabolomic analysis at 2 h (Fig. 3A), clusters of mono treatment tigecycline and combination treatment ceftazidime/avibactam-tigecycline show high similarity within biological replicates but also across treatment groups, indicating similar metabolic perturbations caused by these treatments. Metabolomes of control group and mono ceftazidime/avibactam treatment are significantly different from each other but also show distinct separation from tigecycline and ceftazidime/avibactam-tigecycline treatments (PC1 = 62.6%).

At 4 h (Fig. 3B), the PCA score plot is similar to that at 2 h, but here the clusters for tigecycline and ceftazidime/avibactam-tigecycline are overlapped and within-group variation is increased. Control and mono treatment ceftazidime/avibactam still separate from tigecycline and ceftazidime/avibactam-tigecycline (PC1 = 42.5%). The heatmap profiles (Fig. 4) revealed consistent clustering among the four biological replicates within each treatment group, with only one ceftazidime/avibactam sample differing (CZA_t4_1). The metabolic profiles were significantly depleted in this sample, which caused within-group variation and contributed to the difference between the ceftazidime/avibactam group to the other groups. Comparing the groups, the control and mono ceftazidime/avibactam profiles were different from those of mono tigecycline and the ceftazidime/avibactam-tigecycline combination, with the latter two displaying notably similar metabolic perturbations. This similarity is characterized by an increase in a variety of metabolites, particularly those related to nucleotide metabolism (i.e., purine metabolism: guanine, guanosine, guanosine nucleotides, desoxyguanosine monophosphate, adenosine nucleotides, desoxyadenosine monophosphate, and inosine; pyrimidine metabolism: cytidine nucleotides, uracil and orotic acid), amino acids and their derivatives (i.e., putrescine, N-acetyl-putrescine, lysine, N-acetyl-lysine, threonine, glycine, serine, homoserine, ornithine, arginine, glutamine, glutamic acid, phenylalanine, methionine, N-acetyl-methionine), and certain carbohydrates (i.e., 2,3-Dihydroxybenzoic acid, sorbitol, sorbitol-6-phosphate) and lipids (i.e., glycerol-3-phosphate, palmitoleic acid, linolenic acid, oleic acid, aconitic acid). These metabolites showed notable elevations in the tigecycline and ceftazidime/avibactam-tigecycline treatments, while they remained stable or decreased in the ceftazidime/avibactam treatment. Conversely, metabolites involved in vitamin B metabolism, such as riboflavin, flavin mononucleotide and pantothenic acid, were increased under mono cef-

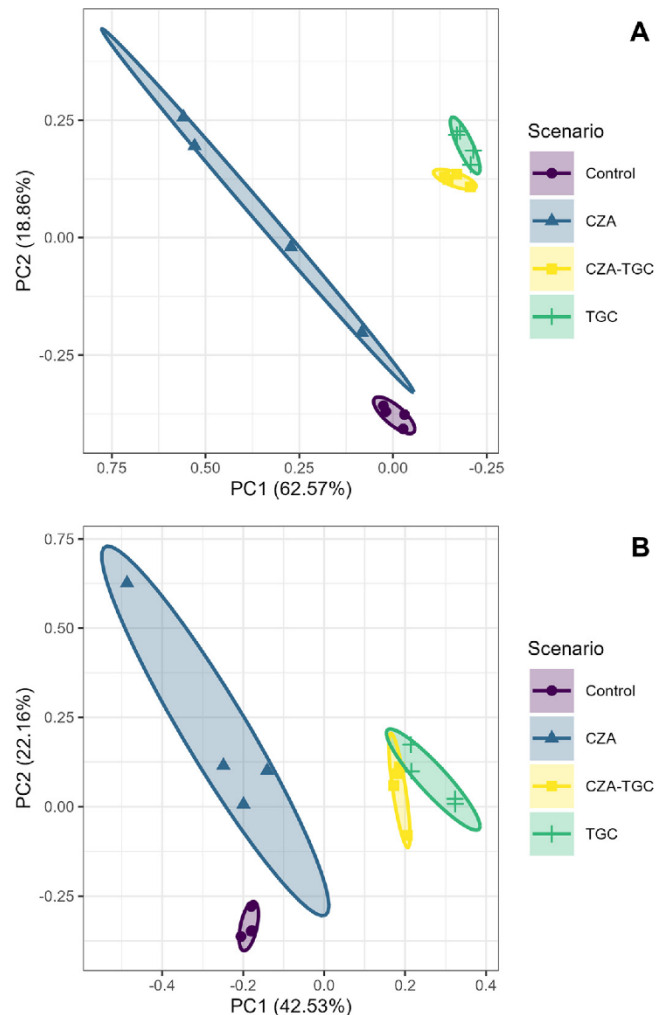


Fig. 3. Multivariate statistical analysis with Principal Component Analysis (PCA) score plots visualising changes in metabolomic profiles. A total of 16 samples with 4 biological replicates under each treatment are displayed for each timepoint. The plots depict clustering based on metabolomic profiles in bacterial cell pellets of *E. coli* JUM_JEA at time points 2 h (A), and 4 h (B). Each cluster represents distinct treatment conditions: Control, 4xMIC ceftazidime/avibactam (CZA), 4xMIC tigecycline (TGC), and their combination (CZA-TGC).

tazidime/avibactam treatment but remained stable or decreased in the tigecycline and ceftazidime/avibactam-tigecycline treatments.

4. Discussion

Ceftazidime/avibactam and tigecycline in susceptible *E. coli* and *K. pneumoniae* exhibited mainly antagonistic interactions (ten of 15 tested strains). Four isogenic *K. pneumoniae* and one *E. coli* strain showed no interaction. Yet in these strains, tigecycline itself showed a bactericidal effect. Hence, our results indicate that the mostly inferior maximum effect of tigecycline limits the possible combined effect and thus antagonizes the effect of ceftazidime/avibactam. The screening experiments in this study were based on the adaptive *in vitro* 'dynamic' checkerboard design using drug potencies as a highly efficient tool to elucidate drug interactions as described by Kroemer et al. [18]. Building on and following these methodologies, this study represents the first attempt to quantitatively characterize the PD interaction between ceftazidime/avibactam and tigecycline, employing a combination

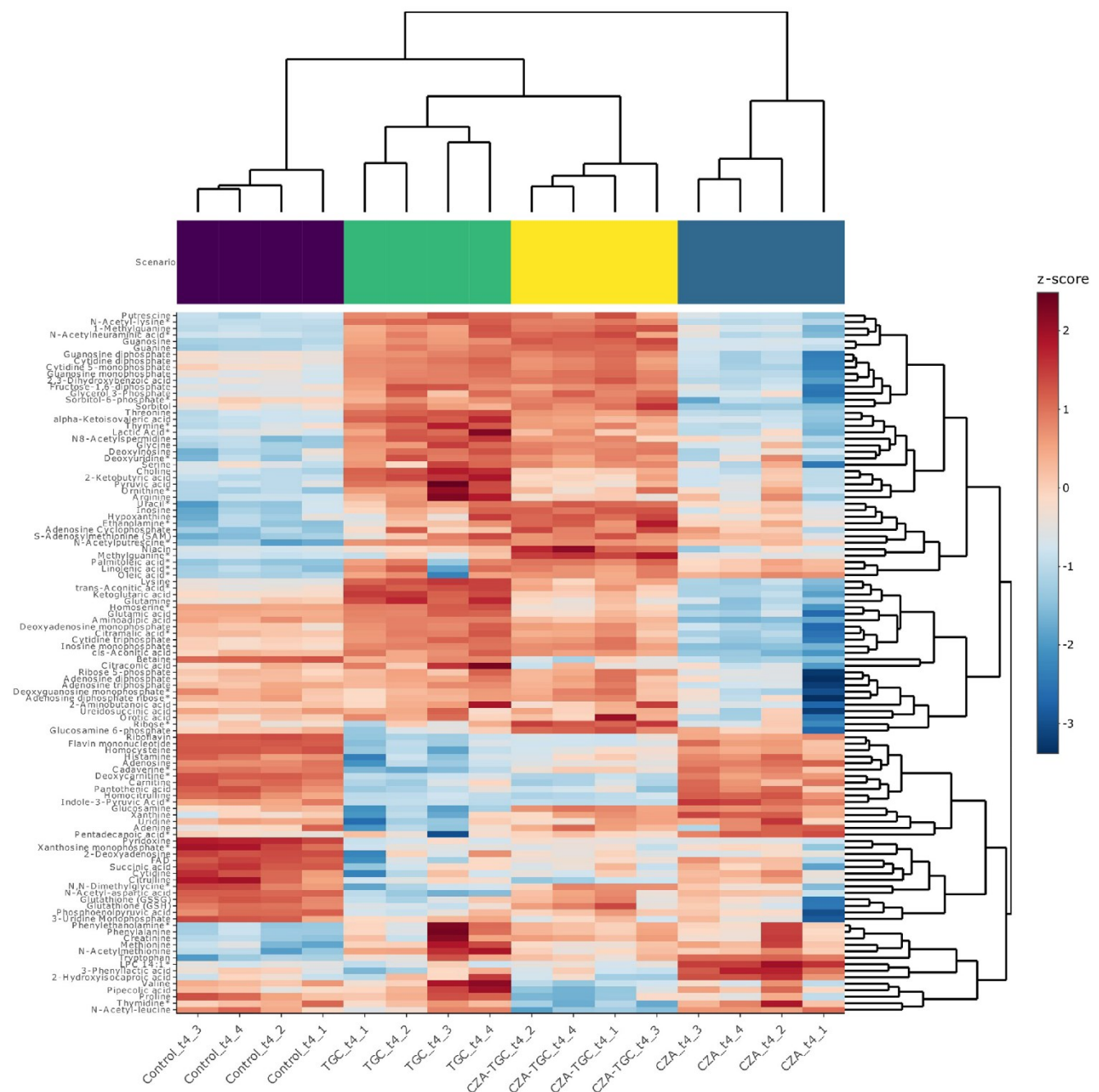


Fig. 4. Heatmap profiles at 4 h showing the commonly affected metabolites in cell pellets associated with all three treatments. A total of 16 samples with 4 biological replicates under each treatment are displayed. The heatmap illustrates significant metabolic changes in *E. coli* JUM_JEA under different scenarios: Control, 4 x MIC ceftazidime/avibactam (CZA), 4 x MIC tigecycline (TGC), and their combination (CZA-TGC). The z-score represents the abundance of each metabolite as the number of standard deviations from the mean, illustrating relative changes across samples.

of precise bacterial counting techniques and pharmacometric approaches.

Time-kill assays conducted on four of the 15 selected strains corroborated these findings, further demonstrating the prevalence of antagonistic interactions. Comparison of the observed killing effect of ceftazidime/avibactam-tigecycline combination and tigecycline alone in time-kill assays over 30 h of exposure resulted in matching killing profiles. The PD interaction models based on time-kill assay data showed that tigecycline antagonistically affects the ceftazidime/avibactam effects across all four strains. This antagonistic relationship outperformed an antagonism with cef-

tazidime/avibactam as perpetrator. Notable reductions in killing rates consistent with checkerboard assay results were estimated. A comparison of the estimated interaction parameters in checkerboard assays (Table 2) versus those estimated in time-kill assays (Table S4) reveals similarities with partly overlapping confidence intervals. In time-kill assays a rather weak antagonism of -67.1% (-85.3 to -28.9%) was identified in *K. pneumoniae* WT, while in checkerboard assays additivity was found. This difference could result from sparse data and identifiability issues in the 'dynamic' checkerboard design. We hypothesize, tigecycline as a protein biosynthesis inhibitor [19–21], could have lowered the expres-

sion of the D-alanyl-D-alanine peptidases, also known as penicillin-binding proteins (PBPs) and the target of beta-lactams such as cephalosporins like ceftazidime/avibactam [22]. This could cause ceftazidime/avibactam to lose its target and therefore its bactericidal activity.

PCA score plots (Fig. 3) and heatmap profiles (Fig. 4) allowed to highlight how the specific treatments alter the metabolome, revealing both similarities and differences in metabolite distribution over time. Our metabolomic analysis indicates significant metabolic disruptions in both tigecycline and ceftazidime/avibactam-tigecycline treatments, strengthening our hypothesis that tigecycline affects metabolic pathways related to protein synthesis, and therefore might also affect expression of ceftazidime/avibactam target. An increase of certain amino acids and their derivatives is expected with exposure to tigecycline, as the inhibition of protein biosynthesis while amino acid synthesis remains unaffected allows for their accumulation. Idrees et al. offer valuable insights into amino acid biosynthesis in microorganisms and its potential as a therapeutic target [23]. This disruption may alter the metabolic environment in a way that influences ceftazidime/avibactam's effectiveness and could possibly explain the observed antagonistic interactions and reduced bactericidal effect of ceftazidime/avibactam when combined with tigecycline. However, the performed metabolomic analysis did not allow to identify intermediate metabolites specific to D-alanyl-D-alanine peptidases catalysed reaction (i.e., components of the stem pentapeptide, D-alanine, D-alanyl-D-alanine) [24]. Further research, possibly incorporating proteomics and similar approaches, could focus on changes in enzyme expression or larger peptides.

The findings of Yeh et al. [25] indicated that antagonistic interactions can narrow the range of drug concentrations where resistance evolves, and slow the rate of evolution through mutations while in synergistic interactions, single-drug resistance mutations could not only remove the effect associated with one of the drugs, but also remove the synergy with the other drug. Our work partly confirms these findings for antagonistic interactions, showing that resistant subpopulations were more prevalent under mono-exposure to ceftazidime/avibactam EC₄₄ or tigecycline EC₄₄ than under combined exposure to ceftazidime/avibactam and tigecycline, as shown in Table 3. However, Kroemer et al. similarly found higher mutation frequencies under mono-exposure of ceftazidime/avibactam and fosfomycin while identifying the combination of these drugs as synergistic.

The concept of collateral sensitivity, as discussed in multiple studies [26–28], describes evolutionary trade-offs between antimicrobial agents when bacteria adapt to different environmental pressures. We hypothesize that collateral sensitivity, particularly related to plasmid-borne tetracycline and chloramphenicol resistance, may have influenced our testing outcomes, and this phenomenon could also be species-dependent. In our study, we noted high E_{max} values for tigecycline monotherapy across all four isogenic *K. pneumoniae* strains, despite tigecycline's typically bacteriostatic effects (Table 1). This was further illustrated by *K. pneumoniae* WT carrying the empty plasmid coding for chloramphenicol and tetracycline resistance, which exhibited high efficacy for tigecycline alone in time-kill assays (Fig. 2). However, similar effects were not observed in the isogenic *E. coli* strains or in clinically isolated strains that do not carry plasmids encoding resistance to tetracycline and chloramphenicol.

The studies of Ojdana et al. and Wang et al. [6,7], which relied on the FICI and turbidity readouts, reported mainly indifferent or additive and synergistic PD interactions between ceftazidime/avibactam and tigecycline. Yet, these results may be misleading since turbidity-based assays do not allow to distinguish between bacteriostatic and bactericidal effects below the turbidity threshold. Similar findings have been observed with meropenem

and linezolid against *Staphylococcus aureus*, where the conventional checkerboard method was unable to detect an antagonistic interaction affecting the maximum killing effect. In this case, linezolid antagonized the effect of meropenem, which was not detectable using the conventional checkerboard approach based on turbidity readouts [29]. Consequently, if the observations from this study had been evaluated using the FICI method instead of time-kill and modelling, they would likely have yielded FICI values interpreted as indifferent.

Several limitations must be acknowledged. The *in vitro* nature of this study restricts the direct translation of these findings to the clinical situation. A fixed concentration of 4 mg/L avibactam was used in the experiments, in line with EUCAST recommendations [13], while changing avibactam concentrations may not only influence the effect of ceftazidime/avibactam [30], but also influence the observed interactions. For the model-based interaction evaluation in the checkerboards, several assumptions were made. The sparse experimental layout identifies the nature of drug interactions but cannot estimate interaction parameters, interaction potencies and interaction directionality at the same time. Due to strong observed antagonistic interactions, the interaction potency (EC_{50INT}) was fixed to very small values to stabilize estimates. When trying to determine the perpetrator and victim in interactions applying the GPD model on the checkerboard screening data, this process proved to be ineffective. Using a single INT parameter for both drugs resolved identifiability issues and avoided selection bias in interaction directionality.

The observed antagonism between tigecycline and ceftazidime/avibactam could significantly impact their clinical use. Higher all-cause mortality with tigecycline standard dose therapy was identified, exceeding what was observed when the drug first entered the market with significantly increased mortality and non-cure rates compared to active comparators [31,32]. In light of this study, combining tigecycline with ceftazidime/avibactam might diminish the high efficacy of ceftazidime/avibactam when used alone [10]. Lin et al. demonstrated in a recent multicentre retrospective study [33] that combination therapy with ceftazidime/avibactam and tigecycline in patients infected with carbapenem-resistant *K. pneumoniae* showed no statistical difference compared to ceftazidime/avibactam monotherapy.

In conclusion, ceftazidime/avibactam and tigecycline combination treatment showed mainly antagonistic PD interactions in 15 clinical and isogenic *E. coli* and *K. pneumoniae* strains, limiting the overall effect of this combination to the effect of tigecycline. Future studies should investigate this combination by incorporating dynamic *in vitro* models such as hollow-fibre infection model experiments, thereby allowing to test different dosing regimens in combination (i.e., prolonged infusion, sequential therapy). These studies could also utilize *in vivo* animal models, expanding the number and diversity of bacterial isolates tested, and including pharmacometric evaluations to better understand the clinical implications of our findings.

Authors contributions

SGW and AF designed the study. BD provided methodological guidance and conducted metabolomics analyses. NK, MH, JI, JWD, DS, JB, NG and SGW provided methodological guidance and contributed to the research. JWD and PN cloned and isolated bacterial isolates. AF wrote the original draft, all authors read, edited and approved the final version of the manuscript.

Declaration of competing interests

None declared.

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Sequence information

Not applicable.

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Data availability

All original data generated during the study are included in the article in the form of figures and tables. All data used in metabolomic analysis can be accessed via Metabolomics Workbench: <http://dx.doi.org/10.21228/M8MJ9D>. For any further inquiries, please contact the corresponding author.

Ethical approval

Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.ijantimicag.2025.107457](https://doi.org/10.1016/j.ijantimicag.2025.107457).

References

- Publicaciones de la Organización Mundial de la Salud. WHO bacterial priority pathogens list, 2024: bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. <https://www.who.int/publications/i/item/9789240093461> (accessed 26.07.2024).
- Doi Y. Treatment options for carbapenem-resistant gram-negative bacterial infections. *Clin Infect Dis* 2019;69:S565–75. doi:10.1093/cid/ciz830.
- Xu T, Guo Y, Ji Y, Wang B, Zhou K. Epidemiology and mechanisms of ceftazidime – avibactam resistance in gram-negative bacteria. *Engineering* 2020;11:138–45. doi:10.1016/j.eng.2020.11.004.
- Sun D, Rubio-Aparicio D, Nelson K, Dudley MN, Lomovskaya O. Meropenem-vaborbactam resistance selection, resistance prevention, and molecular mechanisms in mutants of KPC-producing klebsiella pneumoniae. *Antimicrob Agents Chemother* 2017;61:1–14. doi:10.1128/AAC.01694-17.
- Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. Infectious diseases society of America 2023 guidance on the treatment of antimicrobial resistant gram-negative infections. *Clin Infect Dis Off Publ Infect Dis Soc Am* 2023;ciad428:16–17. doi:10.1093/cid/ciad428.
- Ojdana D, Gutowska A, Sacha P, Majewski P, Wiczorek P, Trzyniewska E. Activity of ceftazidime-avibactam alone and in combination with ertapenem, fosfomicin, and tigecycline against carbapenemase-producing klebsiella pneumoniae. *Microb Drug Resist* 2019;25:1357–64. doi:10.1089/mdr.2018.0234.
- Wang F, Zhou Q, Yang X, Bai Y, Cui J. Evaluation of ceftazidime/avibactam alone and in combination with amikacin, colistin and tigecycline against klebsiella pneumoniae carbapenemase-producing K. pneumoniae by in vitro time-kill experiment. *PLoS ONE* 2021;16:1–10. doi:10.1371/journal.pone.0258426.
- Heizmann WR, Löschmann P-A, Eckmann C, von Eiff C, Bodmann K-F, Petrik C. Clinical efficacy of tigecycline used as monotherapy or in combination regimens for complicated infections with documented involvement of multiresistant bacteria. *Infection* 2015;43:37–43. doi:10.1007/s15010-014-0691-4.
- Yang K-Y, Peng C-K, Sheu C-C, Lin Y-C, Chan M-C, Wang S-H, et al. Clinical effectiveness of tigecycline in combination therapy against nosocomial pneumonia caused by CR-GNB in intensive care units: a retrospective multi-centre observational study. *J Intensive Care* 2023;11:1. doi:10.1186/s40560-022-00647-y.
- Castón JJ, Lacort-Peralta I, Martín-Dávila P, Loeches B, Tabares S, Temkin L, et al. Clinical efficacy of ceftazidime/avibactam versus other active agents for the treatment of bacteremia due to carbapenemase-producing enterobacteriaceae in hematologic patients. *Int J Infect Dis* 2017;59:118–23. doi:10.1016/j.ijid.2017.03.021.
- Kroemer N. Optimized rhombic experimental dynamic checkerboard designs to elucidate pharmacodynamic drug interactions of antibiotics. *Pharm Res* 2022;39:3267–77.
- Chen C, Wicha SG, Nordgren R, Simonsson USH. Comparisons of analysis methods for assessment of pharmacodynamic interactions including design recommendations. *AAPS J* 2018;20:1–12. doi:10.1208/s12248-018-0239-0.
- . Breakpoint tables for interpretation of MICs and zone diameters; 2024. p. 4–6. v 14.0.
- Jitkova Y, Gronda M, Hurren R, Wang X, Goard CA, Jhas B, et al. A novel formulation of tigecycline has enhanced stability and sustained antibacterial and anti-leukemic activity. *PLoS ONE* 2014;9:1–11. doi:10.1371/journal.pone.0095281.
- Amann LF, Vicente ER, Rathke M, Broecker A, Riedner M, Wicha SG. Stability studies with tigecycline in bacterial growth medium and impact of stabilizing agents. *Eur J Clin Microbiol Infect Dis* 2021;40:215–18. doi:10.1007/s10096-020-03970-0.
- Wicha SG, Chen C, Clewe O, Simonsson USH. A general pharmacodynamic interaction model identifies perpetrators and victims in drug interactions. *Nat Commun* 2017;8:1–11. doi:10.1038/s41467-017-01929-y.
- Akaike H. A new look at the statistical model identification. *IEEE Trans Autom Control* 1974;19:716–23. doi:10.1109/TAC.1974.1100705.
- Kroemer N, Martens M, Decusser J-W, Grégoire N, Nordmann P, Wicha SG. Evaluation of *in vitro* pharmacodynamic drug interactions of ceftazidime/avibactam and fosfomicin in *Escherichia coli*. *J Antimicrob Chemother* 2023;78:2524–34. doi:10.1093/jac/dkad264.
- Sum P-E, Petersen P. Synthesis and structure-activity relationship of novel glycylic acid derivatives leading to the discovery of GAR-936. *Bioorg Med Chem Lett* 1999;9:1459–62. doi:10.1016/S0960-894X(99)00216-4.
- Murphy TM, Deitz JM, Petersen PJ, Mikels SM, Weiss WJ. Therapeutic efficacy of GAR-936, a novel glycylic acid, in a rat model of experimental endocarditis. *Antimicrob Agents Chemother* 2000;44:3022–7. doi:10.1128/AAC.44.11.3022-3027.2000.
- Bauer G. Comparison of tetracycline and tigecycline binding to ribosomes mapped by dimethylsulphate and drug-directed Fe²⁺ cleavage of 16S rRNA. *J Antimicrob Chemother* 2004;53:592–9. doi:10.1093/jac/dkh125.
- Zhanell GG, Lawson CD, Adam H, Schweizer F, Zelenitsky S, Lagacé-Wiens PRS, et al. Ceftazidime-avibactam: a novel cephalosporin/β-lactamase inhibitor combination. *Drugs* 2013;73:159–77. doi:10.1007/s40265-013-0013-7.
- Idrees M, Mohammad AR, Karodia N, Rahman A. Multimodal role of amino acids in microbial control and drug development. *Antibiotics* 2020;9:330. doi:10.3390/antibiotics9060330.
- Sauvage E, Kerff F, Terrak M, Ayala JA, Charlier P. The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. *FEMS Microbiol Rev* 2008;32:234–58. doi:10.1111/j.1574-6976.2008.00105.x.
- Yeh PJ, Hegreness MJ, Aiden AP, Kishony R. Drug interactions and the evolution of antibiotic resistance. *Nat Rev Microbiol* 2009;7:460–6. doi:10.1038/nrmicro2133.
- Pál C, Papp B, Lázár V. Collateral sensitivity of antibiotic-resistant microbes. *Trends Microbiol* 2015;23:401–7. doi:10.1016/j.tim.2015.02.009.
- Aulin LBS, Liakopoulos A, van der Graaf PH, Rozen DE, van Hasselt JGC. Design principles of collateral sensitivity-based dosing strategies. *Nat Commun* 2021;12:5691. doi:10.1038/s41467-021-25927-3.
- Yekani M, Azargun R, Sharifi S, Nabizadeh E, Nahand JS, Ansari NK, et al. Collateral sensitivity: an evolutionary trade-off between antibiotic resistance mechanisms, attractive for dealing with drug-resistance crisis. *Health Sci Rep* 2023;6:e1418. doi:10.1002/hsr2.1418.
- Wicha SG, Kees MG, Kuss J, Kloft C. Pharmacodynamic and response surface analysis of linezolid or vancomycin combined with meropenem against *Staphylococcus aureus*. *Pharm Res* 2015;32:2410–18. doi:10.1007/s10995-015-1632-3.
- Palombo M, Secci B, Bovo F, Gatti M, Ambretti S, Gaibani P. In vitro evaluation of increasing Avibactam concentrations on ceftazidime activity against ceftazidime/avibactam-susceptible and resistant KPC-producing *Klebsiella pneumoniae* clinical isolates. *Antibiotics* 2023;12:1707. doi:10.3390/antibiotics12121707.
- Prasad P, Sun J, Danner RL, Natanson C. Excess deaths associated with tigecycline after approval based on noninferiority trials. *Clin Infect Dis* 2012;54:1699–709. doi:10.1093/cid/cis270.
- McGovern PC, Wible M, El-Tahtawy A, Biswas P, Meyer RD. All-cause mortality imbalance in the tigecycline phase 3 and 4 clinical trials. *Int J Antimicrob Agents* 2013;41:463–7. doi:10.1016/j.ijantimicag.2013.01.020.
- Lin J, Zhang L, Zhou M, Tian X, Chen J, Lu M, et al. Combination therapy of ceftazidime/avibactam for the treatment of patients infected with carbapenem-resistant *Klebsiella pneumoniae*: a multicenter retrospective study. *Infect Dis Ther* 2023;12:2165–77. doi:10.1007/s40121-023-00852-8.

5.4 Publication IV

Meropenem and fosfomycin against *Klebsiella pneumoniae*: Towards a combination breakpoint using a pharmacometric approach

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Synopsis

Our previous study investigated and identified the synergy of meropenem and fosfomycin combination therapy and concluded the need of more comprehensive studies to reveal the clinical potential of it. In this study we first pooled the experimental *in vitro* data of 12 MDR *K. pneumoniae* strains across two different dynamic *in vitro* infection models. To propose and establish a rationale for combined clinical breakpoints for meropenem and fosfomycin against infections caused by *K. pneumoniae*, we then developed a pharmacometric model which coupled human population PK models and an *in vitro* data derived PD model. Finally, we performed simulations of 9 distinct dosing regimens across a wide range of meropenem and fosfomycin MICs, and calculated PD-target based PTAs and CFRs for representative bacterial MDR populations. Meropenem and fosfomycin monotherapies achieved PTA $\geq 90\%$ only for low MICs (≤ 4 mg/L for meropenem, ≤ 4 mg/L for fosfomycin), while combination therapy of high-dose combinations (meropenem 2 g + fosfomycin 8 g q8h) achieved PTA $\geq 90\%$ for 1-log and 2-log killing at high MICs (32 mg/L for meropenem, 512 mg/L for fosfomycin). The study reveals robust synergy of meropenem and fosfomycin combination therapy across 12 strains expressing different resistance genes, and across two different *in vitro* infection models. The findings

provide evidence for the reintroduction of a fosfomycin clinical breakpoint in combination with meropenem and paves the way for clinical validation of the proposed dosing regimens in patients with severe infections caused by MDR *K. pneumoniae*.

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Original article

Meropenem and fosfomycin against *Klebsiella pneumoniae*: Towards a combination breakpoint using a pharmacometric approachAneeq Farooq¹, Miklas Martens¹, Marie L.G. Attwood², Patrice Nordmann³,
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ABSTRACT

Objectives: To quantify the antibacterial effects and pharmacodynamic interactions of meropenem and fosfomycin combinations against multidrug-resistant *Klebsiella pneumoniae* through pharmacometric analysis, and to support an evidence-based rationale for combined clinical breakpoints.

Methods: *In vitro* data from 12 clinical *K. pneumoniae* strains (carrying genes such as KPC, NDM, OXA-48, VIM, CTX-M, SHV, etc.), generated in dynamic *in vitro* infection model experiments mimicking human pharmacokinetics of meropenem and fosfomycin, were analysed using pharmacodynamic modelling. A nonlinear mixed-effects model, incorporating MICs as covariates, was developed. Simulations using published population pharmacokinetic models evaluated nine mono and combination dosing regimens (intravenous [i.v.] meropenem 2 g every 8 hours; i.v. fosfomycin 6–24 g total daily dose every 6 hours, every 8 hours, or continuous infusion) and calculated probability of target attainment (PTA) (for bacteriostasis, 1-log, and 2-log killing after 24 hours) for each dosing regimen across wide meropenem and fosfomycin MICs.

Results: Meropenem and fosfomycin monotherapies achieved PTA ≥90% only for low MICs (≤4 mg/L for meropenem, ≤4 mg/L for fosfomycin). In contrast, combination therapy enabled PTA ≥90% for bacteriostasis at MICs up to 32 mg/L (meropenem) and 512 mg/L (fosfomycin), and for 1-log killing at 32 mg/L and 256 mg/L, using low-dose regimens (meropenem 2 g + fosfomycin 2 g every 8 hours). For high-dose combinations (meropenem 2 g + fosfomycin 8 g every 8 hours), PTA ≥90% was achieved for 1-log and 2-log killing at even higher MICs. In total, the model captured time–kill dynamics for all 12 strains and 9 distinct drug regimens.

Conclusions: This study demonstrates that meropenem–fosfomycin combination therapy produces robust synergy against multidrug-resistant *K. pneumoniae* strains, and expands attainable clinical breakpoints compared with monotherapy. These findings provide evidence for reintroducing a fosfomycin breakpoint in combination with meropenem and support further clinical validation of the proposed dosing regimens in patients with severe infections. **Aneeq Farooq, Clin Microbiol Infect 2026;■:1**

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Introduction

The global rise of extended-spectrum β -lactamase-producing and carbapenemase-producing *Klebsiella pneumoniae* poses a substantial public health threat, driven by the decreasing efficacy of available antibiotics and the microorganisms well-documented

propensity for acquiring multidrug resistance [1,2]. *K. pneumoniae* harbours intrinsic fosfomycin resistance genes such as *fosA*, which may be overexpressed [3].

Combination therapy, particularly involving β -lactams and other drug classes, may leverage synergistic pharmacodynamic (PD) interactions and restore antibacterial efficacy against

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resistant pathogens [4,5]. Meropenem remains a first-line option for extended-spectrum β -lactamase-producing infections, whereas intravenous (i.v.) fosfomycin—although less commonly used and typically reserved for complex cases—has demonstrated efficacy in multidrug-resistant (MDR) infections, especially when administered in combination [6–8]. The i.v. fosfomycin European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints for most Enterobacterales were recently removed because of limited evidence for monotherapy. However, many clinicians note the lack of crucial susceptibility testing criteria for fosfomycin in combination therapy, and current Infectious Diseases Society of America guidelines do not routinely recommend i.v. fosfomycin for serious MDR Enterobacterales infections [6]. Despite reported clinical benefits in MDR infections, fosfomycin MICs have not been clearly linked to clinical outcomes [9–12]. Several preclinical studies have reported synergistic antibacterial effects between meropenem and fosfomycin, but the clinical applicability of these findings remain unclear [13–16].

We used published Hollow Fibre and Open Flow *in vitro* data from 12 clinically relevant *K. pneumoniae* isolates [15,16], and developed a pharmacometric model framework describing meropenem and fosfomycin effects and their PD interactions. We then performed simulation studies for clinically relevant dosing regimens to predict the probability of target attainment (PTA) for bacteriostatic and bactericidal endpoints of meropenem–fosfomycin combination therapy across a wide range of MICs. Our analysis offers novel insights to support optimized combination therapy and proposes evidence-based clinical breakpoints for meropenem–fosfomycin combination therapy in *K. pneumoniae*.

Methods

Experimental data

In vitro data of a total of 12 clinically isolated *K. pneumoniae* strains (Table 1) [15,16] were included in this study. PD data for meropenem and fosfomycin—both as monotherapy and in combination—were sourced from previously conducted dynamic *in vitro* experiments designed to mimic human pharmacokinetics (PK) across a range of low- and high-exposure regimens. These included hollow fibre studies with four clinical, KPC-carrying isolates from the University of Hamburg (Hamburg, Germany) and Open Flow *in vitro* model experiments with eight clinical isolates from Bristol Centre for Antimicrobial Research and

Evaluation, Bristol (UK) [15,16]. Additional data are provided in Text S1, Tables S1 and S2, Figures S1 and S2.

Pharmacometric modelling

Nonlinear mixed-effects modelling was conducted on observed bacterial counts over time using NONMEM® 7.5 (Icon Development Solutions, USA). A pharmacometric model was constructed *de novo*, and all potential PD interactions were systematically re-evaluated. The PK of meropenem and fosfomycin in each experimental system were represented by first-order elimination with system-specific half-lives (1 hour and 1.5 hours), and the bacterial response was modelled using the resulting programmed concentration–time profiles, which were verified against measured concentrations in the original experiments [15,16].

The final pharmacometric model (Fig. 1) consisted of two bacterial compartments representing subpopulations with higher and lower susceptibility (S and R in the model), respectively, accounting for the biphasic killing patterns in the experimental data. Although meropenem and fosfomycin MIC values served as covariates on effect- and interaction-potencies, single drug effects of meropenem and fosfomycin were described with separate parameter sets for each subpopulation of bacteria (S and R). Combined effects were quantified using Bliss Independence [17] and the General Pharmacodynamic Interaction model, which quantifies a potential PD interaction as shifts of the potency (half-maximal effect concentration, EC50) or maximum effect (Emax) [18]. To account for differences between the two dynamic *in vitro* systems, system-specific inocula and bacterial capacities were implemented. Details are given in Text S2 and S3, Figures S3 and S4.

Model selection of competing PK/PD models (i.e. interaction on Emax versus EC50) was performed after inspecting biological plausibility of parameters, parameter uncertainty, and the Akaike-Information-Criterion for identifying the one representing the observed data best [19].

Simulation of combination dosing regimens

Previously published population PK models for meropenem and fosfomycin were recoded in R (4.2.1) using mrgsolve [20–23]. Population PK models were then coupled to the PD model developed from the *in vitro* experiments described above. This integrated PK/PD simulation framework enabled predictions of single

Table 1
Tested strains with identified resistance genes and determined MIC values

Strain	Resistance genes	MIC _{MEM} (mg/L)	MIC _{FOF} (mg/L)	<i>In vitro</i> system
<i>Klebsiella pneumoniae</i> 2977	KPC-2, CTX-M-2, SHV-12, OXA-9, TEM-1, SHV-110	32	32	Hollow Fibre ^a
<i>K. pneumoniae</i> N2859	KPC-2, SHV-1	64	4	Hollow Fibre ^a
<i>K. pneumoniae</i> N864	KPC-3, OXA-9, TEM-1, SHV-11	32	16	Hollow Fibre ^a
<i>K. pneumoniae</i> R307	KPC-2, CTX-M-2, OXA-2, OXA-9, SHV-39, TEM-1	128	64	Hollow Fibre ^a
<i>K. pneumoniae</i> C1.184	NMD-1, FosA6	32	32	Open Flow ^b
<i>K. pneumoniae</i> C1.151	KPC-3, FosA6	64	16	Open Flow ^b
<i>K. pneumoniae</i> C1.121	FosA6	0.06	256	Open Flow ^b
<i>K. pneumoniae</i> C1.182	VIM-29, FosA6	>1024	8	Open Flow ^b
<i>K. pneumoniae</i> C1.173	KPC-3, FosA6	>1024	16	Open Flow ^b
<i>K. pneumoniae</i> C1.164	OXA-48, FosA5	1	1024	Open Flow ^b
<i>K. pneumoniae</i> 113321	IMP	256	512	Open Flow ^b
<i>K. pneumoniae</i> KLEB3	—	128	256	Open Flow ^b

MEM, meropenem; FOF, fosfomycin.

^a Farooq et al. [16].

^b MacGowan et al. [15].

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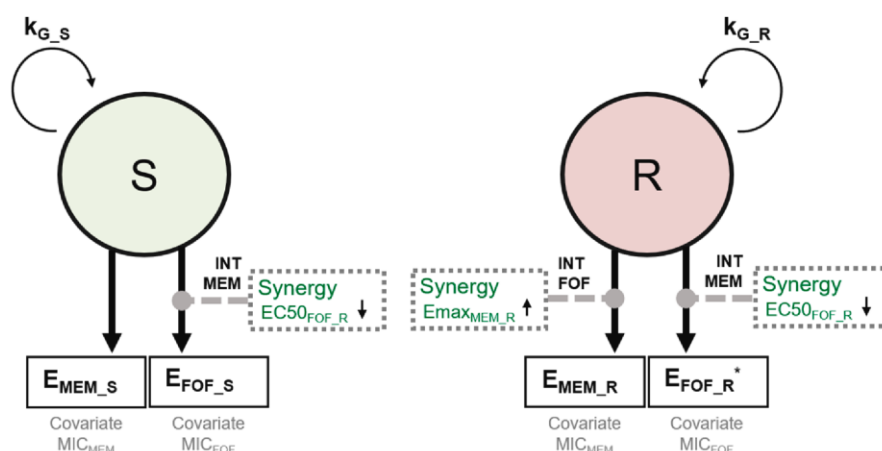


Fig. 1. Schematic representation of the pharmacometric model for dynamic *in vitro* experiments in twelve tested *K. pneumoniae*. (S): compartment addressing subpopulation with higher susceptibility; (R): compartment addressing subpopulation with lower susceptibility; k_{G_S} : growth rate constant; k_{G_R} : growth rate constant; E_{MEM_S} : effect of meropenem on S; E_{MEM_R} : effect of meropenem on R; E_{FOF_S} : effect of fosfomycin on S; E_{FOF_R} : effect of fosfomycin on R; INT MEM: synergistic PD interaction with perpetrator meropenem affecting fosfomycin effect parameter $EC_{50_FOF_S}$ (1) or $EC_{50_FOF_R}$ (1); INT FOF: synergistic PD interaction with perpetrator fosfomycin affecting meropenem effect parameter $E_{max_MEM_R}$ (1).

and combined antibacterial effects over a wide range of clinically relevant scenarios. Simulations were conducted for a wide spectrum of dosing regimens: i.v. meropenem 2 g every 8 hours; and i.v. fosfomycin total daily doses of 6–24 g, administered as every 6 hours, every 8 hours, or continuous infusion. For each regimen, the simulated PK profiles were linked to the *in vitro*-derived PD models to predict the PTA for three predefined efficacy thresholds: bacteriostasis, 1-log killing, and a 2-log killing in bacterial burden after 24 hours of treatment. To assess the impact across the antimicrobial susceptibility range, simulations were performed over a wide distribution of *K. pneumoniae* strains with meropenem and fosfomycin MICs ranging from 0.125 to 1024 mg/L. The PTA threshold for a successful therapy allowing us to derive single and combined breakpoints was predefined as ≥ 0.90 . In addition, the cumulative fraction of response (CFR) was calculated for each *in vitro* system and dosing regimen using PTA predictions across combined MIC population distributions of carbapenemase-producing (mostly New Delhi metallo- β -lactamase [MBL]) *K. pneumoniae* clinical isolates for meropenem and fosfomycin described in the literature [24,25]. Details are provided in Text S2.

Results

Pharmacometric modelling

Table S3 shows the final model parameter estimates with respective confidence intervals. Initial reduction of bacteria was mainly described through effects on subpopulation (S), whereas observed regrowth under drug exposure was mainly driven through subpopulation (R). Three different synergistic PD interactions were identified and estimated: (1) Meropenem reducing the EC_{50} of fosfomycin on (S) by up to 99.99% (95% CI 95.0–99.99%), (2) Meropenem reducing the EC_{50} of fosfomycin on (R) by up to 98.1% (95% CI 91.0–99.9%), and (3) fosfomycin increasing the E_{max} of meropenem on (R) by up to 24.9% (95% CI 18.0–32.0%). The pharmacometric model accurately captured the time course of bacterial concentrations ($\log_{10}$ CFU/mL) in control (drug-free) conditions, as well as under meropenem and fosfomycin monotherapy (Fig. 2) and combination therapy (Fig. 3), with only minor misspecifications.

Model-encoded synergies as visualized in Fig. 1 allowed us to describe strong killing with up to 4 $\log_{10}$ reduction across various

of tested strains and dosing regimen. For KP2977 and KPN864, the combination of meropenem 2 g every 8 hours and fosfomycin 8 g every 8 hours achieved a 4 $\log_{10}$ reduction in bacterial counts with sustained suppression of regrowth for up to 72 hours (Fig. 3). In contrast, monotherapies did not prevent regrowth (Fig. 2). These findings exemplified the model's ability to accurately predict distinct bacterial responses under both single drug and combination treatment scenarios.

Simulation studies

Predictions derived from Monte Carlo simulations based on the developed pharmacometric model—for PTA ≥ 0.90 at selected MIC combinations (referred to as combined breakpoints) and CFR—are summarized for nine different combination dosing regimens and three different predefined PD targets in Table 2 [24,25]. These simulations used the meropenem model of Li et al. [20] and the fosfomycin model of Parker et al. [21]. Results for renal impairment and for alternative fosfomycin PK models are shown in Material S1, with the alternative models producing virtually identical results.

Fig. 4 visualizes predicted PTAs for differently susceptible bacterial strains treated with meropenem 2 g and fosfomycin 8 g every 8 hours in both *in vitro* systems and for predefined PD targets in the shape of heatmaps. Increasing MIC values along each of the axes allows us to identify predicted PTAs for meropenem and fosfomycin under respective monotherapies, whereas any other predicted PTA values are representing combination therapy. The breakpoint of meropenem monotherapy for bacteriostasis, 1-log killing, and 2-log killing was predicted in the Open Flow system to be 4 mg/L, 4 mg/L, and 2 mg/L, respectively. In the Hollow Fibre system on the other hand, a meropenem breakpoint was only identified for bacteriostasis at 2 mg/L. For fosfomycin monotherapy and bacteriostasis, the breakpoint was predicted at 4 mg/L in both *in vitro* systems, whereas for 1-log killing, it was 1 mg/L. For all other PD targets, the predicted single PTA values were too low to define single breakpoints. The predicted combined breakpoints ($MIC_{MEM} | MIC_{FOF}$) for bacteriostasis and 1-log killing in both *in vitro* systems were 1024 mg/L | 1024 mg/L and 512 mg/L | 512 mg/L, respectively. For 2-log killing, breakpoint values differed between the two systems: in the Hollow Fibre system, the combined breakpoint was 32 mg/L | 32 mg/L, whereas in the Open Flow system it was 32 mg/L | 512 mg/L.



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Fig. 3. Stratified visual predictive checks of pharmacometric model characterizing the bacterial load in log 10 cfu/mL for meropenem and fosfomycin combination scenarios in all twelve clinical *K. pneumoniae* strains across both *in vitro* systems. Model predictions incorporate the system-specific PK profiles programmed and validated in each dynamic *in vitro* experiment. Dots, observations; solid green line, median model prediction; shaded green area, 90% prediction intervals; MEM, meropenem; FOF, fosfomycin; AUC/MIC, AUC over MIC; T/MIC, time over MIC.

To enable consistent comparisons of combined breakpoints across the range of bacterial susceptibilities and dosing regimens, Table 2 presents the results by simplifying the MIC_{MEM} dimension and fixes it at MIC_{MEM} = 32 mg/L. Predicted PTA values with the

lowest dosing regimen simulated (meropenem 2 g plus fosfomycin 2 g every 8 hours) identified bacteriostasis in strains with 32 mg/L | 512 mg/L (or higher susceptibility) as achievable. The higher PD targets of 1-log killing and 2-log killing were identified as

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Table 2

Pharmacometric model-derived simulations of combined fosfomycin MIC breakpoints with respective probability of target attainment ≥ 0.90 , and cumulative fraction of response for meropenem/fosfomycin combination therapy in different *in vitro* models, dosing regimens, and pharmacodynamic endpoints

Dosing regimen	<i>In vitro</i> system	Combined FOF breakpoint ^a (mg/L)			Cumulative fraction of response ^b		
		Bacteriostasis	1-log killing	2-log killing	Bacteriostasis	1-log killing	2-log killing
MEM 2 g every 8 h +	Hollow Fibre	512	256	8	0.99	0.97	0.27
FOF 2 g every 8 h	Open Flow	1024	1024	64	1.00	0.99	0.87
MEM 2 g every 8 h +	Hollow Fibre	1024	1024	16	1.00	0.98	0.68
FOF 4 g every 8 h	Open Flow	1024	1024	128	1.00	0.99	0.90
MEM 2 g every 8 h +	Hollow Fibre	1024	1024	16	1.00	0.99	0.72
FOF 5 g every 8 h	Open Flow	1024	1024	1024	1.00	1.00	0.90
MEM 2 g every 8 h +	Hollow Fibre	1024	1024	16	1.00	0.99	0.72
FOF 8 g every 12 h	Open Flow	1024	1024	512	1.00	0.99	0.90
MEM 2 g every 8 h +	Hollow Fibre	1024	1024	16	1.00	0.99	0.72
FOF 4 g every 6 h	Open Flow	1024	1024	256	1.00	1.00	0.90
MEM 2 g every 8 h +	Hollow Fibre	1024	1024	32	1.00	1.00	0.75
FOF 8 g bolus + 8 g cont.	Open Flow	1024	1024	512	1.00	1.00	0.90
MEM 2 g every 8 h +	Hollow Fibre	1024	1024	16	1.00	0.99	0.75
FOF 6 g every 8 h	Open Flow	1024	1024	1024	1.00	1.00	0.90
MEM 2 g every 8 h +	Hollow Fibre	1024	1024	32	1.00	0.99	0.77
FOF 8 g every 8 h	Open Flow	1024	1024	512	1.00	1.00	0.91
MEM 2 g every 8 h +	Hollow Fibre	1024	1024	32	1.00	1.00	0.79
FOF 8 g bolus + 16 g cont.	Open Flow	1024	1024	1024	1.00	1.00	0.90

Combined breakpoints are based on a meropenem MIC of 32 mg/L. Each PTA was based on pharmacometric predictions for virtual patients with serum creatinine clearance >60 mL/min, and calculated from 1000 simulations per MIC pair in both *in vitro* systems (Hollow Fibre and Open Flow) at 24 hours of therapy for three different pharmacodynamic targets: bacteriostasis, 1-log kill, and 2-log kill. CFR, cumulative fraction of response; FOF, fosfomycin; MEM, meropenem; PTA, probability of target attainment.

^a PTA was calculated using 1000 simulations for each combination of MIC_{MEM}/MIC_{FOF} pair with MIC_{MEM} = 32 mg/L, for each *in vitro* system, dosing regimen, and pharmacodynamic target.

^b CFR was calculated using PTA and combined MIC population distributions of meropenem and fosfomycin from Della Rocca et al. [24] and Erturk Sengel et al. [25].

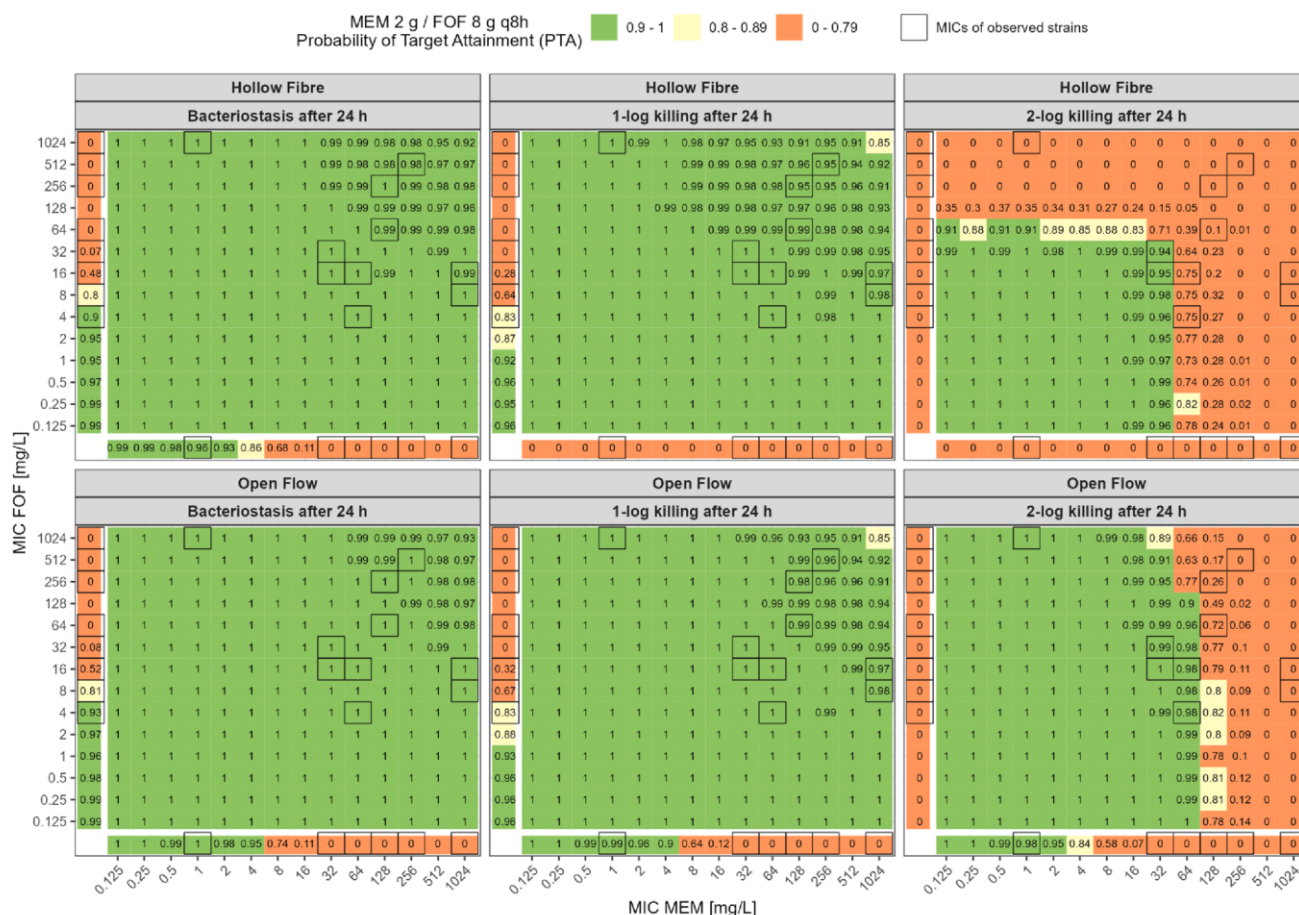


Fig. 4. Probability of target attainment (PTA) heatmap for meropenem 2 g (short infusion) fosfomycin 8 g (1-hour infusion) q8h, based on pharmacometric model predictions for virtual patients with serum creatinine clearance >60 mL/min and infected by differently susceptible bacterial strains. Each PTA was calculated from $n = 1,000$ simulations per MIC pair in both *in vitro* systems (Hollow Fibre and Open Flow) at 24 hours of therapy for three different pharmacodynamic targets: bacteriostasis, 1-log killing, and 2-log killing. Single MIC values along the axes represent predicted PTA under monotherapy. Single MIC values and MIC combinations in which PTA ≥ 0.9 is predicted, are highlighted in green, facilitating the identification of single and combined breakpoints. Tiles outlined with a border indicate MIC values of observed clinical strains present in the experimental datasets. MEM, meropenem; FOF, fosfomycin.

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achievable for strains with 32 mg/L | 256 mg/L and 32 mg/L | 8 mg/L, respectively. For the high-dose regimen (meropenem 2 g plus fosfomycin 8 g every 8 hours), simulations based on the Hollow Fibre system suggested a combined breakpoint at 32 mg/L | 32 mg/L, whereas based on the Open Flow system at 32 mg/L | 512 mg/L. A simulated continuous-infusion regimens offered no or minor additional benefit over intermittent dosing. We note that multiple MIC pairs can satisfy $PTA \geq 0.90$; a single representative pair is reported for clarity.

The calculated CFRs were close to 1 for bacteriostasis and at least 0.97 for 1-log killing, both across all simulated dosing regimens—indicating target attainment across almost the entire study population of strains. In contrast, CFRs for achieving 2-log killing varied depending on both the dosing regimen and the *in vitro* system, ranging from 0.27 to 0.79 in the Hollow Fibre system and from 0.87 to 0.90 in the Open Flow system.

Discussion

This study developed a novel pharmacometric model describing the antibacterial effects of meropenem and fosfomycin, both as mono and combination therapy, against clinically relevant, MDR *K. pneumoniae* in two dynamic *in vitro* systems. Significant synergistic interactions were identified, and population PK models were integrated to perform extensive PK/PD simulations across clinically relevant dosing regimens, assessing bacteria with different susceptibilities. We predicted PTA for bacteriostasis, 1-log, and 2-log endpoints, assessed CFR, and determined evidence-based single and combined breakpoints for meropenem–fosfomycin therapy. Predicted PTAs for low and high-dose combination therapy indicate the potential of these regimens, whereas monotherapies at similar doses are expected to fail. CFRs based on PTA and MIC distributions from the literature were high for bacteriostasis and 1-log killing, and moderate to high for 2-log killing depending on total daily doses [24,25]. These findings may allow for the use of meropenem when combined with i.v. fosfomycin for the treatment of carbapenemase-producing *K. pneumoniae* infections with elevated meropenem MICs, including MBL producers. This is particularly interesting, as there are limited options available for the therapy of MBL-producing Enterobacterales. Notably, synergy of fosfomycin-containing combinations has also been demonstrated in a human-simulated neutropenic murine infection model, supporting the potential translatability of our *in vitro* findings [26].

Our results align with previous preclinical studies documenting synergy between meropenem and fosfomycin, which also discuss possible mechanistic explanations for this interaction. [13–16]. Moreover, we extended previous static modelling by integrating dynamic PK/PD data from two complementary *in vitro* systems. Our single-agent breakpoints align with EUCAST clinical standards, which are based on bacteriostasis. In both *in vitro* systems, a fosfomycin breakpoint of 4 mg/L was identified; although EUCAST does not provide a general Enterobacterales breakpoint for fosfomycin, it sets a susceptibility threshold of $S \leq 8$ mg/Lin *Escherichia coli* urinary infections. Simulations based on the Hollow Fibre system, which provided a more conservative prediction, suggested high PTA and CFR for combination therapy, with combined breakpoints (e.g. $MIC_{MEM} = 32$ mg/L | $MIC_{FOF} = 1024$ mg/L for 1-log killing, $MIC_{MEM} = 32$ mg/L | $MIC_{FOF} = 32$ mg/L for 2-log killing) that reflect synergy. The higher breakpoint predictions in the Open Flow system likely reflect its lower inoculum, lower bacterial capacity, and dilution-driven PK, which result in less regrowth pressure than the longer and higher-density Hollow Fibre experiments. These differences mean that Hollow Fibre generally provides the more conservative, and thus clinically cautionary

predictions. Importantly, our simulated high-dose regimens achieved high PTA at or above the epidemiological cut-off (128 mg/L), ensuring the proposed combination breakpoint does not bisect the wild-type MIC distribution for *K. pneumoniae* [27]. Furthermore, our simulations indicate that favourable PD outcomes are achievable with combination therapy, even in strains exhibiting high-level resistance to both meropenem and fosfomycin. Such combined breakpoints could be implemented in practice by applying a standardized MIC_{MEM} | MIC_{FOF} interpretive matrix to routine single-agent MIC results. Considering potential fosfomycin side effects [28], this may support combination strategies using lower fosfomycin doses in some clinical situations. In line with this, the lowest tested regimen (meropenem 2 g + fosfomycin 2 g every 8 hours) still achieved a combined 2-log-killing breakpoint of 32 mg/L | 8 mg/L in the Hollow Fibre. On the basis of our findings, monotherapy is likely appropriate only when the MIC of meropenem or fosfomycin is ≤ 4 mg/L, whereas higher MICs would favour the use of combination therapy, although disease severity or deep-seated infections may further influence this decision.

A key innovation of our study is the novel insight into combined breakpoints for meropenem and fosfomycin, which exceed single-agent breakpoints because of proven synergy. Our use of pharmacometric modelling and simulation, integrating real-world human population PK and a range of robust *in vitro* PD data, expands PTA predictions far beyond traditional PK/PD indices [29,30]. In addition, drawing from two complementary *in vitro* systems enhances external validity and enables cross-system comparison over a wide MIC range. Mechanistically, our model demonstrates that synergy arises from shifts in both EC_{50} and $Emax$ in the combinations, providing a strong scientific basis for combination therapy.

Limitations mainly arise from reliance on *in vitro* data, which cannot fully represent clinical infection settings. Although we included 12 *K. pneumoniae* strains of varying susceptibilities, the sample does not capture the full clinical diversity, and clinical infection-site bacterial burdens remain unknown. MIC distributions for CFR calculations were literature-based and may not fully generalize to clinical populations, and although the model was built using experimental MICs spanning meropenem 0.06 to >1028 mg/L and fosfomycin 4–1024 mg/L, these ranges still do not encompass all clinically observed combinations. In addition, several MIC values used in simulations required interpolation or extrapolation beyond the experimental range, which should be interpreted cautiously. Although dynamic *in vitro* models provide quantitative, human PK-linked time–kill data, they still cannot replicate host immunity or disease-site PD, and the magnitude of the observed PD interactions may differ *in vivo* and in clinic.

The findings provide a rational for a fosfomycin breakpoint as a combined breakpoint with meropenem. However, as the observed synergy represents an *in vitro* finding, further confirmation *in vivo* and in clinical studies is needed.

CRediT authorship contribution statement

Aneeq Farooq: Conceptualization, Data Curation, Formal Analysis, Methodology, Software, Visualization, Writing - Original draft preparation, Writing - Reviewing and Editing. Miklas Martens: Formal Analysis, Visualization, Writing - Reviewing and Editing. Marie L.G. Attwood: Data Curation, Writing - Reviewing and Editing. Patrice Nordmann: Data Curation, Writing - Reviewing and Editing. Alasdair MacGowan: Data Curation, Writing - Reviewing and Editing. Sebastian G. Wicha: Conceptualization, Data Curation, Formal Analysis, Methodology, Writing - Reviewing and Editing.

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Transparency declaration

Potential conflict of interest

A.M. holds research grants/activities with Merck, Shionogi, InfectoPharm, GSK, Roche, BioVersys, iFAST, Oxford Drug Design, JPIAMR, and NIHR; A.M. provides consultancy advice to Shionogi, Roche, Bicycle Therapeutics, and BioVersys. S.G.W. holds research grants/activities with AqVida, Boehringer Ingelheim, Merck KGaA, Medicines for Malaria Venture, InfectoPharm, and Shionogi. All other authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cmi.2026.02.024>.

References

- [1] Effah CY, Sun T, Liu S, Wu Y. *Klebsiella pneumoniae*: an increasing threat to public health. *Ann Clin Microbiol Antimicrob* 2020;19:1. <https://doi.org/10.1186/s12941-019-0343-8>.
- [2] Doi Y. Treatment options for carbapenem-resistant Gram-negative bacterial infections. *Clin Infect Dis* 2019;69:S565–75. <https://doi.org/10.1093/cid/ciz830>.
- [3] Elliott ZS, Barry KE, Cox HL, Stoesser N, Carroll J, Vegesana K, et al. The role of *fosA* in challenges with fosfomycin susceptibility testing of multispecies *Klebsiella pneumoniae* carbapenemase-producing clinical isolates. *J Clin Microbiol* 2019;57:e00634–19. <https://doi.org/10.1128/jcm.00634-19>.
- [4] Tyers M, Wright GD. Drug combinations: a strategy to extend the life of antibiotics in the 21st century. *Nat Rev Microbiol* 2019;17:141–55. <https://doi.org/10.1038/s41579-018-0141-x>.
- [5] Tamma PD, Cosgrove SE, Maragakis LL. Combination therapy for treatment of infections with Gram-negative bacteria. *Clin Microbiol Rev* 2012;25:450–70. <https://doi.org/10.1128/CMR.05041-11>.
- [6] Tamma PD, Heil EL, Justo JA, Mathers AJ, Satlin MJ, Bonomo RA. Infectious Diseases Society of America 2024 Guidance on the treatment of antimicrobial-resistant gram-negative infections. *Clin Infect Dis* 2024; ciae403. <https://doi.org/10.1093/cid/ciae403>.
- [7] Tedeschi S, Giannitsioti E, Mayer C. Emerging concepts for the treatment of biofilm-associated bone and joint infections with IV fosfomycin: a literature review. *Microorganisms* 2025;13:963. <https://doi.org/10.3390/microorganisms13050963>.
- [8] Butler DA, Patel N, O'Donnell JN, Lodise TP. Combination therapy with IV fosfomycin for adult patients with serious Gram-negative infections: a review of the literature. *J Antimicrob Chemother* 2024;79:2421–59. <https://doi.org/10.1093/jac/dkac253>.
- [9] Paul M, Carrara E, Retamar P, Tängdén T, Bitterman R, Bonomo RA, et al. European society of clinical microbiology and infectious diseases (ESCMID) guidelines for the treatment of infections caused by multidrug-resistant gram-negative bacilli (endorsed by European society of intensive care medicine). *Clin Microbiol Infect* 2022;28:521–47. <https://doi.org/10.1016/j.cmi.2021.11.025>.
- [10] Bodmann K-F, Hagel S, Oliva A, Kluge S, Mularoni A, Galfo V, et al. Real-world use, effectiveness, and safety of intravenous fosfomycin: the FORTRESS study. *Infect Dis Ther* 2025;14:765–91. <https://doi.org/10.1007/s40121-025-01125-2>.
- [11] The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, version 15.0. 2025. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_15.0_Breakpoint_Tables.pdf.
- [12] Bassetti M, Graziano E, Berruti M, Giacobbe DR. The role of fosfomycin for multidrug-resistant gram-negative infections. *Curr Opin Infect Dis* 2019;32: 617–25. <https://doi.org/10.1097/QCO.0000000000000597>.
- [13] Albiero J, Sy SKB, Mazucheli J, Caparroz-Assef SM, Costa BB, Alves JLB, et al. Pharmacodynamic evaluation of the potential clinical utility of fosfomycin and meropenem in combination therapy against KPC-2-producing *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2016;60:4128–39. <https://doi.org/10.1128/AAC.03099-15>.
- [14] Al-Quraini M, Rizvi M, Al-Jabri Z, Sami H, Al-Muzahmi M, Al-Muharrmi Z, et al. Assessment of in-vitro synergy of fosfomycin with meropenem, amikacin and tigecycline in whole genome sequenced extended and pan drug resistant *Klebsiella pneumoniae*: exploring a colistin sparing protocol. *Antibiotics (Basel)* 2022;11:153. <https://doi.org/10.3390/antibiotics11020153>.
- [15] MacGowan AP, Griffin P, Attwood MLG, Daum AM, Avison MB, Noel AR. The pharmacodynamics of fosfomycin in combination with meropenem against *Klebsiella pneumoniae* studied in an in vitro model of infection. *J Antimicrob Chemother* 2025;80:967–75. <https://doi.org/10.1093/jac/dkaf020>.
- [16] Farooq A, Martens M, Kroemer N, Pfaffendorf C, Decousser J-W, Nordmann P, et al. Pharmacokinetic/pharmacodynamic analysis of meropenem and fosfomycin combinations in in vitro time–kill and hollow-fibre infection models against multidrug-resistant and carbapenemase-producing *Klebsiella pneumoniae*. *J Antimicrob Chemother* 2025;80:701–12. <https://doi.org/10.1093/jac/dkaf459>.
- [17] Bliss CI. The toxicity of poisons applied jointly. *Ann Appl Biol* 1939;26: 585–615. <https://doi.org/10.1111/j.1744-7348.1939.tb06990.x>.
- [18] Wicha SG, Chen C, Clewe O, Simonsson USH. A general pharmacodynamic interaction model identifies perpetrators and victims in drug interactions. *Nat Commun* 2017;8:2129. <https://doi.org/10.1038/s41467-017-01929-y>.
- [19] Akaike H. A new look at the statistical model identification. *IEEE Trans Autom Control* 1974;19:716–23. <https://doi.org/10.1109/TAC.1974.1100705>.
- [20] Li C, Kutij JL, Nightingale CH, Nicolau DP. Population pharmacokinetic analysis and dosing regimen optimization of meropenem in adult patients. *J Clin Pharmacol* 2006;46:1171–8. <https://doi.org/10.1177/0091270006291035>.
- [21] Parker SL, Frantzeskaki F, Wallis SC, Diakaki C, Giamarellou H, Koulenti D, et al. Population pharmacokinetics of fosfomycin in critically ill patients. *Antimicrob Agents Chemother* 2015;59:6471–6. <https://doi.org/10.1128/aac.01321-15>.
- [22] Merino-Bohórquez V, Docobo-Pérez F, Sojo J, Morales I, Lupión C, Martín D, et al. Population pharmacokinetics and pharmacodynamics of fosfomycin in non-critically ill patients with bacteremic urinary infection caused by multidrug-resistant *Escherichia coli*. *Clin Microbiol Infect* 2018;24:1177–83. <https://doi.org/10.1016/j.cmi.2018.02.005>.
- [23] Pfausler B, Spiss H, Ditttrich P, Zeitlinger M, Schmutzhard E, Joukhadar C. Concentrations of fosfomycin in the cerebrospinal fluid of neurointensive care patients with ventriculostomy-associated ventriculitis. *J Antimicrob Chemother* 2004;53:848–52. <https://doi.org/10.1093/jac/dkh158>.
- [24] Della Rocca MT, Di Caprio G, Colucci F, Merola F, Panetta V, Cordua E, et al. Fosfomycin–meropenem synergistic combination against NDM carbapenemase-producing *Klebsiella pneumoniae* strains. *New Microbiol* 2023;46:264–70.
- [25] Erturk Sengel B, Altinkanat Gelmez G, Soylerir G, Korten V. In vitro synergistic activity of fosfomycin in combination with meropenem, amikacin and colistin against OXA-48 and/or NDM-producing *Klebsiella pneumoniae*. *J Chemother* 2020;32:237–43. <https://doi.org/10.1080/1120009X.2020.1745501>.
- [26] Papp-Wallace KM, Zeiser ET, Becka SA, Park S, Wilson BM, Winkler ML, et al. Ceftazidime–Avibactam in combination with fosfomycin: a novel therapeutic strategy against multidrug-resistant *Pseudomonas aeruginosa*. *J Infect Dis* 2019;220:666–76. <https://doi.org/10.1093/infdis/jiz149>.
- [27] European Committee on Antimicrobial Susceptibility Testing (EUCAST). Antimicrobial wild type distributions of microorganisms. 2025. <https://mic.eucast.org/search/show-registration/39183?back=https://mic.eucast.org/search/?search%255Bmethod%255D%3Dmic%26search%255Bantibiotic%255D%3D100%26search%255Bspecies%255D%3D-1%26search%255Bdisk%255D%3D-1%26search%255Blimit%255D%3D50> (accessed November 4, 2025).
- [28] Cojutti PG, Tedeschi S, Zamparini E, Fornaro G, Zagarrigo M, De Paolis M, et al. Could a reduced dose of 8 g of continuous infusion fosfomycin be considered as effective as and safer than a standard 16 g dose when combined with high-dose daptomycin in the treatment of Staphylococcal osteoarticular infections? *Antibiotics (Basel)* 2025;14:139. <https://doi.org/10.3390/antibiotics14020139>.
- [29] Nielsen EI, Cars O, Friberg LE. Pharmacokinetic/Pharmacodynamic (PK/PD) indices of antibiotics predicted by a semimechanistic PKPD model: a step toward model-based dose optimization. *Antimicrob Agents Chemother* 2011;55:4619–30. <https://doi.org/10.1128/aac.00182-11>.
- [30] Rodriguez-Gonzalez RA, Leung CY, Chan BK, Turner PE, Weitz JS. Quantitative models of phase-antibiotic combination therapy. *mSystems* 2020;5:e00756-19. <https://doi.org/10.1128/mSystems.00756-19>.

6 Discussion

The presented publications I – IV cover three different combination therapies, including meropenem-fosfomycin (**Publication I** and **IV**), phage pK8-polymyxin B (**Publication II**), and ceftazidime/avibactam-tigecycline (**Publication III**), against MDR Gram-negative bacteria in *in vitro* studies, as well as the application of pharmacometric modelling for identifying and characterising their PD interactions. Meropenem, fosfomycin, ceftazidime/avibactam and polymyxin B are approved last-resort antibiotics recommended to be used in different infectious diseases^{30,31}, while phage pK8 is an investigational drug¹⁴². Resistance against each of these antibiotics has been already reported with the overall global situation worsening. IV fosfomycin is recommended to be used in combination with other antibiotics, but at the same time, suitable combination partners following a rationale are not given⁴⁷. Additionally, there are currently no breakpoints for its use in patients defined by EUCAST²⁴. Phage therapy is a promising option in times of escalating AMR and sparse innovation in the field of antimicrobials, but suitable applications for overcoming its limitations are not clear yet^{70,84}. *In vitro* investigations on the combination of ceftazidime/avibactam and tigecycline based on the FICI showed mainly indifferent results, leaving the effectiveness of this combination unclear. Each study effectively met the three main objectives that were initially set. Firstly, appropriate *in vitro* methods were successfully developed and applied for a comprehensive quantitative evaluation of combined effects. Secondly, suitable pharmacometric models were developed that accurately captured the combined effects by integrating PD interactions, fully characterizing their directionality, type, and potency. Thirdly, additional *in vitro* and *in silico* experimental data was collected, validating the findings through HFIM experiments, by utilizing metabolomic analyses, or by performing extensive simulations.

The presented results of the four works highlight the potential of these combination therapies, thereby clarifying their possible applications and evaluating the rationale for expanded investigations.

7.1 Promising strategies for rational combination therapies

7.1.1 Meropenem-fosfomycin combination therapy

Publication I used the integration of static time-kill assays, pharmacometric modelling and human PK mimicking HFIM experiments to identify significant synergistic PD interactions between meropenem and fosfomycin, particularly against *K. pneumoniae* strains expressing resistance against meropenem and partially even against fosfomycin. The findings reveal that this combination therapy significantly suppressed bacterial regrowth at clinically achievable concentrations, even when single therapy of meropenem or fosfomycin failed to do so. By applying the GPDI model in pharmacometric models, PD interactions in all four tested strains could successfully be categorised into additive and synergistic, while gaining knowledge regarding the directionality of the identified synergy. Furthermore, actual drug levels from static time-kill assays were fed into the development of PK/PD models, consequently addressing the degradation of instable antibiotics such as meropenem, bacteria-induced degradation and the time-dependent effect sizes. A comparable approach, which incorporates actual concentrations of antibiotics alongside bacteria-independent drug degradation solely due to thermodynamic effects, was described by Wicha *et al.*¹⁴³. Bacteria-induced degradation rate constants were successfully estimated to exceed total meropenem degradation to more than thrice the size of the basal meropenem degradation without bacteria. This additional degradation can be explained through expression of KPC-2 or KPC-3, which as a result actively hydrolysed meropenem. When translating this into the clinical situation, one could hypothesize that similar enzymatic degradation of carbapenems happens in the infected tissues of the patients, resulting in lower actual exposure to the drug. In two of four strains, synergy was best described with a substantial reduction of the EC_{50} of fosfomycin on subpopulation B_R , driven by the concentration of meropenem as perpetrator of the interaction. This suggests that the combination therapy could serve as a protective measure against the emergence of resistant subpopulations. In fact, a lower mutation frequency was proven in an *in vitro* study of ceftazidime/avibactam, a beta-lactam/beta-lactamase combination similar to meropenem, and fosfomycin⁴⁸. The synergy between meropenem-fosfomycin was observed not only in static models but also in dynamic HFIM experiments over a period of three days, which better reflects the clinical situation. Moreover, it can be argued that in animals or humans with an intact immune system, the tested combination therapies simulating human PK might have an even higher likelihood of successfully reducing bacterial burden¹⁴⁴. A study by Drusano *et al.* examined tedizolid, a bacteriostatic drug, using a thigh infection model in both healthy and neutropenic mice¹⁴⁵. Their

results showed that granulocytes significantly boosted the efficacy of tedizolid treatment, with healthy animals experiencing a much greater reduction in bacterial load compared to granulocytopenic animals. These results underline the importance of an intact immune system in maximizing the clinical efficacy observed with combination therapies in preclinical models.

Our findings suggest that leveraging meropenem-fosfomycin combination therapy could enhance treatment outcomes in clinical settings where monotherapy might be expected to be insufficient. Simultaneous consideration of MIC values for meropenem and fosfomycin planned for combined application could be a primary decision factor, as the only combination therapy that failed in the HFIM was the one associated with the highest MIC values. The combination of meropenem and fosfomycin might serve as an alternative to the recommended treatments for infections caused by KPC-producers, such as meropenem/vaborbactam or ceftazidime/avibactam³⁰. Additionally, sufficient evidence showing the suppression of resistance through combination therapy could encourage its use not only in severe cases but also as a standard method to prevent resistant strains from emerging, thereby providing another tool against the worsening AMR situation.

An important extension to these findings is provided by **Publication IV**, which analysed *in vitro* data from twelve MDR *K. pneumoniae* strains across two dynamic infection model systems (HFIM and Open Flow). Using a pharmacometric approach that included MIC values as covariates and employed the GPD1 model, synergistic interactions were quantitatively characterized. *In silico* simulations with published population PK models were performed, evaluating a diverse range of dosing regimens. **Publication IV** demonstrated that while monotherapy achieved PTA $\geq 90\%$ only for low MICs, meropenem-fosfomycin combination therapy enabled PTA $\geq 90\%$ for bacteriostasis and 1-log killing at substantially higher MIC combinations—specifically up to MIC_{MER} | MIC_{FOF} of 32 mg/L | 512 mg/L, depending on the treatment regimen and efficacy endpoint. Based on these simulations, **Publication IV** proposed rational, evidence-based combined breakpoints for meropenem-fosfomycin therapy, such as MIC_{MER} | MIC_{FOF} values of 32 mg/L | 512 mg/L for 1-log killing, and 32 mg/L | 32 mg/L for 2-log killing, depending on the dosing strategy and *in vitro* model. This approach provides a scientific rationale for reintroducing a fosfomycin breakpoint in the context of combination therapy, guiding both susceptibility testing and optimal dosing for severe MDR *K. pneumoniae* infections and addressing the gap in clinical practice after the EUCAST removal of the standalone IV fosfomycin breakpoint.

Considering safety, Abdallah *et al.* showed in their study investigating the efficacy and safety of IV fosfomycin for the treatment of MDR Gram-negative infections that hypernatremia (23.3%) and hypokalemia (43.3%) were the most frequently observed adverse events¹⁴⁶. Depending on their severity, these side effects can limit the use of IV fosfomycin. Here the rationale could be combining fosfomycin with meropenem, subsequently reducing the need of fosfomycin dose escalation and its side effects.

A recent retrospective observational study conducted by Oliva *et al.* compared fosfomycin-containing combination regimens to those without fosfomycin for treating infections caused by carbapenem-resistant *K. pneumoniae* and *A. baumannii*¹⁴⁷. The study, which analysed 78 critically ill patients, showed promising results: patients receiving fosfomycin-containing regimens experienced higher rates of clinical cure, early 3-day improvement, microbiological eradication, and lower 7-, 14- and 30-day mortality. The combination regimens also included meropenem-fosfomycin and meropenem/vaborbactam-fosfomycin in six patients infected by *K. pneumoniae* expressing KPC.

7.1.2. Phage-polymyxin B combination therapy

Publication II implemented a pharmacometric modelling approach to elucidate the killing kinetics and PD interaction dynamics of bacteriophage pK8 in combination with polymyxin B against a polymyxin-resistant *K. pneumoniae* strain. Key findings demonstrate that while polymyxin B alone was ineffective, its combination with phage pK8 resulted in notable bactericidal effects and reduced bacterial burden, particularly at higher initial MOI. Even though phage pK8 alone significantly reduced bacterial concentrations, it was not sufficient to prevent regrowth beyond initial levels across all scenarios tested. The study builds upon earlier findings highlighting the synergistic potential of phage pK8 and polymyxin B, supported by profound metabolic changes and morphological differences observed in bacterial cells under drug exposure¹⁴². In their study, distinct differences in cell surface morphology and membrane potential were observed in the resistant *K. pneumoniae* strain with polymyxin B, phage pK8, or their combination. Polymyxin B alone disorganized the outer membrane without killing the cells, while the combination caused lysis and significant metabolic perturbations. Metabolomic analysis showed similar changes from phage pK8 and polymyxin B at 4 hours, but not at 24 h due to resistance, indicating initial synergy driven by the phage and later resistance prevention to pK8 or polymyxin B., indicating initial synergy and later resistance prevention After evaluating

four biologically plausible mechanisms to describe the PD interaction and a comparison of our *in silico* findings with the mechanistic discoveries made by Han *et al.*, we can affirm agreement. The pharmacometric model effectively captured these dynamics, identifying resensitization of polymyxin B as the driver of the observed synergy. This work enhances our understanding of PD interactions by moving beyond traditional checkerboard assays, instead applying time-dissolved sampling for a precise exploration of interaction dynamics.

Although limited by its *in vitro* nature, this study provides a strong rationale for advancing phage-antibiotic combination strategies to *in vivo* models, thereby informing potential clinical applications against MDR pathogens. Future investigations are needed to translate these *in vitro* results into clinical practice, considering crucial factors such as infection site specificity, administration routes, and phage PK. Given phages' high specificity, strategic frameworks are essential to ensure the appropriate phage or phage-antibiotic combination is applied accurately to target bacterial strains. A randomised, controlled and double-blind clinical trial investigated the efficacy and tolerability of a cocktail of phages for treating burn wound infections caused by *Pseudomonas aeruginosa*¹⁴⁸. This study was the first clinical trial that followed good manufacturing and good clinical practice when comparing phage therapy to standard of care, in this case sulfadiazine silver. In conclusion, this study showed that phage therapy can effectively reduce bacterial burden in infected wounds, despite taking longer than standard care. Initial susceptibility of the infecting strain to applied phages was crucial for success, highlighting the need for phage susceptibility testing instead of pure presumptive therapy. Additionally, the phage treatment group experienced fewer serious septic complications, suggesting an extra benefit of phage therapy. These findings encourage further research into the use of phages alongside other phages or even combining phage cocktails with antibiotics. Looking ahead, numerous clinical trials are actively exploring phage therapy, particularly among patients with cystic fibrosis, offering promising insights into its future applications¹⁴⁹.

Polymyxins are last-resort antibiotics, but their use is largely restricted because of their unfavourable safety profile. They are linked to adverse effects such as nephrotoxicity and neurotoxicity^{150,151}. Different strategies have been recommended for minimizing polymyxin-induced toxicity, including dose adjustments according to patient's renal function estimates and discontinuation with use of alternative antibiotic therapy. As already proposed for the synergistic combination meropenem-fosfomycin, polymyxin therapy could be supplemented

with phages resulting in a combination therapy with known synergistic effects, hence allowing to potentially reduce polymyxin doses.

This work demonstrates how personalized phage therapy could be further advanced in clinical settings. The workflow in this work involved the isolation of a clinical strain from a patient, cultivating and amplifying phage pK8 against this specific bacterium, and conducting both mono and combination testing. Since extensive testing with time-kill assays is not feasible for clinical real-life situations, these could be replaced by experiments similar to traditional checkerboards. Checkerboard assays for testing potential phage-antibiotic combinations were already developed based on the FICI-criterion¹⁵². This approach could help not only to cultivate effective phages but also to test combinations known to be synergistic in *in vitro* experiments, following the example of Antimicrobial Susceptibility Testing via MIC, which is usually present in hospitals.

7.1.3. Mechanistic insights into synergistic PD interactions

Publication I, **II**, and **IV** present two promising strategies for rational combination therapies by showcasing enhanced treatment efficacy through synergistic interactions. They show how a broad range of *in vitro* assays coupled with pharmacometric modelling can serve as tools to analyse the underlying mechanisms of PD interactions and define them quantitatively.

Pharmacometric modelling included the testing of different structural models for describing the often observed two-phasic bacterial growth under drug exposure. Initial decline of bacterial burden was modelled with a drug-susceptible bacterial subpopulation, while regrowth of bacteria was mainly driven by a less susceptible or resistant subpopulation. A commonly used structural model for describing time-kill data of antibiotics describes the second bacterial compartment to be resistant, completely drug-insensitive and resting, with an adaptive resistance reducing the effect of the drug on the susceptible population²⁹. Since phenotypical resistance to meropenem, fosfomycin, or both was already proven through MIC testing in all four strains, the structure of the developed PK/PD models in **Publication I** and **IV** consists of two bacterial compartments (susceptible (S) and less-susceptible (R)), with the R subpopulation assumed to be present at the beginning of each experiment, with its size being estimated as a model parameter ('inoculum resistant bacteria'). On the other hand, the developed PK/PD model in **Publication II** also consists of two structural compartments, but resistance was described to develop rapidly following an estimated rate constant ('resistance to phages rate constant') with no phage-resistant subpopulation present at the beginning of the experiments. This most likely

describes the reality of these experiments, proven by two facts: i. Eradication of bacteria under phage pK8 exposure was so substantial, that in most of the cases no bacteria were quantifiable and ii. Han *et al.* proved rapid emergence of resistance in their investigation of this specific phage against the tested *K. pneumoniae* strain. In published studies, phage kinetics have been described using an *in vitro* measured time-delay in the lysis of bacteria after infection of phages¹⁵³, or with an estimated lysis rate constant. Following an approach similar to that of Delattre *et al.*¹⁵⁴, in this work phage-bacterial infection-lysis kinetics were modelled with a phage binding rate constant to phage-susceptible bacteria (B_s), resulting in infected bacteria (B_i), which undergo lysis following an estimated lysis rate constant. Both structural models of bacterial compartments used in **Publication I, II, and IV** are commonly used and have been described in the literature^{155–157}.

Furthermore, experiments conducted in both studies used clinical *K. pneumoniae* strains isolated from patients. This approach allows for more clinically relevant insights and enhances the potential for translation to in-human situations. Specifically, the four strains included in meropenem-fosfomycin testing were resistant to meropenem, with varying fosfomycin susceptibility. The *K. pneumoniae* strain included in phage pK8-polymyxin B testing showed high resistance to polymyxin. As thoroughly explained, pathogens of this type are classified by the WHO as the most challenging and difficult to treat²⁰. Accordingly, both studies represent crucial edge cases. In clinical practice, an antibiotic would not typically be administered to a patient if the strain causing the infection was proven resistant to it. Following this idea, it is arguable that in infections caused by pathogens classified as susceptible, with or without increased exposure to both combination partners (EUCAST classifications: S|S, S|I, I|I), successful therapy is more likely.

7.2 Limitations and risks in combination therapies

Publication III highlighted the antagonistic interactions between ceftazidime/avibactam and tigecycline in the tested clinical and isogenic *E. coli* and *K. pneumoniae* strains. This antagonism was observed in ten of the fifteen strains, underscoring the limitations of this combination in effective bacterial killing. The adaptive *in vitro* 'dynamic' checkerboard design, as utilized in this study, provided a systematic approach to interaction testing, revealing that tigecycline's limited bactericidal effect could reduce the efficacy of ceftazidime/avibactam. The time-kill assays further confirmed these antagonistic dynamics, showing that the killing effects of the

combination were similar to tigecycline alone. This outcome highlights the risks of using this combination, where the action of ceftazidime/avibactam is impaired, limiting therapeutic effectiveness against MDR pathogens. While the study provides valuable insights, it is limited by its *in vitro* nature, which does not fully capture the complexities of clinical situations. *In vitro* models lack the influence of an immune response and do not replicate the dynamic conditions in patients.

However, the findings indicate that the combination of ceftazidime/avibactam and tigecycline may not provide a clinical advantage, as antagonistic interactions could reduce treatment efficacy and promote resistance development. Prior studies have pointed out the risks associated with tigecycline, including increased MICs and less favourable clinical outcomes^{55,158–160}.

Therefore, future studies should explore other combinatory approaches that focus on leveraging synergistic interactions, ideally employing dynamic *in vitro* and *in vivo* models to enhance our understanding and improve outcomes for MDR infections. Compared to the synergistic findings in **Publications I, II, and IV**, where investigated combinations demonstrated enhanced bacterial suppression and potential clinical benefits, **Publication III's** results highlight the risks and challenges associated with antagonistic drug interactions. While synergy in the former two studies suggested promising strategies to combat resistant pathogens, the antagonistic dynamics observed in this study caution against certain combinations that could undermine therapeutic success.

7 Perspectives

Antimicrobial combination therapy is a promising approach, already showing effectiveness against MDR Gram-negative bacteria under certain conditions. Studies suggest that tailoring antibiotic strategies based on factors like illness severity and immune status can optimize the use of combination therapy for high-risk patients, while monotherapy can suffice for less severe infections^{71,161}. The presented research into various combination therapies highlights their potential applications and underlying mechanisms. Some combinations, such as those involving meropenem and fosfomycin, have demonstrated significant synergistic effects, successfully suppressing bacterial growth in resistant strains. These findings suggest that such combinations could serve as alternative treatments for difficult infections. Phage therapy also holds potential, particularly when combined with antibiotics like polymyxin B. Phage therapy could play a crucial role in future clinical applications, especially when customized to target specific bacterial strains. However, not all combinations are beneficial. Some, like ceftazidime/avibactam and tigecycline, have shown antagonistic interactions, highlighting the risks of reduced efficacy and increased resistance. This underscores the importance of careful selection and further exploration to identify effective synergies.

Future research should focus on both pre-clinical and clinical investigations. Utilizing dynamic *in vitro* and *in vivo* models to test meropenem-fosfomycin and phage pK8-polymyxin B combinations across more clinical strains of different susceptibility, simultaneously employing pharmacometric techniques to refine their application, would be more representative for the biological variability of pathogens found in clinics. Furthermore, gaining *in vivo* data could allow for *in vitro-in vivo*-translation, which would be especially important in the case of phage therapy. Pharmacometric models investigating on quadruple dynamics of host-bacterium-phage-antibiotic would allow considering physiological factors like distribution and excretion of phages, effects of the immune system, and possibly many more. In light of these findings, combined MIC breakpoints for meropenem-fosfomycin, as established in this work, should be considered by EUCAST and other international committees for potential implementation and further clinical validation.

To ensure effective real-world applications, conducting clinical studies will be essential. In the case of meropenem and fosfomycin, both already marketed and clinically used for decades the presented *in vitro* evidence, along with the clinical trials already conducted and discussed, indicates significant potential for this combination in future clinical studies.

The worsening AMR situation urges specialists worldwide to come up with new drugs, new approaches, but also new ideas on how to handle problems with already existing structures and medicines. Ultimately, a coordinated effort in advancing research and revising clinical guidelines is crucial to effectively tackle the challenges.

8 References

1. van Seventer JM, Hochberg NS. Principles of Infectious Diseases: Transmission, Diagnosis, Prevention, and Control. *International Encyclopedia of Public Health* 2017: 22–39.
2. World Health Organisation. WHO EMRO | Infectious diseases | Health topics. *World Health Organization - Regional Office for the Eastern Mediterranean*. Available at: <http://www.emro.who.int/health-topics/infectious-diseases/index.html>. Accessed September 23, 2024.
3. GBD 2019 Antimicrobial Resistance Collaborators. Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet* 2022; **400**: 2221–48.
4. Bartholomew JW, Mittwer T. The gram stain. *Bacteriological Reviews* 1952; **16**: 1–29.
5. Magiorakos A-P, Srinivasan A, Carey RB, *et al*. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection* 2012; **18**: 268–81.
6. Murray CJ, Ikuta KS, Sharara F, *et al*. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet* 2022; **399**: 629–55, Creative Commons user license: <https://creativecommons.org/licenses/by/4.0/>.
7. O'Neill J. *Tackling drug-resistant infections globally: final report and recommendations*. Government of the United Kingdom. London: Reviews on Antimicrobial Resistance; 2016. Available at: <https://apo.org.au/node/63983>. Accessed September 23, 2024.
8. Reygaert WC. An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiol* 2018; **4**: 482–501.
9. Kumar A, Schweizer HP. Bacterial resistance to antibiotics: Active efflux and reduced uptake. *Advanced Drug Delivery Reviews* 2005; **57**: 1486–513.
10. Blair JM, Richmond GE, Piddock LJ. Multidrug Efflux Pumps in Gram-Negative Bacteria and Their Role in Antibiotic Resistance. *Future Microbiology* 2014; **9**: 1165–77.
11. Lambert P a. Cellular impermeability and uptake of biocides and antibiotics in Gram-positive bacteria and mycobacteria. *Journal of Applied Microbiology* 2002; **92**: 46S-54S.
12. Gill MJ, Simjee S, Al-Hattawi K, Robertson BD, Easmon CSF, Ison CA. Gonococcal Resistance to β -Lactams and Tetracycline Involves Mutation in Loop 3 of the Porin Encoded at the penB Locus. *Antimicrobial Agents and Chemotherapy* 1998; **42**: 2799–803.
13. Hawkey PM. Mechanisms of quinolone action and microbial response. *Journal of Antimicrobial Chemotherapy* 2003; **51**: 29–35.
14. Redgrave LS, Sutton SB, Webber MA, Piddock LJV. Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success. *Trends in Microbiology* 2014; **22**: 438–45.

15. Stogios PJ, Savchenko A. Molecular mechanisms of vancomycin resistance. *Protein Sci* 2020; **29**: 654–69.
16. Bush K, Jacoby GA. Updated Functional Classification of β -Lactamases. *Antimicrobial Agents and Chemotherapy* 2010; **54**: 969–76.
17. Bush K, Bradford PA. β -Lactams and β -Lactamase Inhibitors: An Overview. *Cold Spring Harb Perspect Med* 2016; **6**: a025247.
18. Thomson KS. Extended-Spectrum- β -Lactamase, AmpC, and Carbapenemase Issues. *Journal of Clinical Microbiology* 2010; **48**: 1019–25.
19. Pandey AK, Cohn J, Nampoothiri V, *et al.* A systematic review of antibiotic drug shortages and the strategies employed for managing these shortages. *Clinical Microbiology and Infection* 2024. Available at: <https://www.sciencedirect.com/science/article/pii/S1198743X24004555>. Accessed September 30, 2024.
20. Publicaciones de la Organización Mundial de la Salud. WHO bacterial priority pathogens list, 2024: Bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. Available at: <https://www.who.int/publications/i/item/9789240093461>. Accessed July 26, 2024.
21. Nicolaou KC, Rigol S. A brief history of antibiotics and select advances in their synthesis. *J Antibiot* 2018; **71**: 153–84.
22. Dhingra S, Rahman NAA, Peile E, *et al.* Microbial Resistance Movements: An Overview of Global Public Health Threats Posed by Antimicrobial Resistance, and How Best to Counter. *Front Public Health* 2020; **8**: 535668.
23. European Committee on Antimicrobial Susceptibility Testing (EUCAST). MIC determination. 2024. Available at: https://www.eucast.org/ast_of_bacteria/mic_determination. Accessed September 25, 2024.
24. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, version 14.0. 2024. Available at: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_14.0_Breakpoint_Tables.pdf.
25. Eagle H, Fleischman R, Levy M. Continuous vs. Discontinuous Therapy with Penicillin. *New England Journal of Medicine* 1953; **248**: 481–8.
26. Eagle H. THE BINDING OF PENICILLIN IN RELATION TO ITS CYTOTOXIC ACTION. *J Exp Med* 1954; **100**: 103–15.
27. Craig WA. Pharmacokinetic/Pharmacodynamic Parameters: Rationale for Antibacterial Dosing of Mice and Men. *Clinical Infectious Diseases* 1998; **26**: 1–10.
28. Barbour A, Scaglione F, Derendorf H. Class-dependent relevance of tissue distribution in the interpretation of anti-infective pharmacokinetic/pharmacodynamic indices. *International Journal of Antimicrobial Agents* 2010; **35**: 431–8.

29. Nielsen EI, Cars O, Friberg LE. Pharmacokinetic/Pharmacodynamic (PK/PD) Indices of Antibiotics Predicted by a Semimechanistic PKPD Model: a Step toward Model-Based Dose Optimization. *Antimicrobial Agents and Chemotherapy* 2011; **55**: 4619–30.
30. Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. Infectious Diseases Society of America 2023 Guidance on the Treatment of Antimicrobial Resistant Gram-Negative Infections. *Clin Infect Dis* 2023: ciad428.
31. WHO Expert Committee. AWaRe classification of antibiotics for evaluation and monitoring of use, 2023. Available at: <https://www.who.int/publications/i/item/WHO-MHP-HPS-EML-2023.04>. Accessed September 23, 2024.
32. Wiseman LR, Wagstaff AJ, Brogden RN, Bryson HM. Meropenem. *Drugs* 1995; **50**: 73–101.
33. Hutchison M, Faulkner KL, Turner PJ, *et al.* A compilation of meropenem tissue distribution data. *J Antimicrob Chemother* 1995; **36 Suppl A**: 43–56.
34. Harris PNA, Tambyah PA, Lye DC, *et al.* Effect of Piperacillin-Tazobactam vs Meropenem on 30-Day Mortality for Patients With E coli or Klebsiella pneumoniae Bloodstream Infection and Ceftriaxone Resistance. *JAMA* 2018; **320**: 984–94.
35. Gadallah MI, Ali HRH, Askal HF, Saleh GA. Towards understanding of the interaction of certain carbapenems with protein via combined experimental and theoretical approach. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 2021; **246**: 119005.
36. Sayed ARM, Shah NR, Basso KB, *et al.* First Penicillin-Binding Protein Occupancy Patterns for 15 β -Lactams and β -Lactamase Inhibitors in Mycobacterium abscessus. *Antimicrobial Agents and Chemotherapy* 2020; **65**: 10.1128/aac.01956-20.
37. Paterson DL, Bonomo RA. Extended-Spectrum β -Lactamases: a Clinical Update. *Clin Microbiol Rev* 2005; **18**: 657–86.
38. Sader HS, Flamm RK, Carvalhaes CG, Castanheira M. Comparison of ceftazidime-avibactam and ceftolozane-tazobactam in vitro activities when tested against gram-negative bacteria isolated from patients hospitalized with pneumonia in United States medical centers (2017-2018). *Diagn Microbiol Infect Dis* 2020; **96**: 114833.
39. Humphries RM, Yang S, Hemarajata P, *et al.* First report of ceftazidime-avibactam resistance in a KPC-3-expressing Klebsiella pneumoniae isolate. *Antimicrobial Agents and Chemotherapy* 2015; **59**: 6605–7.
40. Dijkmans AC, Ortiz Zacarías NV, Burggraaf J, *et al.* Fosfomycin: Pharmacological, Clinical and Future Perspectives. *Antibiotics (Basel)* 2017; **6**: 24.
41. Kahan FM, Kahan JS, Cassidy PJ, Kropp H. The mechanism of action of fosfomycin (phosphonomycin). *Ann N Y Acad Sci* 1974; **235**: 364–86.
42. Popovic M, Steinort D, Pillai S, Joukhadar C. Fosfomycin: an old, new friend? *Eur J Clin Microbiol Infect Dis* 2010; **29**: 127–42.
43. Kirby WM. Pharmacokinetics of fosfomycin. *Chemotherapy* 1977; **23 Suppl 1**: 141–51.

44. Pfausler B, Spiss H, Dittrich P, Zeitlinger M, Schmutzhard E, Joukhadar C. Concentrations of fosfomycin in the cerebrospinal fluid of neurointensive care patients with ventriculostomy-associated ventriculitis. *Journal of Antimicrobial Chemotherapy* 2004; **53**: 848–52.
45. Frossard M, Joukhadar C, Erovic BM, *et al.* Distribution and Antimicrobial Activity of Fosfomycin in the Interstitial Fluid of Human Soft Tissues. *Antimicrobial Agents and Chemotherapy* 2000; **44**: 2728–32.
46. Anastasia A, Bonura S, Rubino R, *et al.* The Use of Intravenous Fosfomycin in Clinical Practice: A 5-Year Retrospective Study in a Tertiary Hospital in Italy. *Antibiotics* 2023; **12**: 971.
47. Bassetti M, Graziano E, Berruti M, Giacobbe DR. The role of fosfomycin for multidrug-resistant gram-negative infections. *Current Opinion in Infectious Diseases* 2019; **32**: 617.
48. Kroemer N, Martens M, Decousser J-W, Grégoire N, Nordmann P, Wicha SG. Evaluation of in vitro pharmacodynamic drug interactions of ceftazidime/avibactam and fosfomycin in *Escherichia coli*. *Journal of Antimicrobial Chemotherapy* 2023; **78**: 2524–34.
49. Kroemer N, Amann LF, Farooq A, *et al.* Pharmacokinetic/pharmacodynamic analysis of ceftazidime/avibactam and fosfomycin combinations in an *in vitro* hollow fiber infection model against multidrug-resistant *Escherichia coli* Asempa TE, ed. *Microbiol Spectr* 2024; **12**: e03318-23.
50. Bakthavatchalam YD, Shankar A, Muthuirulandi Sethuvel DP, Asokan K, Kanthan K, Veeraraghavan B. Synergistic activity of fosfomycin–meropenem and fosfomycin–colistin against carbapenem resistant *Klebsiella pneumoniae*: an in vitro evidence. *Future Sci OA* **6**: FSO461.
51. Stein GE, Babinchak T. Tigecycline: an update. *Diagnostic Microbiology and Infectious Disease* 2013; **75**: 331–6.
52. Yaghoubi S, Zekiy AO, Krutova M, *et al.* Tigecycline antibacterial activity, clinical effectiveness, and mechanisms and epidemiology of resistance: narrative review. *European Journal of Clinical Microbiology and Infectious Diseases* 2021.
53. Tombs NL. Tissue distribution of GAR-936, a broad-spectrum antibiotic, in male rats. *Program and Abstracts of the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, American Society for Microbiology* 1999; **302**: abstract 413.
54. Dixit D, Madduri RP, Sharma R. The role of tigecycline in the treatment of infections in light of the new black box warning. *Expert Review of Anti-infective Therapy* 2014; **12**: 397–400.
55. Amann LF, Broeker A, Riedner M, *et al.* Pharmacokinetic/pharmacodynamic evaluation of tigecycline dosing in a hollow fiber infection model against clinical bla-KPC producing *Klebsiella pneumoniae* isolates. *Diagnostic Microbiology and Infectious Disease* 2024; **108**: 116153.
56. Zavaski AP, Goldani LZ, Li J, Nation RL. Polymyxin B for the treatment of multidrug-resistant pathogens: a critical review. *Journal of Antimicrobial Chemotherapy* 2007; **60**: 1206–15.
57. Manchandani P, Zhou J, Ledesma KR, *et al.* Characterization of Polymyxin B Biodistribution and Disposition in an Animal Model. *Antimicrobial Agents and Chemotherapy* 2016; **60**: 1029–34.

58. Kubin CJ, Ellman TM, Phadke V, Haynes LJ, Calfee DP, Yin MT. Incidence and predictors of acute kidney injury associated with intravenous polymyxin B therapy. *Journal of Infection* 2012; **65**: 80–7.
59. Majumdar A, Das PS. Safety and efficacy of polymyxin B in multidrug resistant gram-negative severe sepsis and septic shock. *Indian Journal of Critical Care Medicine* 2008; **12**: 153–7.
60. Sulakvelidze A, Alavidze Z, J Glenn Morris J. Bacteriophage Therapy. *Antimicrobial Agents and Chemotherapy* 2001; **45**: 649.
61. Casey A, Coffey A, McAuliffe O. Genetics and Genomics of Bacteriophages: The Evolution of Bacteriophage Genomes and Genomic Research. In: Harper DR, Abedon ST, Burrowes BH, McConville ML, eds. *Bacteriophages*. Cham: Springer International Publishing, 2021; 193–218. Available at: https://link.springer.com/10.1007/978-3-319-41986-2_5. Accessed October 29, 2024.
62. Pertics BZ, Kovács T, Schneider G. Characterization of a Lytic Bacteriophage and Demonstration of Its Combined Lytic Effect with a K2 Depolymerase on the Hypervirulent *Klebsiella pneumoniae* Strain 52145. *Microorganisms* 2023; **11**: 669.
63. Lin DM, Koskella B, Lin HC. Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. *WJGPT* 2017; **8**: 162.
64. Diallo K, Dublanchet A. A Century of Clinical Use of Phages: A Literature Review. *Antibiotics (Basel)* 2023; **12**: 751.
65. Nang SC, Lin Y-W, Petrovic Fabijan A, *et al.* Pharmacokinetics/pharmacodynamics of phage therapy: a major hurdle to clinical translation. *Clinical Microbiology and Infection* 2023; **29**: 702–9.
66. Międzybrodzki R, Borysowski J, Weber-Dąbrowska B, *et al.* Clinical Aspects of Phage Therapy. In: Łobocka M, Szybalski W, eds. *Advances in Virus Research*. Vol 83. Bacteriophages, Part B, Chapter 3. Academic Press, 2012; 73–121. Available at: <https://www.sciencedirect.com/science/article/pii/B9780123944382000037>. Accessed September 27, 2024.
67. Liu D, Van Belleghem JD, de Vries CR, *et al.* The Safety and Toxicity of Phage Therapy: A Review of Animal and Clinical Studies. *Viruses* 2021; **13**: 1268.
68. Friman V, Soanes-Brown D, Sierocinski P, *et al.* Pre-adapting parasitic phages to a pathogen leads to increased pathogen clearance and lowered resistance evolution with *Pseudomonas aeruginosa* cystic fibrosis bacterial isolates. *Journal of Evolutionary Biology* 2016; **29**: 188–98.
69. Pirnay J-P, Kutter E. Bacteriophages: it's a medicine, Jim, but not as we know it. *The Lancet Infectious Diseases* 2021; **21**: 309–11.
70. Uyttendaele S, Chen B, Onsea J, *et al.* Safety and efficacy of phage therapy in difficult-to-treat infections: a systematic review. *The Lancet Infectious Diseases* 2022; **22**: e208–20.
71. Schmid A, Wolfensberger A, Nemeth J, Schreiber PW, Sax H, Kuster SP. Monotherapy versus combination therapy for multidrug-resistant Gram-negative infections: Systematic Review and Meta-Analysis. *Sci Rep* 2019; **9**: 15290.

72. Tamma PD, Cosgrove SE, Maragakis LL. Combination Therapy for Treatment of Infections with Gram-Negative Bacteria. *Clin Microbiol Rev* 2012; **25**: 450–70.
73. Tyers M, Wright GD. Drug combinations: a strategy to extend the life of antibiotics in the 21st century. *Nat Rev Microbiol* 2019; **17**: 141–55.
74. Tängdén T. Combination antibiotic therapy for multidrug-resistant Gram-negative bacteria. *Ups J Med Sci* 2014; **119**: 149–53.
75. Yeh PJ, Hegreness MJ, Aiden AP, Kishony R. Drug interactions and the evolution of antibiotic resistance. *Nature Reviews Microbiology* 2009; **7**: 460–6.
76. Yekani M, Azargun R, Sharifi S, *et al.* Collateral sensitivity: An evolutionary trade-off between antibiotic resistance mechanisms, attractive for dealing with drug-resistance crisis. *Health Science Reports* 2023; **6**: e1418.
77. Roemhild R, Andersson DI. Mechanisms and therapeutic potential of collateral sensitivity to antibiotics. *PLoS Pathog* 2021; **17**: e1009172.
78. World Health Organisation. Comprehensive TB Guidelines for National Tuberculosis Program V Edition. 2023. Available at: <https://www.who.int/publications/i/item/TLS-NTP-manual-2023>. Accessed September 27, 2024.
79. Aumpan N, Mahachai V, Vilaichone R. Management of Helicobacter pylori infection. *JGH Open* 2023; **7**: 3–15.
80. Abedon ST, Danis-Wlodarczyk KM, Wozniak DJ. Phage Cocktail Development for Bacteriophage Therapy: Toward Improving Spectrum of Activity Breadth and Depth. *Pharmaceuticals* 2021; **14**: 1019.
81. Fungo GBN, Uy JCW, Porciuncula KLJ, *et al.* “Two Is Better Than One”: The Multifactorial Nature of Phage-Antibiotic Combinatorial Treatments Against ESKAPE-Induced Infections. *PHAGE* 2023; **4**: 55–67.
82. Torres-Barceló C, Hochberg ME. Evolutionary Rationale for Phages as Complements of Antibiotics. *Trends in Microbiology* 2016; **24**: 249–56.
83. Gu Liu C, Green SI, Min L, *et al.* Phage-Antibiotic Synergy Is Driven by a Unique Combination of Antibacterial Mechanism of Action and Stoichiometry. *mBio* 2020; **11**: 10.1128/mbio.01462-20.
84. Pirnay J-P, Djebara S, Steurs G, *et al.* Personalized bacteriophage therapy outcomes for 100 consecutive cases: a multicentre, multinational, retrospective observational study. *Nat Microbiol* 2024; **9**: 1434–53.
85. Van Nieuwenhuysse B, Van der Linden D, Chatzis O, *et al.* Bacteriophage-antibiotic combination therapy against extensively drug-resistant Pseudomonas aeruginosa infection to allow liver transplantation in a toddler. *Nat Commun* 2022; **13**: 5725.
86. A. I. Sabra (ed., Trans.). *The Optics Of Ibn Al Haytham, Books I - III, On Direct Vision*. Available at: <http://archive.org/details/A.I.Sabraed.Trans.TheOpticsOfIbnAlHaythamBooksIIIONDirectVision>. Accessed October 8, 2024.

87. Voit EO. Perspective: Dimensions of the scientific method. *PLOS Computational Biology* 2019; **15**: e1007279.
88. CLSI. Performance Standards for Antimicrobial Susceptibility Testing, 34th Edition M100 Ed34. *Clinical & Laboratory Standards Institute* 2024.
89. Hall MJ, Middleton RF, Westmacott D. The fractional inhibitory concentration (FIC) index as a measure of synergy. *Journal of Antimicrobial Chemotherapy* 1983; **11**: 427–33.
90. Wicha SG, Kees MG, Kuss J, Kloft C. Pharmacodynamic and response surface analysis of linezolid or vancomycin combined with meropenem against *Staphylococcus aureus*. *Pharm Res* 2015; **32**: 2410–8.
91. Kroemer N. Optimized Rhombic Experimental Dynamic Checkerboard Designs to Elucidate Pharmacodynamic Drug Interactions of Antibiotics. *Pharmaceutical Research* 2022.
92. EMA. Use of pharmacokinetics and pharmacodynamics in the development of antibacterial medicinal products - Scientific guideline | European Medicines Agency. 2017. Available at: <https://www.ema.europa.eu/en/use-pharmacokinetics-pharmacodynamics-development-antibacterial-medicinal-products-scientific-guideline>. Accessed October 1, 2024.
93. Blaser J, Zinner SH. In Vitro Models for the Study of Antibiotic Activities. In: Jucker E, Meyer U, eds. *Progress in Drug Research/Fortschritte der Arzneimittelforschung/Progrès des recherches pharmaceutiques*. Basel: Birkhäuser, 1987; 349–81. Available at: https://doi.org/10.1007/978-3-0348-9289-6_11. Accessed October 2, 2024.
94. Bilello JA, Bauer G, Dudley MN, Cole GA, Drusano GL. Effect of 2',3'-didehydro-3'-deoxythymidine in an in vitro hollow-fiber pharmacodynamic model system correlates with results of dose-ranging clinical studies. *Antimicrobial Agents and Chemotherapy* 1994; **38**: 1386–91.
95. Drusano GL. EMA Workshop Non-Clinical Models to Identify PK/PD Indices and PD Targets. *European Medicines Agency* 2015. Available at: https://www.ema.europa.eu/en/documents/presentation/presentation-non-clinical-models-identify-pkpd-indices-and-pd-targets-vitro-george-drusano_en.pdf.
96. Benet LZ, Rowland M. Pharmacometrics: A new journal section. *Journal of Pharmacokinetics and Biopharmaceutics* 1982; **10**: 349–50.
97. Bonate PL. *Pharmacokinetic-Pharmacodynamic Modeling and Simulation*. Boston, MA: Springer US; 2011. Available at: <https://link.springer.com/10.1007/978-1-4419-9485-1>. Accessed October 4, 2024.
98. Ette EI, Williams PJ eds. *Pharmacometrics: the science of quantitative pharmacology*. Hoboken, NJ: John Wiley & Sons; 2007.
99. Gabrielsson J, Weiner D. Non-compartmental Analysis. In: Reisfeld B, Mayeno AN, eds. *Computational Toxicology*. Vol 929. Methods in Molecular Biology. Totowa, NJ: Humana Press, 2012; 377–89. Available at: https://link.springer.com/10.1007/978-1-62703-050-2_16. Accessed October 4, 2024.

100. Garrett ER. Classical pharmacokinetics to the frontier. *Journal of Pharmacokinetics and Biopharmaceutics* 1973; **1**: 341–61.
101. Greim H. Oswald Schmiedeberg (1838–1921). *Naunyn-Schmiedeberg's Arch Pharmacol* 2024. Available at: <https://doi.org/10.1007/s00210-024-03306-1>. Accessed October 8, 2024.
102. Salahudeen MS, Nishtala PS. An overview of pharmacodynamic modelling, ligand-binding approach and its application in clinical practice. *Saudi Pharm J* 2017; **25**: 165–75.
103. Peck CC, Beal SL, Sheiner LB, Nichols AI. Extended least squares nonlinear regression: A possible solution to the “choice of weights” problem in analysis of individual pharmacokinetic data. *Journal of Pharmacokinetics and Biopharmaceutics* 1984; **12**: 545–58.
104. Laird NM, Ware JH. Random-Effects Models for Longitudinal Data. *Biometrics* 1982; **38**: 963–74.
105. Jennrich RI, Schluchter MD. Unbalanced Repeated-Measures Models with Structured Covariance Matrices. *Biometrics* 1986; **42**: 805–20.
106. Lindstrom MJ, Bates DM. Nonlinear Mixed Effects Models for Repeated Measures Data. *Biometrics* 1990; **46**: 673–87.
107. Aldrich J. R.A. Fisher and the making of maximum likelihood 1912-1922. *Statistical Science* 1997; **12**: 162–76.
108. Akaike H. A New Look at the Statistical Model Identification. *IEEE Transactions on Automatic Control* 1974; **19**: 716–23.
109. Ziegler I, Lindström S, Källgren M, Strålin K, Mölling P. 16S rDNA droplet digital PCR for monitoring bacterial DNAemia in bloodstream infections. *PLOS ONE* 2019; **14**: e0224656.
110. Wang X, Howe S, Deng F, Zhao J. Current Applications of Absolute Bacterial Quantification in Microbiome Studies and Decision-Making Regarding Different Biological Questions. *Microorganisms* 2021; **9**: 1797.
111. Bliss CI. The Toxicity of Poisons Applied Jointly. *Annals of Applied Biology* 1939; **26**: 585–615.
112. Farooq A, Martens M, Kroemer N, *et al.* Pharmacokinetic/pharmacodynamic analysis of meropenem and fosfomycin combinations in in vitro time–kill and hollow-fibre infection models against multidrug-resistant and carbapenemase-producing *Klebsiella pneumoniae*. *Journal of Antimicrobial Chemotherapy* 2024: dkae459.
113. Farooq A, Nang SC, Yu HH, *et al.* A pharmacometric model assessing the *in vitro* synergistic effect of a bacteriophage-polymyxin B combination in a clinical multidrug-resistant *K. pneumoniae* isolate. *International Journal of Antimicrobial Agents* 2025; **66**: 107628.
114. Farooq A, Drotleff B, Kroemer N, *et al.* Evaluation of *in vitro* pharmacodynamic drug interactions of ceftazidime-avibactam with tigecycline in ESBL- and carbapenemase producing *Escherichia coli* and *Klebsiella pneumoniae*. *International Journal of Antimicrobial Agents* 2025; **65**: 107457.

115. Farooq A, Martens M, Attwood MLG, Nordmann P, MacGowan A, Wicha SG. Meropenem and fosfomycin against *Klebsiella pneumoniae*: Towards a combination breakpoint using a pharmacometric approach. *Clinical Microbiology and Infection* 2026; **0**. Available at: [https://www.clinicalmicrobiologyandinfection.org/article/S1198-743X\(26\)00102-3/fulltext](https://www.clinicalmicrobiologyandinfection.org/article/S1198-743X(26)00102-3/fulltext). Accessed April 20, 2026.
116. Effah CY, Sun T, Liu S, Wu Y. *Klebsiella pneumoniae*: an increasing threat to public health. *Annals of Clinical Microbiology and Antimicrobials* 2020; **19**: 1.
117. Doi Y. Treatment Options for Carbapenem-resistant Gram-negative Bacterial Infections. *Clinical Infectious Diseases* 2019; **69**: S565–75.
118. Elliott ZS, Barry KE, Cox HL, *et al.* The Role of *fosA* in Challenges with Fosfomycin Susceptibility Testing of Multispecies *Klebsiella pneumoniae* Carbapenemase-Producing Clinical Isolates. *Journal of Clinical Microbiology* 2019; **57**: 10.1128/jcm.00634-19.
119. Mączyńska B, Paleczny J, Oleksy-Wawrzyniak M, Choroszy-Król I, Bartoszewicz M. In Vitro Susceptibility of Multi-Drug Resistant *Klebsiella pneumoniae* Strains Causing Nosocomial Infections to Fosfomycin. A Comparison of Determination Methods. *Pathogens* 2021; **10**: 512.
120. Baltas I, Patel T, Soares AL. Resistance profiles of carbapenemase-producing Enterobacterales in a large centre in England: are we already losing cefiderocol? *J Antimicrob Chemother* 2025; **80**: 59–67.
121. Tamma PD, Heil EL, Justo JA, Mathers AJ, Satlin MJ, Bonomo RA. Infectious Diseases Society of America 2024 Guidance on the Treatment of Antimicrobial-Resistant Gram-Negative Infections. *Clin Infect Dis* 2024: ciae403.
122. Tedeschi S, Giannitsioti E, Mayer C. Emerging Concepts for the Treatment of Biofilm-Associated Bone and Joint Infections with IV Fosfomycin: A Literature Review. *Microorganisms* 2025; **13**: 963.
123. Butler DA, Patel N, O'Donnell JN, Lodise TP. Combination therapy with IV fosfomycin for adult patients with serious Gram-negative infections: a review of the literature. *J Antimicrob Chemother* 2024; **79**: 2421–59.
124. Pascale R, Giannella M, Bartoletti M, Viale P, Pea F. Use of meropenem in treating carbapenem-resistant Enterobacteriaceae infections. *Expert Review of Anti-infective Therapy* 2019; **17**: 819–27.
125. European Committee on Antimicrobial Susceptibility Testing (EUCAST). EUCAST guidance on use of fosfomycin i.v. breakpoints. 2024. Available at: https://www.eucast.org/fileadmin/eucast/pdf/guidance_documents/Use_of_fosfomycin_iv_breakpoints_General_advice_20240528.pdf.
126. Paul M, Carrara E, Retamar P, *et al.* European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines for the treatment of infections caused by multidrug-resistant Gram-negative bacilli (endorsed by European society of intensive care medicine). *Clinical Microbiology and Infection* 2022; **28**: 521–47.

127. Bodmann K-F, Hagel S, Oliva A, et al. Real-World Use, Effectiveness, and Safety of Intravenous Fosfomycin: The FORTRESS Study. *Infect Dis Ther* 2025; **14**: 765–91.
128. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, version 15.0. 2025. Available at: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_15.0_Breakpoint_Tables.pdf. Accessed July 27, 2025.
129. Albiero J, Sy SKB, Mazucheli J, et al. Pharmacodynamic Evaluation of the Potential Clinical Utility of Fosfomycin and Meropenem in Combination Therapy against KPC-2-Producing Klebsiella pneumoniae. *Antimicrob Agents Chemother* 2016; **60**: 4128–39.
130. AL-Quraini M, Rizvi M, AL-Jabri Z, et al. Assessment of In-Vitro Synergy of Fosfomycin with Meropenem, Amikacin and Tigecycline in Whole Genome Sequenced Extended and Pan Drug Resistant Klebsiella Pneumoniae: Exploring A Colistin Sparing Protocol. *Antibiotics (Basel)* 2022; **11**: 153.
131. MacGowan AP, Griffin P, Attwood MLG, Daum AM, Avison MB, Noel AR. The pharmacodynamics of fosfomycin in combination with meropenem against Klebsiella pneumoniae studied in an in vitro model of infection. *Journal of Antimicrobial Chemotherapy* 2025; **80**: 967–75.
132. Wicha SG, Chen C, Clewe O, Simonsson USH. A general pharmacodynamic interaction model identifies perpetrators and victims in drug interactions. *Nature Communications* 2017; **8**: 1–11.
133. Akaike H. A New Look at the Statistical Model Identification. *IEEE Transactions on Automatic Control* 1974; **19**: 716–23.
134. Li C, Kuti JL, Nightingale CH, Nicolau DP. Population Pharmacokinetic Analysis and Dosing Regimen Optimization of Meropenem in Adult Patients. *The Journal of Clinical Pharmacology* 2006; **46**: 1171–8.
135. Parker SL, Frantzeskaki F, Wallis SC, et al. Population Pharmacokinetics of Fosfomycin in Critically Ill Patients. *Antimicrobial Agents and Chemotherapy* 2015; **59**: 6471–6.
136. Merino-Bohórquez V, Docobo-Pérez F, Sojo J, et al. Population pharmacokinetics and pharmacodynamics of fosfomycin in non-critically ill patients with bacteremic urinary infection caused by multidrug-resistant Escherichia coli. *Clinical Microbiology and Infection* 2018; **24**: 1177–83.
137. Della Rocca MT, Di Caprio G, Colucci F, et al. Fosfomycin-meropenem synergistic combination against NDM carbapenemase-producing Klebsiella pneumoniae strains. *New Microbiol* 2023; **46**: 264–70.
138. Erturk Sengel B, Altinkanat Gelmez G, Soyletir G, Kortan V. In vitro synergistic activity of fosfomycin in combination with meropenem, amikacin and colistin against OXA-48 and/or NDM-producing Klebsiella pneumoniae. *Journal of Chemotherapy* 2020; **32**: 237–43.
139. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Antimicrobial wild type distributions of microorganisms. 2025. Available at: <https://mic.eucast.org/search/show-registration/39183?back=https://mic.eucast.org/search/?search%25Bmethod%25D%3Dmic%>

- 26search%255Bantibiotic%255D%3D100%26search%255Bspecies%255D%3D-1%26search%255Bdisk_content%255D%3D-1%26search%255Blimit%255D%3D50. Accessed November 4, 2025.
140. Cojutti PG, Tedeschi S, Zamparini E, *et al.* Could a Reduced Dose of 8 g of Continuous Infusion Fosfomycin Be Considered as Effective as and Safer than a Standard 16 g Dose When Combined with High-Dose Daptomycin in the Treatment of Staphylococcal osteoarticular Infections? *Antibiotics* 2025; **14**: 139.
 141. Rodriguez-Gonzalez RA, Leung CY, Chan BK, Turner PE, Weitz JS. Quantitative Models of Phage-Antibiotic Combination Therapy. *mSystems* 2020; **5**.
 142. Han ML, Nang SC, Lin YW, *et al.* Comparative metabolomics revealed key pathways associated with the synergistic killing of multidrug-resistant *Klebsiella pneumoniae* by a bacteriophage-polymyxin combination. *Computational and Structural Biotechnology Journal* 2022; **20**: 485–95.
 143. Wicha S, Huisinga W, Kloft C. Translational Pharmacometric Evaluation of Typical Antibiotic Broad-Spectrum Combination Therapies Against *Staphylococcus Aureus* Exploiting In Vitro Information. *CPT: Pharmacometrics & Systems Pharmacology* 2017; **6**: 512–22.
 144. Andes D, Craig WA. Animal model pharmacokinetics and pharmacodynamics: a critical review. *International Journal of Antimicrobial Agents* 2002; **19**: 261–8.
 145. Drusano GL, Liu W, Kulawy R, Louie A. Impact of Granulocytes on the Antimicrobial Effect of Tedizolid in a Mouse Thigh Infection Model. *Antimicrobial Agents and Chemotherapy* 2011; **55**: 5300–5.
 146. Abdallah TAK, Elajezi R, Ibrahim TB, Alimam AB, Omrani AS. Efficacy and safety of intravenous fosfomycin for the treatment of difficult-to-treat Gram-negative bacterial infections. *Journal of Infection and Public Health* 2021; **14**: 1620–2.
 147. Oliva A, Curtolo A, Falletta A, *et al.* Efficacy of fosfomycin-containing regimens in treating severe infections caused by KPC-producing *Klebsiella pneumoniae* and carbapenem-resistant *Acinetobacter baumannii* in critically ill patients. *International Journal of Antimicrobial Agents* 2024: 107365.
 148. Jault P, Leclerc T, Jennes S, *et al.* Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by *Pseudomonas aeruginosa* (PhagoBurn): a randomised, controlled, double-blind phase 1/2 trial. *The Lancet Infectious Diseases* 2019; **19**: 35–45.
 149. Hitchcock NM, Devequi Gomes Nunes D, Shiach J, *et al.* Current Clinical Landscape and Global Potential of Bacteriophage Therapy. *Viruses* 2023; **15**: 1020.
 150. Falagas ME, Kasiakou SK. Toxicity of polymyxins: a systematic review of the evidence from old and recent studies. *Crit Care* 2006; **10**: R27.
 151. Hartzell JD, Neff R, Ake J, *et al.* Nephrotoxicity Associated with Intravenous Colistin (Colistimethate Sodium) Treatment at a Tertiary Care Medical Center. *Clinical Infectious Diseases* 2009; **48**: 1724–8.

152. Nikolic I, Vukovic D, Gavric D, *et al.* An Optimized Checkerboard Method for Phage-Antibiotic Synergy Detection. *Viruses* 2022; **14**: 1542.
153. Cairns BJ, Timms AR, Jansen VAA, Connerton IF, Payne RJH. Quantitative models of in vitro bacteriophage-host dynamics and their application to phage therapy. *PLoS Pathogens* 2009; **5**.
154. Delattre R, Seurat J, Haddad F, *et al.* Combination of in vivo phage therapy data with in silico model highlights key parameters for pneumonia treatment efficacy. *Cell Reports* 2022; **39**.
155. Nielsen EI, Friberg LE. Pharmacokinetic-Pharmacodynamic Modeling of Antibacterial Drugs Andersson D, ed. *Pharmacol Rev* 2013; **65**: 1053–90.
156. Jacobs M, Grégoire N, Couet W, Bulitta JB. Distinguishing Antimicrobial Models with Different Resistance Mechanisms via Population Pharmacodynamic Modeling. *PLoS Computational Biology* 2016; **12**: e1004782.
157. Minichmayr IK, Aranzana-Climent V, Friberg LE. Pharmacokinetic/pharmacodynamic models for time courses of antibiotic effects. *International Journal of Antimicrobial Agents* 2022; **60**: 106616.
158. Prasad P, Sun J, Danner RL, Natanson C. Excess Deaths Associated With Tigecycline After Approval Based on Noninferiority Trials. *Clinical Infectious Diseases* 2012; **54**: 1699–709.
159. McGovern PC, Wible M, El-Tahtawy A, Biswas P, Meyer RD. All-cause mortality imbalance in the tigecycline phase 3 and 4 clinical trials. *International Journal of Antimicrobial Agents* 2013; **41**: 463–7.
160. Falagas ME, Vardakas KZ, Tsiveriotis KP, Triarides NA, Tansarli GS. Effectiveness and safety of high-dose tigecycline-containing regimens for the treatment of severe bacterial infections. *International Journal of Antimicrobial Agents* 2014; **44**: 1–7.
161. Chow JW, Yu VL. Combination antibiotic therapy versus monotherapy for gram-negative bacteraemia: a commentary. *International Journal of Antimicrobial Agents* 1999; **11**: 7–12.
162. Cadwell JJS. The hollow fiber infection model: Principles and practice. *Clin Microbiol* 2018; **07**. Available at: <https://www.omicsonline.org/conference-proceedings/clinical-microbiology-2018-scientifictracks.digital>. Accessed January 16, 2023.
163. Wicha SG, Chen C, Clewe O, Simonsson USH. A general pharmacodynamic interaction model identifies perpetrators and victims in drug interactions. *Nature Communications* 2017; **8**: 1–11.
164. Han ML, Zhu Y, Creek DJ, *et al.* Alterations of Metabolic and Lipid Profiles in Polymyxin-Resistant *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy* 2018; **62**: 1–14.
165. Tsugawa H, Cajka T, Kind T, *et al.* MS-DIAL: data-independent MS/MS deconvolution for comprehensive metabolome analysis. *Nat Methods* 2015; **12**: 523–6.
166. Drotleff B, Lämmerhofer M. Guidelines for Selection of Internal Standard-Based Normalization Strategies in Untargeted Lipidomic Profiling by LC-HR-MS/MS. *Anal Chem* 2019; **91**: 9836–43.

167. Merino-Bohórquez V, Docobo-Pérez F, Sojo J, *et al.* Population pharmacokinetics and pharmacodynamics of fosfomycin in non-critically ill patients with bacteremic urinary infection caused by multidrug-resistant *Escherichia coli*. *Clin Microbiol Infect* 2018; **24**: 1177–83.

9 Appendix

9.1 Supplementary data Publication I

Journal of Antimicrobial Chemotherapy

Electronic Supplementary Material

Pharmacokinetic/pharmacodynamic analysis of meropenem and fosfomycin combinations in *in vitro* time-kill and hollow fibre infection model assays against multidrug-resistant ESBL- and carbapenemase producing *Klebsiella pneumoniae*

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Text S1: Methods: Bacterial strains and antimicrobial susceptibility testing**Origin of bacterial strains**

Bacterial strains were analyzed at Medical and Molecular Microbiology, University of Fribourg, Switzerland. Detailed information regarding their geographic origin and isolation is given in **Tab. S1**.

Table S1 Characteristics of *Klebsiella pneumoniae* strains, their geographic origins, and isolation sources.

Strain	Origin	Isolation
<i>K. pneumoniae</i> 2977 _{KPC-2}	Liebefeld, Switzerland	Urine
<i>K. pneumoniae</i> N2859 _{KPC-2}	Bellinzona, Switzerland	-
<i>K. pneumoniae</i> N864 _{KPC-3}	Lausanne, Switzerland	Rectal swab
<i>K. pneumoniae</i> R307 _{KPC-2}	Brazil	Blood culture

Antimicrobial susceptibility testing

The minimal inhibitory concentration (MIC) of meropenem was determined as triplicates using the broth microdilution method according to the EUCAST recommendation.²⁴ Bacterial isolates (approx. 5×10^5 cfu/mL) were inoculated in cation-adjusted Mueller-Hinton-broth II (ca-MHB, NutriSelect® Plus, Sigma-Aldrich, USA) using 48-well-plates that contained a series of 2-fold dilutions of each antibiotic. The plates were incubated at 37 °C in ambient air for 24 h and were then evaluated visually. The MIC was defined as the lowest concentration that showed no visible growth of bacteria. The MIC of fosfomycin was determined in using the agar dilution method in accordance EUCAST recommendation²⁴. For this Columbia agar plates supplemented with 2-fold concentrations of fosfomycin and 25 mg/L glucose-6-phosphate were prepared. Bacterial inocula were set to 0.5 McFarland and then diluted to achieve the desired colony count. A standardized inoculum of 10^4 cfu per spot was inoculated onto the agar plates. The plates were then incubated for 18 to 24 h at 37 °C ambient air, after which the MIC was read as the lowest concentration of fosfomycin that completely inhibited visible bacterial growth, neglecting small colonies within the spot area.

Text S2: Methods: Static time-kill assays and Hollow-fibre Infection Model (HFIM)**Static time-kill assays**

All four clinical *K. pneumoniae* isolates were tested in static time-kill assays against meropenem and fosfomycin alone and in combination to obtain comprehensive understanding of the killing

kinetics. Briefly, time-kill assays were performed by inoculating and pre-incubating the bacterial isolate (approx. 10^6 cfu/mL) in freshly prepared cation-adjusted Mueller-Hinton-broth II (ca-MHB, NutriSelect® Plus, Sigma-Aldrich, USA) for 2 h at 37 °C ambient air. Following pre-incubation, meropenem and/or fosfomycin were added at 2-fold serial dilutions. Studied concentrations ranged from low levels, below the clinical breakpoints defined for both antimicrobials by EUCAST,²⁴ to high levels, exceeding clinically achievable concentrations. All tested scenarios including growth controls were studied over 24 h. Samples were collected at 0, 2, 4, 6, 8 and 24 h to determine the bacterial count. Regrowth was defined as the bacterial concentration exceeding the inoculum at time zero used to initiate the culture. Each experiment was performed with a minimum of $n = 2$ replicates with a final volume of 10 mL and a lower limit of quantification of 10^2 cfu/mL. Additionally, samples of selected scenarios for each strain as well as in ca-MHB without bacteria were collected at 0, 4 and 24 h, and stored at -80 °C for later high-performance liquid chromatography-mass spectrometry (LC-MS/MS) analysis to investigate meropenem degradation in absence (non-enzymatic degradation) and presence of KPC-expressing bacteria. Degradation rate constants were later estimated in PK/PD modelling to enhance model accuracy by accounting for enzyme-driven changes in meropenem concentration over time.

Experimental setup HFIM

The HFIM setup followed what was described previously,¹⁶² with a central compartment connected to a cellulosic hollow-fibre cartridge (catalogue no. C8008, FiberCell® Systems Inc., USA) via tubing, pre-programmed peristaltic pumps (ISMATEC®, ISM 4412 Reglo ICC Pump; Masterflex® Drive MFLEX 115/230; Darmstadt, Germany) and syringe pumps (Cole-Parmer Syringe Pump SKU 7490554). The required infusion volumes, concentrations and pump rates to achieve the target concentration-time profiles were calculated using R. HFIM experiments were performed by inoculating and pre-incubating the bacterial isolate (approx. 10^6 cfu/mL) in ca-MHB for 2 h at 37 °C ambient air. Following pre-incubation, meropenem, mimicking 2 g q8h, and/or fosfomycin, mimicking 8 g q8h, were administered over 72 h. Killing kinetics were observed after collecting samples at 0, 0.75, 2, 4, 6, 8, 8.75, 24, 24.75, 28, 32, 48, 52, 56 and 72 h from the extra-capillary space of the cartridge and determination of the bacterial count. Additionally, samples from the central compartment were collected at 0, 0.75, 4, 8, 8.75, 24, 24.75, 28, 32, 32.75, 48, 48.75, 52, 56, 56.75, and 72 h, and stored at -80 °C for later LC-MS/MS analysis to verify if target profiles were achieved.

Pharmacokinetics HFIM

In order to streamline the experimental procedure, both antibiotics were administered as bolus infusions while maintaining the clinically expected minimum (C_{min}) and maximum (C_{max}) concentrations **Tab. S2** shows target C_{min} and C_{max} of meropenem and fosfomycin in the HFIM.

Table S2 Overview of target minimum (C_{min}) and maximum (C_{max}) concentrations of meropenem (MEM) and fosfomycin (FOF) in the Hollow-Fibre Infection Model experiments. 50th: median patients as virtual individuals providing a benchmark for typical drug exposure within the studied population.

Dosing Hollow Fibre Experiment	C_{min} [mg/L]	C_{max} [mg/L]
MEM 2 g q8h 50 th	11.1	50.9
FOF 8 g q8h 50 th	84.8	388

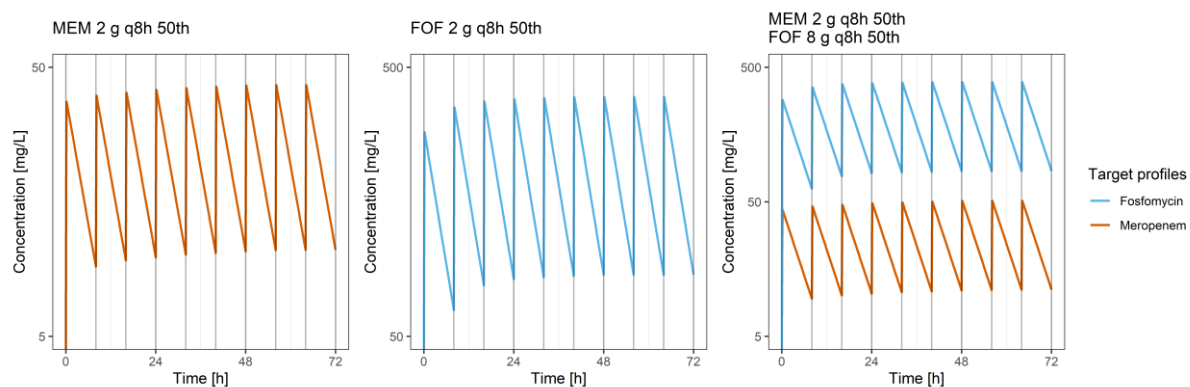


Figure S7 Target concentration-time profiles for high-dose meropenem and high-dose fosfomycin dosing regimen mimicked in dynamic Hollow-Fibre Infection Model experiments. MEM: meropenem; FOF: fosfomycin; 50th: simulated median patient.

Text S3: Methods: Pharmacometric Modelling

Mathematical modelling of single drug effects

Single drug effects were quantified using the slope effect model or (sigmoidal) maximum effect model (Eq. S1, S2) describing the observed effect E as functions of its concentration c and the model parameters β (slope parameter in $L/mg \times h^{-1}$), EC_{50} (half-effective concentration in mg/L), E_{max} (maximal effect in h^{-1}) and H (Hill factor of sigmoidicity).

$$E(c) = \beta * c^H \quad (\text{Eq. S1})$$

$$E(c) = \frac{E_{\max} \times c^H}{EC_{50}^H + c^H} \quad (\text{Eq. S2})$$

Nonlinear-mixed-effects modelling was conducted on observed bacterial counts over time using NONMEM® 7.5 (Icon Development Solutions, USA). First-order-conditional-estimation with interaction (FOCE-I) was used to obtain maximum-likelihood estimations by minimizing the negative log-likelihood objective function criterion $-2LL$.

For better comparison of models with different numbers of parameters the Akaike-Information-Criterion AIC was calculated from $-2LL$ with k representing the number of estimated (non-fixed) parameters:

$$AIC = -2LL + 2 \times k \quad (\text{Eq. S3})$$

General Pharmacodynamic Interaction (GPDI) model¹⁶³

The GPDI model is given by Eq. S4 - S11 for two drugs as interaction partners with an interaction on the PD parameters $E_{\max}$ or EC_{50} . The GPDI term is a function of the perpetrator drug concentration, which affects a PD parameter θ (either EC_{50} or $E_{\max}$) of the victim drug with INT as maximum possible fractional shift of the PD parameter θ , $EC_{50_{INT}}$ as potency of the perpetrator and H_{INT} representing the sigmoidicity of the interaction (Eq. S4). The fractional shift of the victim's PD parameter caused by the perpetrator drug allowed the directional quantification of the PD interaction, i.e., either synergy, antagonism or additivity. Whenever $-1 < INT < 0$ applies to $E_{\max}$ based PD interactions, antagonism is described, and whenever $-1 < INT < 0$ applies to EC_{50} based PD interactions, synergy is described (vice versa for $INT > 0$). In the case of bidirectional PD interactions, a drug could be perpetrator and victim at the same time. Whenever synergism or antagonism were not significant (confidence interval of INT not including 0), the interaction was described as null-interaction model by Bliss Independence with E_A and E_B representing the effect of drug A and B (Eq. 8). Since Bliss Independence was derived from the probabilistic approach, the probabilistic Bliss Independence term was calculated using the single drug effects, which were normalized to the maximum drug effect possible with a single drug, and afterwards scaled back to the effect scale (Eq. S9 - S11).

$$\theta \times \left(1 + \frac{\text{INT} \times C^{\text{H}_{\text{INT}}}}{\text{EC50}_{\text{INT}}^{\text{H}_{\text{INT}}} + C^{\text{H}_{\text{INT}}}} \right) \quad (\text{Eq. S4})$$

$$E_A = \frac{\text{Emax}_A \times \left(1 + \frac{\text{INT}_{AB} \times C_B^{\text{H}_{\text{INT},AB}}}{\text{EC50}_{\text{INT},AB}^{\text{H}_{\text{INT},AB}} + C_B^{\text{H}_{\text{INT},AB}}} \right) \times C_A^{\text{H}_A}}{\left(\text{EC50}_A^{\text{H}_A} \right)^{\text{H}_A} + C_A^{\text{H}_A}} \quad (\text{Eq. S5})$$

$$E_B = \frac{\text{Emax}_B \times \left(1 + \frac{\text{INT}_{BA} \times C_A^{\text{H}_{\text{INT},BA}}}{\text{EC50}_{\text{INT},BA}^{\text{H}_{\text{INT},BA}} + C_B^{\text{H}_{\text{INT},AB}}} \right) \times C_B^{\text{H}_B}}{\left(\text{EC50}_B^{\text{H}_B} \right)^{\text{H}_B} + C_B^{\text{H}_B}} \quad (\text{Eq. S6})$$

$$E_A = \frac{\text{Emax}_A \times C_A^{\text{H}_A}}{\left(\text{EC50}_A \times \left(1 + \frac{\text{INT}_{AB} \times C_B^{\text{H}_{\text{INT},AB}}}{\text{EC50}_{\text{INT},AB}^{\text{H}_{\text{INT},AB}} + C_B^{\text{H}_{\text{INT},AB}}} \right) \right)^{\text{H}_A} + C_A^{\text{H}_A}} \quad (\text{Eq. S7})$$

$$E_B = \frac{\text{Emax}_B \times C_B^{\text{H}_B}}{\left(\text{EC50}_B \times \left(1 + \frac{\text{INT}_{BA} \times C_B^{\text{H}_{\text{INT},BA}}}{\text{EC50}_{\text{INT},BA}^{\text{H}_{\text{INT},BA}} + C_B^{\text{H}_{\text{INT},BA}}} \right) \right)^{\text{H}_B} + C_B^{\text{H}_B}} \quad (\text{Eq. S8})$$

$$E = E_A + E_B - E_A \times E_B \quad (\text{Eq. S9})$$

$$\text{Max}_E = \max (E_{\text{max},A}, E_{\text{max},B}) \quad (\text{Eq. S10})$$

$$E = \left(\frac{E_A}{\text{Max}_E} + \frac{E_B}{\text{Max}_E} - \frac{E_A}{\text{Max}_E} \cdot \frac{E_B}{\text{Max}_E} \right) \cdot \text{Max}_E \quad (\text{Eq. S11})$$

Text S4: Methods: Bioanalysis

Instruments used were 1290 Infinity HPLC II system (Agilent Technologies, California, USA) equipped with a reverse-phase Accucore C18 column (Thermo Fisher, Dreieich, Germany) coupled with a QTRAP 5500 mass spectrometer (SCIEX, Framingham, Massachusetts, USA). Firstly, samples were diluted using ca-MHB (1:20) before further preparation. Briefly, sample preparation was performed by mixing 50 µL of sample with 50 µL of ice-cold internal standard ceftazidime (1 mg/L) in methanol and 200 µL of ice-cold acetonitrile. The mixtures were vortexed for 10 s and subsequently centrifuged for 20 min at 4 °C and 17.968 x g after which 75 µL of the

supernatant was transferred into an HPLC vial. The same procedure was used for quality controls and calibration solutions.

The mobile phase consisted of 10 mM ammonium formate + 0.1% formic acid buffer in H₂O (component A) and acetonitrile with 0.1% formic acid (component B) running following gradient elution protocol: Initially, 95% A and 5% B was maintained from 0 to 0.8 min. The proportion of component B was then increased linearly to 20% from 0.8 to 2 min and further increased to 40% by 3.5 min, maintaining this composition until 4.50 min. At 4.60 min, the initial conditions of 95% A and 5% B were re-established and maintained until 6 min. Detailed information about the ion source parameters of the instrument, method specifications, retention times and analysed fragments are given in **Tab. S3** and **S4**.

Table S3 Ion source parameters for two distinct ESI phases in the mass spectrometer method.

	Curtain Gas (psi)	Collision Gas	Ion Spray Voltage (volt)	Temperature (°C)	Nebulizer Gas (psi)	Heater Gas (psi)
Phase 1 (0-3.5 min)	40	Medium	-3500	450	50	50
Phase 2 (3.5-7 min)	20	Medium	4000.0	450	50	50

Table S4 Fragments and parameters for each antibiotic and internal standard (IS) in the bioanalysis method. Optimization of mass spectrometry parameters was performed by direct injection of individual standard solutions for each analyte and IS. FOF: fosfomycin; MEM: meropenem; CAZ: ceftazidime; Coll: Collision.

Antibiotic	Identifier	ESI mode	Retention time [min]	Q1 [Da]	Q3 [Da]	Dwell Time [msec]	Declustering Potential [V]	Entrance Potential [V]	Coll. Energy [V]	Coll. Cell Exit Potential [V]
FOF	Quantification	-	1.47	136.884	78.9	150	-80	-10	-38	-11
FOF	Qualification	-	1.47	136.884	81.1	150	-80	-10	-20	-9
MEM	Quantification	+	4.65	385.109	67.9	150	96	10	85	8
MEM	Qualification	+	4.65	385.109	114.1	150	96	10	35	10
CAZ	Internal Standard	+	4.67	546.957	468	150	96	10	17	20

Concentration ranges of calibration standards and quality controls (**Tab. S5**) were derived from expected target concentrations in the experiment samples. Results of the validation of this method over a period of three consecutive days using shown quality control levels are summarized in **Tab. S6**.

Table S5 Concentration ranges of meropenem and fosfomycin calibration and quality standards with ^a measured concentration on LC-MS/MS and ^b resulting actual concentrations after recalculation of sample dilution step (1:20). MEM: meropenem FOF: fosfomycin; LLOQ: lower limit of quantification; LQC: low-level quality control; MQC: mid-level quality control; HQC: high-level quality control; ULOQ: upper limit of quantification.

Compound	MEM [mg/L]		FOF [mg/L]		Quality Controls
Level	a	b	a	b	
1 (LLOQ)	0.35	7	2.50	50	LLOQ
2	1.05	21	7.50	150	LQC
3	1.75	35	12.5	250	MQC
4	2.10	42	15.0	300	
5	2.80	56	20.0	400	HQC
6 (ULOQ)	3.50	70	25.0	500	

Table S6 Accuracy and precision of meropenem (MEM) and fosfomycin (FOF) across individual validation days (samples each level n = 15) for the parallel bioanalytical determination of MEM and FOF in bacterial growth medium (ca-MHB) using HPLC-MS/MS. All values are within the described EMA limits.

Quality Control Level	Calculated MEM concentration [mg/L]	Accuracy [%]	Precision [%CV]	Calculated FOF concentration [mg/L]	Accuracy [%]	Precision [%CV]
Day 1						
LLOQ	0.38	109.5	3.2	2.39	95.4	7.2
Low	1.05	100.3	1.6	6.83	91.0	4.6
Middle	1.81	103.2	3.0	11.3	90.4	4.6
High	2.76	98.6	1.3	17.6	88.1	3.6
Day 2						
LLOQ	0.39	110.9	4.0	2.45	98.0	3.9
Low	1.07	101.8	3.6	7.41	98.8	6.0
Middle	1.90	108.1	1.3	13.3	106.5	3.3
High	2.90	103.5	3.6	20.9	104.3	2.8

Day 3						
LLOQ	0.38	109.6	1.5	2.08	83.1	1.9
Low	1.01	96.5	2.5	6.57	87.6	2.6
Middle	1.72	98.1	3.7	11.9	95.0	6.1
High	2.94	105.1	4.6	20.9	104.4	5.9
All Days						
LLOQ	0.385	110.0	2.9	2.304	92.2	4.3
Low	1.045	99.5	2.6	6.935	92.5	4.4
Middle	1.805	103.1	2.7	12.16	97.3	4.7
High	2.867	102.4	3.2	19.79	98.9	4.1

Text S5: Results: Bioanalysis of meropenem in static time-kill assays

The observed concentrations of meropenem under specific scenarios for each tested strain, including expected degradation in the absence of bacteria (meropenem without bacteria) and predicted degradation based on the final pharmacometric models (meropenem with bacteria), are presented in **Fig. S2**.

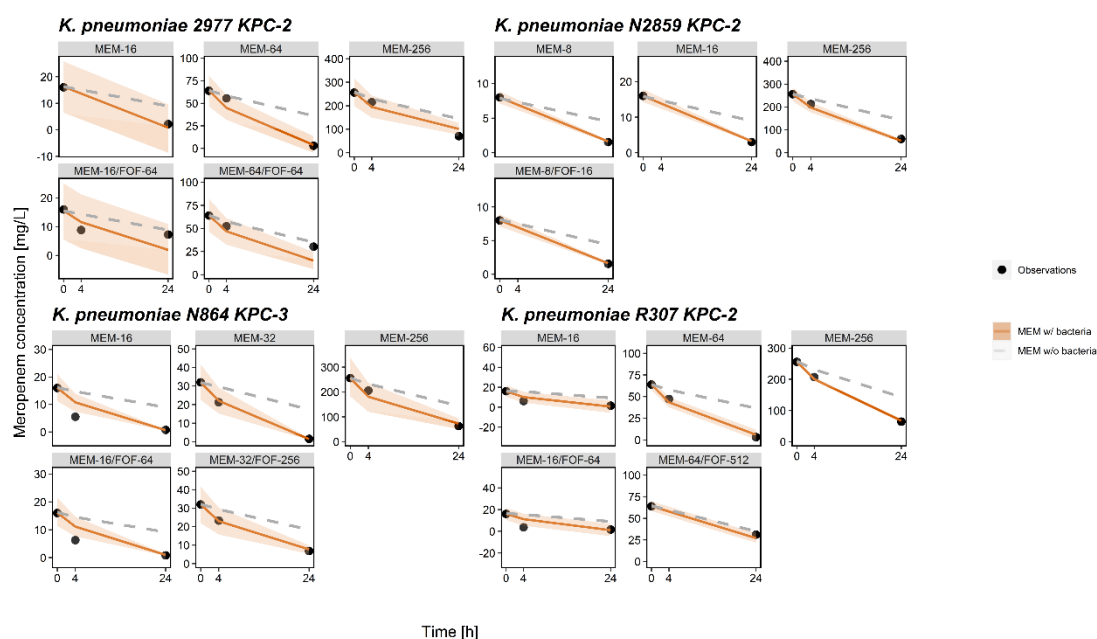


Figure S8 Observed concentrations of meropenem in selected scenarios per tested strain, with expected degradation in the absence of bacteria (MEM w/o bacteria) and predicted degradation using final pharmacometric models (MEM w/ bacteria), are shown. Dots: observations; solid line: median prediction; dashed line: expected degradation w/o bacteria; shaded area: 90% prediction intervals; MEM: meropenem.

Text S5: Example control stream run102.8.mod from *K. pneumoniae* 2977

;; 1. Based on: run102.6

;; 2. Description: PKPD Model *K. pneumoniae* 2977 GPDI EC50_Synergy_FOFaffectedbyMER
Bacteria-depedent-KPC_deg Comb.Err

;; x1. Author: farooq

;; 3. Label:

\$PROB Modelling of TKE MER/FOF in KP 2977

\$INPUT

ID ; experiment ID

TIME ; h

DV ; lncfu/mL

EVID ; event identifier

MDV ; MDV

AMT ; amount of dose in mg

C_A ; concentration of Meropenem in mg/L

C_B ; concentration of fosfomycin in mg/L

CMT ; 1-5

FLAG_SCENARIOS ; 1-7

\$DATA NONMEM_MER-VAB-FOF_TKC_DATA_both_PKMERK.pneumoniae-2977.csv IGNORE=@

ACCEPT = (FLAG_SCENARIOS.EQ.1) ;growth scenarios PD

ACCEPT = (FLAG_SCENARIOS.EQ.2) ;MER scenarios PD

ACCEPT = (FLAG_SCENARIOS.EQ.4) ;FOF scenarios PD

ACCEPT = (FLAG_SCENARIOS.EQ.5) ;MER-FOF scenarios PD

ACCEPT = (FLAG_SCENARIOS.EQ.7) ;MER_PK_LCMS PK

\$SUBROUTINE ADVAN13 TOL=8

\$MODEL

COMP (OBS_S)

COMP (DRUG_A) ;A = MER

COMP (DRUG_B) ;B = FOF

COMP (OBS_R)

\$PK

;Bacterial system with bacterium-specific parameters

KG_AB_S = THETA(1) ; 1/h growth constant susceptible population

KG_AB_R = THETA(2) ; 1/h growth constant resistant population

INOC_AB_S = 10**(THETA(3)*exp(ETA(1))) ; cfu/mL start inoculum susceptible population

INOC_AB_R = 10**(THETA(4)*exp(ETA(2))) ; cfu/mL start inoculum resistant population

BMAX = 10**THETA(5) ; cfu/mL maximal bacterial concentration

;Drug-specific parameters

;Effect model drug A

SLOPE_A_S = THETA(6)

H_A_S = THETA(7) ; Hill-coefficient

EMAX_A_R = THETA(8) ; 1/h maximal effect

EC50_A_R = THETA(9) ; mg/L

H_A_R = THETA(10) ; Hill-coefficient

;MER degradation rates

KDEG = THETA(11) ; basal without bacteria from separate experiment

KPC_DEG = THETA(12) ; bacteria-dependent degradation rate constant

;Effect model drug B

EMAX_B_S = THETA(13) ; 1/h maximal effect

EC50_B_S = THETA(14); mg/L

H_B_S = THETA(15) ; Hill-coefficient

EMAX_B_R = THETA(16) ; 1/h maximal effect

EC50_B_R = THETA(17); mg/L

$H_B_R = \text{THETA}(18)$; Hill-coefficient

;Combined effects

$EC50_INT_BA_R = \exp(\text{THETA}(19)) - 1$;GPDI INT-parameter log transformed

$EC50_EC50INT_BA_R = \text{THETA}(20)$;GPDI EC50 of interaction

$EC50_H_INT_BA_R = \text{THETA}(21)$;GPDI Hill of interaction

; Initial conditions of ODE

$A_0(1) = \text{INOC_AB_S}$

$A_0(2) = 0$; Drug A

$A_0(3) = 0$; Drug B

$A_0(4) = \text{INOC_AB_R}$

\$DES

$B_AB_S = A(1)$

$C_DA = A(2)$

$C_DB = A(3)$

$B_AB_R = A(4)$

$CA = C_DA$; concentration solved in ODE with MER degradation

$CB = C_B$;concentration as covariate from dataset drug B

;Power model

$$E_A_S = \text{SLOPE_A_S} * (CA^{**}H_A_S)$$

;Sigmoidal Emax model

$$E_A_R = (EMAX_A_R * (CA^{**}H_A_R)) / ((EC50_A_R^{**}H_A_R) + (CA^{**}H_A_R))$$

$$E_B_S = (EMAX_B_S * (CB^{**}H_B_S)) / ((EC50_B_S^{**}H_B_S) + (CB^{**}H_B_S))$$

$$E_B_R = (EMAX_B_R * (CB^{**}H_B_R)) / ((EC50_B_R^{**}H_B_R) + (CB^{**}H_B_R))$$

;Combined effects (INT EC50 A effected by B on both populations)

;GPDl term

;Interaction on resistant population

$$GPDl_BA_R_EC50 = EC50_B_R * (1 + ((EC50_INT_BA_R * (CA^{**}EC50_H_INT_BA_R)) / ((EC50_EC50INT_BA_R^{**}EC50_H_INT_BA_R) + (CA^{**}EC50_H_INT_BA_R))))$$

;Recalculation of E_B_R with GPDl term

$$E_B_R = (EMAX_B_R * (CB^{**}H_B_R)) / ((GPDl_BA_R_EC50^{**}H_B_R) + (CB^{**}H_B_R))$$

;Bliss Independence

$$E_AB_S_MAX = \max(EMAX_A_S, EMAX_B_S)$$

$$E_AB_R_MAX = \max(EMAX_A_R, EMAX_B_R)$$

$$E_AB_S = ((E_A_S/E_AB_S_MAX) + (E_B_S/E_AB_S_MAX) - ((E_A_S/E_AB_S_MAX) * (E_B_S/E_AB_S_MAX))) * E_AB_S_MAX \quad ;\text{Effect scaled with EMAX}$$

$E_AB_R = ((E_A_R/E_AB_R_MAX) + (E_B_R/E_AB_R_MAX) - ((E_A_R/E_AB_R_MAX) * (E_B_R/E_AB_R_MAX))) * E_AB_R_MAX$;Effect scaled with EMAX

;Bacterial system under drug exposure

$DADT(1) = B_AB_S * KG_AB_S * (1 - ((B_AB_S + B_AB_R) / BMAX)) - E_AB_S * B_AB_S$

$DADT(2) = -(KDEG + KPC_DEG * LOG(A(1) + A(4))) * A(2)$

$DADT(3) = 0$

$DADT(4) = B_AB_R * KG_AB_R * (1 - ((B_AB_S + B_AB_R) / BMAX)) - E_AB_R * B_AB_R$

\$ERROR

;PD

IF(FLAG_SCENARIOS.LE.5) THEN

$IPRED = LOG(A(1) + A(4))$; total bacterial concentration

$W1 = SQRT(THETA(22)**2)$; ADDITIVE ERROR

$RESV = EPS(1)$

$IRES = DV - IPRED$

$W = W1$

$IWRES = IRES / W$

$Y = IPRED + W * RESV$

ENDIF

;PK

IF(FLAG_SCENARIOS.EQ.7) THEN

$IPRED = A(2)$; MER concentration

$W1 = SQRT(THETA(23)**2 + (THETA(24)*IPRED)**2)$; PROPORTIONAL AND ADDITIVE ERROR

$RESV = EPS(1)$

IRES = DV-IPRED

W=W1

IWRES = IRES/W

Y= IPRED+W*RESV

ENDIF

\$THETA

(0, 2.43) ;1_KG_AB_S

(0, 0.906) ;2_KG_AB_R

(0, 6.42) ;3_INOC_AB_S

(0, 1.8) ;4_INOC_AB_R

(0, 9.22) ;5_BMAX

(0, 1.42) ;6_SLOPE_A_S

(0, 0.304) ;7_H_A_S

(0, 1.1) ;8_EMAX_A_R

(0, 91.7) ;9_EC50_A_R

(0, 20) FIX ;10_H_A_R

(0, 0.0242) FIX ;11_kdeg_MER

(0, 0.001) ;12_KPC_deg_MER

(0, 6.06) ;13_EMAX_B_S

(0, 7.28) ;14_EC50_B_S

(0, 1.29) ;15_H_B_S

(0, 0.986) ;16_EMAX_B_R

(0, 529) ;17_EC50_B_R

(0, 1) FIX ;18_H_B_R

(-20, -3.92,-0.01) ;19_EC50_INT_BA_R

(0, 8.7) ;20_EC50_EC50INT_BA_R

(20) FIX ;21_EC50_H_INT_BA_R

(0, 1.33) ;22_Add.Res.Err_PD

(0, 5.49) ;23_Add.Res.Err_PK

(0, 0.137) ;24_Prop.Res.Err_PK

\$OMEGA ;no IIV implemented

0 FIX ;IIV_INOC_AB_S

0 FIX ;IIV_INOC_AB_R

\$SIGMA

1 FIX ;'Uppsala dialect': Modeled as THETAS

\$EST METHOD=1 INTER SLOW MAXEVAL=9999 NOABORT NSIG=3 PRINT=5

\$COV PRINT=E MATRIX=R

\$TABLE ID TIME DV MDV EVID IPRED C_A C_DA C_B C_DB B_AB_S B_AB_RE_A_S E_A_RE_B_S
E_B_R B_AB_S B_AB_R SPOP RPOP IWRES BMAX FLAG_SCENARIOS ONEHEADER NOPRINT
FILE=sdtab102.8

\$TABLE INOC_AB_S INOC_AB_R BMAX KG_AB_S KG_AB_R FIRSTONLY ONEHEADER NOPRINT
FILE=patab102.8

References

1. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, version 14.0. Published online 2024. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_14.0_Breakpoint_Tables.pdf
2. Wicha SG, Chen C, Clewe O, Simonsson USH. A general pharmacodynamic interaction model identifies perpetrators and victims in drug interactions. *Nature Communications*. 2017;8(1):1-11. doi:10.1038/s41467-017-01929-y
3. Cadwell JJS. The hollow fiber infection model: Principles and practice. *Clin Microbiol*. 2018;07. doi:10.4172/2327-5073-C2-036

9.2 Supplementary data Publication II

International Journal of Antimicrobial Agents

Electronic Supplementary Material

Title: A pharmacometric model assessing the *in vitro* synergistic effect of a bacteriophage-polymyxin B combination in a clinical multidrug-resistant *K. pneumoniae* isolate.

Authors: Aneeq Farooq^{1,*}, Sue C. Nang^{2,*,#}, Heidi H. Yu², Hasini Wickremasinghe², Mei-Ling Han², Yu-Wei Lin², Sebastian G. Wicha^{1,*}, Jian Li^{2,*}

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* Equal contributions to this work

Running Title: PK/PD analysis of phage-polymyxin B in MDR *K. pneumoniae*

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Text S1: Mathematical modelling of single drug effects

Single drug effects were quantified using the slope effect model or (sigmoidal) maximum effect model (Eq. S1 and S2) describing the observed effect E as functions of its concentration c and the model parameters β (slope parameter in $L/mg \times h^{-1}$), EC_{50} (half-effective concentration in mg/L), E_{max} (maximal effect in h^{-1}) and H (Hill factor of sigmoidicity).

$$E(c) = \beta * c^H \quad (\text{Eq. S1})$$

$$E(c) = \frac{E_{max} \times c^H}{EC_{50}^H + c^H} \quad (\text{Eq. S2})$$

Nonlinear-mixed-effects modelling was conducted on observed bacterial counts over time using Monolix® 2023R1 (Lixsoft, France). Stochastic Approximation Estimation Maximization (SAEM) algorithm was used to obtain maximum-likelihood estimations by minimizing the negative log-likelihood objective function criterion ($-2LL$).

For better comparison of models with different numbers of parameters the Akaike-Information-Criterion AIC was calculated from $-2LL$ with k representing the number of estimated (non-fixed) parameters:

$$AIC = -2LL + 2 \times k.^{108} \quad (\text{Eq. S3})$$

Text S2: General Pharmacodynamic Interaction (GPDI) model¹⁶³

The GPDI model is given by Eq. S4-S8 for two drugs as interaction partners with an interaction on the PD parameters E_{max} or EC_{50} . The fractional shift of the victim's PD parameter caused by the perpetrator drug allowed the directional quantification of the PD interaction, i.e., either synergy, antagonism or additivity. In the case of asymmetric PD interactions, a drug could be perpetrator and victim at the same time. Since the nature of phage killing effects can not be

described with classical pharmacodynamic parameters as $EC50$ or E_{max} , the GPDl model was applied on binding kinetics rate constant k_{bind} or phage resistance development rate constant k_{mut} (Eq. S8).

$$E_A = \frac{E_{max_A} \times \left(1 + \frac{INT_{AB} \times C_B^{H_{INT,AB}}}{EC50_{INT,AB}^{H_{INT,AB}} + C_B^{H_{INT,AB}}} \right) \times C_A^{H_A}}{\left(EC50_A^{H_A} \right)^{H_A} + C_A^{H_A}} \quad (\text{Eq. S4})$$

$$E_B = \frac{E_{max_B} \times \left(1 + \frac{INT_{BA} \times C_A^{H_{INT,BA}}}{EC50_{INT,BA}^{H_{INT,BA}} + C_A^{H_{INT,BA}}} \right) \times C_B^{H_B}}{\left(EC50_B^{H_B} \right)^{H_B} + C_B^{H_B}} \quad (\text{Eq. S5})$$

$$E_A = \frac{E_{max_A} \times C_A^{H_A}}{\left(EC50_A \times \left(1 + \frac{INT_{AB} \times C_B^{H_{INT,AB}}}{EC50_{INT,AB}^{H_{INT,AB}} + C_B^{H_{INT,AB}}} \right) \right)^{H_A} + C_A^{H_A}} \quad (\text{Eq. S6})$$

$$E_B = \frac{E_{max_B} \times C_B^{H_B}}{\left(EC50_B \times \left(1 + \frac{INT_{BA} \times C_A^{H_{INT,BA}}}{EC50_{INT,BA}^{H_{INT,BA}} + C_A^{H_{INT,BA}}} \right) \right)^{H_B} + C_B^{H_B}} \quad (\text{Eq. S7})$$

$$\theta \times \left(1 + \frac{INT \times C^{H_{INT}}}{EC50_{INT}^{H_{INT}} + C^{H_{INT}}} \right) \quad \text{for } \theta \in \{k_{mut}, k_{bind}, k_g\} \quad (\text{Eq. S8})$$

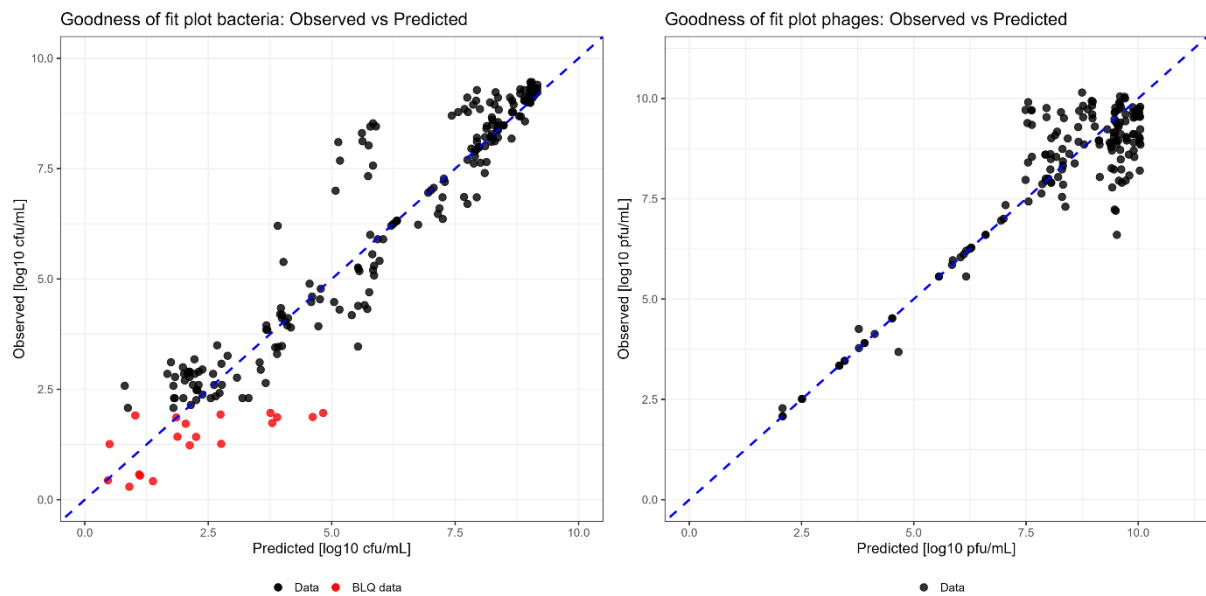
Text S3: Monolix final model: Diagnostic plots and control stream

Figure S9. Goodness of fit plots for individual bacteria and phage predictions of final pharmacokinetic/pharmacodynamic model.

Model control stream

DESCRIPTION:

[LONGITUDINAL]

input={KG, BMAX, INOC_B,

C_PMB,

KMUT, KBIND, BSIZE, KDECAY, KLYSIS, INOC_P,

SLOPE_PMB, H_PMB,

H_INT}

C_PMB = {use=regressor}

INOC_B = {use=regressor}

INOC_P = {use=regressor}

PK:

EQUATION:

; retransformation of ln data from dataset to normal scale for modeling

if INOC_P == 0

 INOC_P_ln = 0

else

 INOC_P_ln = exp(INOC_P)

End

INOC_B_ln = exp(INOC_B)

; PMB effect model

; Interaction: E_PMB resensitization by Phages

if C_PMB == 0

 E_PMB = 0

elseif INOC_P == 0

 E_PMB = 0

else

 E_PMB = SLOPE_PMB * ((INOC_P/INOC_B)^H_INT) * C_PMB^H_PMB

end

; Initials

PHAGE_0 = INOC_P_In

B_S_0 = INOC_B_In

B_R_0 = 0

B_I_0 = 0

; ODE

ddt_PHAGE = BSIZE*KLYSIS*B_I - (10[^](-KBIND))*PHAGE*B_S- KDECAY*PHAGE

ddt_B_S = KG*B_S * (1-((B_S + B_R + B_I) / 10[^]BMAX)) - (10[^](-KMUT)) *B_S - (10[^](-KBIND))*B_S*PHAGE - E_PMB * B_S

ddt_B_R = KG*B_R * (1-((B_S + B_R + B_I) / 10[^]BMAX)) + (10[^](-KMUT)) *B_S - E_PMB * B_R

ddt_B_I = (10[^](-KBIND))*B_S*PHAGE - KLYSIS * B_I

B = log(B_S + B_R + B_I)

P = log(PHAGE)

OUTPUT:

output = {B, P}

References

1. Akaike H. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 1974; **19**: 716–23.
2. Wicha SG, Chen C, Clewe O, *et al.* A general pharmacodynamic interaction model identifies perpetrators and victims in drug interactions. *Nat Commun* 2017; **8**: 1–11.

9.3 Supplementary data Publication III

International Journal of Antimicrobial Agents

Electronic Supplementary Material

Title: Evaluation of in vitro pharmacodynamic drug interactions of ceftazidime-avibactam with tigecycline in ESBL- and carbapenemase producing *Escherichia coli* and *Klebsiella pneumoniae*.

Authors: Aneeq Farooq¹, Bernhard Drotleff², Niklas Kroemer¹, Mei-Ling Han³, Jian Li³, Jean-Winoc Decousser⁴, David Schrey¹, Julien Buyck⁵, Nicolas Grégoire⁵, Patrice Nordmann⁶, Sebastian G. Wicha^{1, #}

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Text S1: Antimicrobial susceptibility testing and quantification of viable bacteria

Bacterial isolates (approx. 5×10^5 CFU/mL) were inoculated in freshly prepared cation-adjusted Mueller-Hinton-broth II (ca-MHB, NutriSelect® Plus, Sigma-Aldrich) using 48-well-plates that contained a series of 2-fold dilutions of each antibiotic. The plates were incubated at 37° C in ambient air for a full 24 h and were then evaluated visually. The MIC was defined as the lowest concentration that showed no visible growth of bacteria. All experiments were performed with fixed concentrations of avibactam (4 mg/L) according to EUCAST recommendation ²⁴.

Text S2: Mathematical modeling of EC₅₀ and derivation of EC₀₈, EC₄₄, EC₈₂

Single drug effects were quantified using the sigmoidal maximum effect model (Eq. S1) describing the observed effect E ($\log_{10}$ CFU/mL reduction compared to control) as function of its concentration C and the model parameters $Base$ (bacterial count in control), EC_{50} (half-effective concentration), E_{max} (maximum $\log_{10}$ CFU/mL reduction) and H (Hill factor of sigmoidicity).

$$E(C) = Base - \frac{E_{max} \times C^H}{EC_{50}^H + C^H} \quad (\text{Eq. S1})$$

The mathematical modeling of the concentration-effect-relationship was performed in R using the 'stats' (v. 4.2.1) package. The 'optim' function was called to perform parameter estimation by minimizing the negative log-likelihood objective function criterion $-LL$:

$$-LL = 0.5 \cdot \sum_{i=1}^n \left[\frac{(obs_i - pred_i)^2}{\sigma_i^2} + \ln \sigma_i^2 \right] \quad (\text{Eq. S2})$$

where obs_i is representing the i^{th} observation, $pred_i$ the i^{th} prediction value and σ_i^2 the residual variability. Minimization methods used in 'optim' function were the Nelder and Mead (NM), the Broyden-Fletcher-Goldfarb-Shanno (BFGS) method, and a hybrid method that first runs NM, using its result as the starting point for BFGS. The method that resulted in the lowest $-LL$ was selected for the final parameter estimation. Four different parameter estimation scenarios were tested: 1) no fixed parameters, 2) fixing the $Base$ to the median bacterial count of control

experiments, 3) fixing the *Base* and *E_{max}* value to the median bacterial count of control experiments and 4) *Base* and *E_{max}* estimated as one parameter. Initial values for the parameter estimation were based on the experimental data of the respective antibiotic and strain. The selection of the final model was based on the Akaike-Information-Criterion¹⁰⁸, inspection of the graphical model fit and biological plausibility of parameters. Minimization methods and scenarios are presented in **Table S1**. Parameters of the final model were used to calculate *EC₀₈/EC₄₄/EC₈₂* by solving the sigmoidal maximum effect model for *c*:

$$c = \frac{H \sqrt{EC_{XX} * E_{max} * EC_{50}^H}}{\sqrt{E_{max} - EC_{XX} * E_{max}}} \quad (\text{Eq. S3})$$

Calculated *EC₀₈/EC₄₄/EC₈₂* values used for dynamic checkerboard testing are presented in **Table S2**.

Table S2 Calculated EC₀₈, EC₄₄ and EC₈₂ values according to Eq. S3, for ceftazidime/avibactam and tigecycline for each strain.

Strain	CZA			TGC		
	EC ₀₈	EC ₄₄	EC ₈₂	EC ₀₈	EC ₄₄	EC ₈₂
	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]
<i>E. coli</i> WT	0.045	0.101	0.191	0.111	0.128	0.143
<i>E. coli</i> OXA-181	0.082	0.166	0.294	0.088	0.151	0.233
<i>E. coli</i> OXA-48	0.063	0.138	0.258	0.084	0.098	0.111
<i>E. coli</i> KPC-2	0.076	0.097	0.119	0.072	0.097	0.122
<i>E. coli</i> KPC-3	0.283	1.922	8.859	0.052	0.068	0.084
<i>E. coli</i> YAL_AMA	0.044	0.092	0.167	0.084	0.107	0.130
<i>E. coli</i> JUM_JEA	0.030	0.039	0.048	0.082	0.199	0.407
<i>E. coli</i> OLA_HAM	0.110	0.197	0.313	0.111	0.163	0.221
<i>K. pneumoniae</i> WT	0.043	0.067	0.094	0.026	0.080	0.194
<i>K. pneumoniae</i> OXA-48	0.037	0.091	0.185	0.028	0.104	0.296
<i>K. pneumoniae</i> KPC-2	0.053	0.068	0.083	0.039	0.107	0.240
<i>K. pneumoniae</i> KPC-3	0.012	0.081	0.374	0.037	0.049	0.060
<i>K. pneumoniae</i> 2977	0.136	0.572	1.805	0.107	0.145	0.184
<i>K. pneumoniae</i> N864	0.696	1.834	3.979	0.103	0.302	0.715
<i>K. pneumoniae</i> R307	0.094	0.470	1.694	0.515	1.062	1.894

Abbreviations: CZA: ceftazidime/avibactam; TGC: tigecycline. Avibactam fixed to 4 mg/L.

Text S3: General Pharmacodynamic Interaction (GPDl) model ¹⁶³

The GPDl model is given by Eq. S4 - S10 for two drugs as interaction partners with an interaction on the PD parameters E_{max} or EC₅₀. The GPDl term is a function of the perpetrator drug concentration, which affects a PD parameter θ (either EC₅₀ or E_{max}) of the victim drug with INT as maximum possible fractional shift of the PD parameter θ , EC₅₀_{INT} as potency of the perpetrator and H_{INT} representing the sigmoidicity of the interaction (Eq. S4). The fractional shift of the victim's PD parameter caused by the perpetrator drug allowed the directional quantification of the PD interaction, i.e., either synergy, antagonism or additivity. Whenever $-1 < \text{INT} < 0$ applies to E_{max} based PD interactions, antagonism is described, and whenever $-1 < \text{INT} < 0$ applies to EC₅₀ based PD interactions, synergy is described (vice versa for INT > 0). In the case of bidirectional PD interactions, a drug could be perpetrator and victim at the same time. Whenever synergism or antagonism were not significant (confidence interval of INT not

including 0), the interaction was described as null-interaction model by Bliss Independence with E_A and E_B representing the effect of drug A and B (Eq. 8). Since Bliss Independence was derived from the probabilistic approach, the probabilistic Bliss Independence term was calculated using the single drug effects, which were normalized to the maximum drug effect possible with a single drug, and afterwards scaled back to the effect scale (Eq. S8 - S10). Confidence intervals of estimated INT parameters were calculated in R using the 'stats' (v. 4.2.1) package by first deriving standard errors from the square root of the diagonal elements of the inverse Hessian matrix, then applying ± 1.96 times these errors to the parameter estimates to establish the bounds.

$$E_A = \frac{E_{\max A} \times \left(1 + \frac{INT_{AB} \times C_B^{H_{INT,AB}}}{EC50_{INT,AB}^{H_{INT,AB}} + C_B^{H_{INT,AB}}} \right) \times C_A^{H_A}}{(EC50_A^{H_A})^{H_A} + C_A^{H_A}} \quad (\text{Eq. S4})$$

$$E_B = \frac{E_{\max B} \times \left(1 + \frac{INT_{BA} \times C_A^{H_{INT,BA}}}{EC50_{INT,BA}^{H_{INT,BA}} + C_A^{H_{INT,BA}}} \right) \times C_B^{H_B}}{(EC50_B^{H_B})^{H_B} + C_B^{H_B}} \quad (\text{Eq. S5})$$

$$E_A = \frac{E_{\max A} \times C_A^{H_A}}{\left(EC50_A \times \left(1 + \frac{INT_{AB} \times C_B^{H_{INT,AB}}}{EC50_{INT,AB}^{H_{INT,AB}} + C_B^{H_{INT,AB}}} \right) \right)^{H_A} + C_A^{H_A}} \quad (\text{Eq. S6})$$

$$E_B = \frac{E_{\max B} \times C_B^{H_B}}{\left(EC50_B \times \left(1 + \frac{INT_{BA} \times C_A^{H_{INT,BA}}}{EC50_{INT,BA}^{H_{INT,BA}} + C_A^{H_{INT,BA}}} \right) \right)^{H_B} + C_B^{H_B}} \quad (\text{Eq. S7})$$

$$E_{comb} = E_A + E_B - E_A \times E_B \quad (\text{Eq. S8})$$

$$Max_E = \max (E_{\max A}, E_{\max B}) \quad (\text{Eq. S9})$$

$$E_{comb} = \left(\frac{E_A}{Max_E} + \frac{E_B}{Max_E} - \frac{E_A}{Max_E} \cdot \frac{E_B}{Max_E} \right) \cdot Max_E \quad (\text{Eq. S10})$$

Text S4: Methods of PD interaction modelling of time-kill assay data

Nonlinear-mixed-effects modelling was conducted on observed bacterial counts over time in time-kill assays for all four strains, using NONMEM® 7.5 (Icon Development Solutions, USA). Quantitative analyses were performed using first-order-conditional-estimation with interaction (FOCE-I) for maximum-likelihood estimations and executed through PsN 5.4 (Uppsala University, Uppsala, Sweden). All pharmacometric models consisted of two bacterial compartments representing subpopulations of antibiotic-susceptible bacteria (S) and less susceptible bacteria (R), respectively. Such a two-compartment PD effect model was used to represent biphasic bacterial killing over time because it assumed the presence of two distinct bacterial subpopulations allowing for a more accurate depiction of how the bacteria responded differently to the treatment. The susceptible compartment (S) captured the bacteria that were initially killed by the antibiotic, while the less susceptible compartment (R) modelled the subpopulation that survived and were killed more slowly despite the antibiotic presence.

Single drug effects of ceftazidime/avibactam and tigecycline were described with separate killing rate constants for both subpopulations of bacteria (S and R). Ceftazidime/avibactam was treated as a single drug, with avibactam set at a fixed concentration of 4 mg/L in all experiments. PD interaction analysis was conducted on growth control, 4 x MIC_{CZA}, 4 x MIC_{TGC} and 4 x MIC_{CZA} – 4 x MIC_{TGC} data as the sparse data prevented accurate estimation of parameters for concentration-dependent effect models.

The resulting differential equations for growth kinetics of susceptible bacteria B_S and less susceptible (here termed “resistant” for clarity) bacteria B_R without drug exposure are

$$\frac{dB_S}{dt} = k_g \times \left(1 - \frac{B_S + B_R}{B_{MAX}}\right) \times B_S, \quad (\text{Eq. S12})$$

$$\frac{dB_R}{dt} = k_g \times \left(1 - \frac{B_S + B_R}{B_{MAX}}\right) \times B_R, \quad (\text{Eq. S13})$$

$$B = B_S + B_R, \quad (\text{Eq. S14})$$

with k_g growth rate constants and B_{MAX} representing maximum bacterial capacity.

PD interactions were evaluated as follows: Similarly, as in the GPDI model, a perpetrator drug was modelled to shift the PD parameter (here: killing rate constant) of the victim drug.

Monodirectional PD interactions on the killing rate constant of ceftazidime/avibactam k_{KILL}^{CZA} in presence of tigecycline was quantified using the interaction parameter INT :

$$k_{KILL}^{CZA} \times (1 + INT) \quad (\text{Eq. S15})$$

Monodirectional PD interactions on the killing rate constant of tigecycline k_{KILL}^{TGC} in presence of ceftazidime/avibactam was calculated quantified using the interaction parameter INT :

$$k_{KILL}^{TGC} \times (1 + INT) \quad (\text{Eq. S16})$$

PD interactions were tested exclusively on the (R) subpopulation, as it is responsible for driving regrowth behaviour at later time points.

Single drug effects were quantified as described above while combined effects were described using Bliss Independence as null-interaction model with E_A and E_B representing the effect of drug A and B following Eq. S8 - S10. Therefore, the resulting differential equations for the subpopulations of susceptible bacteria B_S and resistant bacteria B_R under drug exposure are

$$\frac{dB_S}{dt} = k_g \times \left(1 - \frac{B_S + B_R}{B_{MAX}}\right) \times B_S - E_{comb,S} \times B_S, \quad (\text{Eq. S17})$$

$$\frac{dB_R}{dt} = k_g \times \left(1 - \frac{B_S + B_R}{B_{MAX}}\right) \times B_R - E_{comb,R} \times B_R. \quad (\text{Eq. S18})$$

An interaction was statistically significant if the confidence intervals of INT did not overlap with zero ($p \leq 0.05$). Model selection of competing models (i. e. PD interaction directionality TGC $\rightarrow$

CZA, CZA $\rightarrow$ TGC, or bidirectional) was performed after inspecting biological plausibility of parameters, parameter uncertainty and the Akaike-Information-Criterion for identifying the one representing the observed data best ¹⁰⁸.

Text S5: Results of PD interaction modelling of time-kill assay data

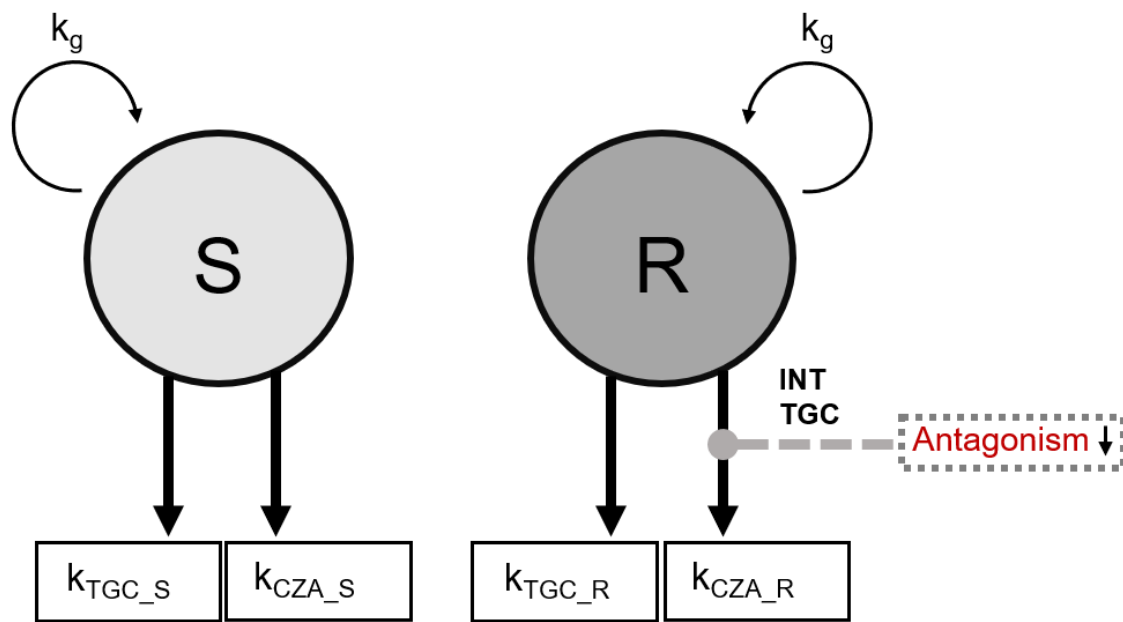


Figure S10. Schematic representation of pharmacodynamic models for time-kill assays in four tested strains. (S): compartment addressing subpopulation of susceptible bacteria; (R): compartment addressing subpopulation of resistant bacteria; k_g : growth rate constant; k_{TGC_S} : effect of tigecycline on susceptible bacteria; k_{TGC_R} : effect of tigecycline on resistant bacteria; k_{CZA_S} : effect of ceftazidime/avibactam on susceptible bacteria; k_{CZA_R} : effect of ceftazidime/avibactam on resistant bacteria; INT TGC: antagonistic PD interaction with perpetrator tigecycline negatively affecting ceftazidime/avibactam killing parameter $\downarrow$.

A schematic representation of all PD models with implemented antagonistic interaction is given in **Fig. S1**. AIC values of competing models with different directionalities of PD interactions are presented in **Table S3**. Antagonism with perpetrator tigecycline shifting the killing rate constant of ceftazidime/avibactam (k_{KILL}^{CZA}) on the (R) population outperformed the opposite directionality model in all four strains. Implementing the PD interaction as one interaction parameter, applied as a bidirectional relationship, did not further improve model fit to the observed data.

Table S3 Comparison of Akaike Information Criterion (AIC) values pharmacodynamic models with different directionalities.

Strain	Model comparison (AIC)	
	TGC → CZA	CZA → TGC
<i>E. coli</i> JUM_JEA	248.58	381.10
<i>E. coli</i> WT	122.55	192.88
<i>K. pneumoniae</i> 2977	136.37	181.44
<i>K. pneumoniae</i> WT	115.66	131.09

Stratified visual predictive checks of final PD models are visualized in **Fig. S2**. The models accurately describe single and combined effects of ceftazidime/avibactam and tigecycline over the 30-h period across all strains. Comparison of $4 \times \text{MIC}_{\text{TGC}}$ and $4 \times \text{MIC}_{\text{CZA}} - 4 \times \text{MIC}_{\text{TGC}}$ reveals similar effect sizes in all four strains for tigecycline alone and the combination of both antibiotics. Although the killing rate constants for the less susceptible subpopulation S were

likely not accurately estimated due to identifiability challenges arising from sparse data, the early-phase killing dynamics were well captured by all models.

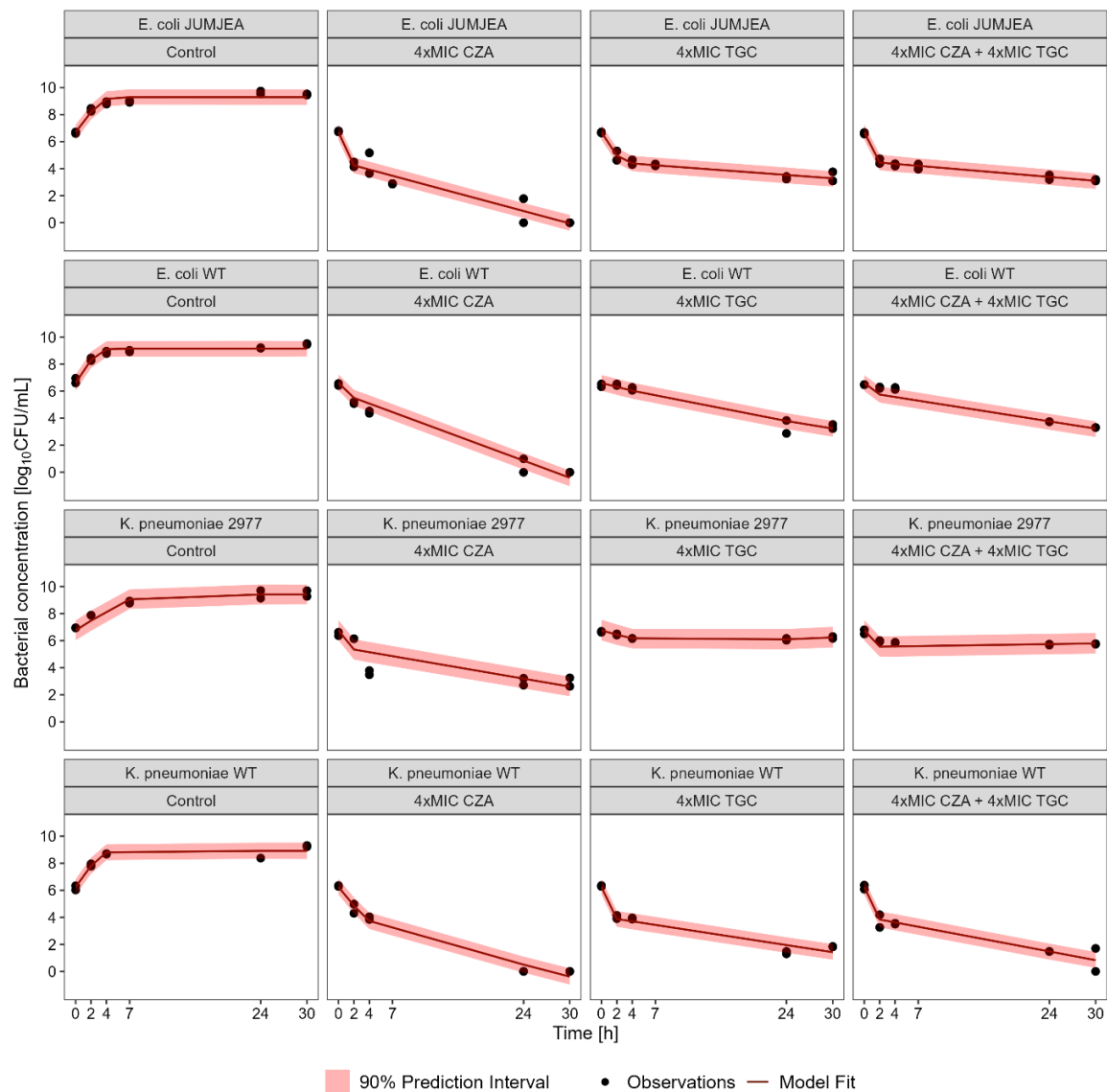


Figure S11 Stratified visual predictive checks of final pharmacodynamic models characterizing the bacterial concentration in $\log_{10}$ cfu/mL for ceftazidime/avibactam (CZA) and tigecycline (TGC) against two *E. coli* and two *K. pneumoniae* isolates. Avibactam fixed to 4 mg/L.

Table S4 shows the final parameter estimates of all models. Estimated shifts of k_{KILL}^{CZA} on (R) range from - 67.1% (- 85.3 - - 28.9%) in *K. pneumoniae* WT to a complete reduction of - 99.99% (- 99.999 - - 99.9%) in *E. coli* WT.

Table S4 Parameter estimates for final pharmacodynamic models of ceftazidime/avibactam (CZA) and tigecycline (TGC) against four tested *K. pneumoniae*. Confidence intervals (95% CI) were calculated using the Sampling Importance Resampling (SIR) method. (S): bacteria of susceptible compartment; (R): bacteria of resistant compartment.

Parameter	Final estimate (95% CI)			
	<i>E. coli</i> JUMJEA	<i>E. coli</i> WT	<i>K. pneumoniae</i> 2977	<i>K. pneumoniae</i> WT
Bacterial system				
Growth rate constant susceptible bacteria (h^{-1})	1.76 (1.39-2.08)	2.05 (1.50-2.46)	0.83 (0.51-1.19)	1.85 (1.25-2.41)
Inoculum susceptible bacteria (S) ($\log_{10}$ cfu/mL)	6.69 (6.53-6.83)	6.50 (6.20-6.73)	6.74 (6.43-7.04)	6.24 (5.99-6.46)
Inoculum resistant bacteria (R) ($\log_{10}$ cfu/mL)	4.54 (4.42-4.69)	5.92 (5.62-6.16)	5.55 (5.22-5.89)	4.05 (3.78-4.31)
Maximum bacterial capacity ($\log_{10}$ cfu/mL)	9.29 (9.10-9.49)	9.15 (8.88-9.36)	9.43 (8.92-9.90)	8.91 (8.56-9.29)
Mono drug effect parameter				
Killing rate constant of CZA on (S) (h^{-1})	134 (88-182)	270 (130-387)	140 (64.8-214)	3.48 (2.63-4.37)
Killing rate constant of TGC on (S) (h^{-1})	3.91 (3.22-4.61)	2.42 (1.87-2.86)	1.23 (0.76-1.80)	181 (41.2-339)
Killing rate constant of CZA on (R) (h^{-1})	2.11 (1.70-2.47)	2.53 (1.98-2.97)	1.06 (0.71-1.41)	2.19 (1.57-2.79)

	1.85	2.26	0.78	2.04
Killing rate constant of TGC on (R) (h^{-1})	(1.47-2.20)	(1.72-2.65)	(0.44-1.13)	(1.43-2.64)
GPDI interaction-model parameters				
Interaction-type	Antagonism	Antagonism	Antagonism	Antagonism
Interaction TGC affecting killing rate constant of	-94.4	-99.9	-87.6	-67.1
CZA on (R) (%)	(-99.1 - -70.0)	(-99.999 - -99.99)	(-96.9 - -55.1)	(-85.3 - -28.9)
Additive residual variability PD ($\log_{10}$ cfu/mL)	0.35	0.36	0.46	0.36
	(0.30-0.39)	(0.29-0.43)	(0.36-0.56)	(0.28-0.44)

Text S6: Metabolomics: Sample preparation of cell pellet samples and data processing

The methodology for sampling and preparation was adapted from the protocol described by Han *et al.*¹⁶⁴. For cell pellet analysis samples, tubes were quenched in an ethanol/dry ice bath for 30 s, centrifuged at 3,220 x *g* for 10 min to obtain cell pellets. After discarding the supernatant, cell pellets were resuspended twice in 0.5 mL of 4 °C saline, centrifuged at 2,576 x *g* for 3 min, and stored at -80 °C until analysis. For liquid chromatography-mass spectrometry (LC-MS/MS) analysis cell pellets were resuspended in 300 μL of a cold chloroform/methanol/water extraction solvent (1:3:0.9 v/v/v) augmented with isotope labelled amino acids (MSK-A2-1.2; Cambridge Isotope Laboratories, MA, USA) as internal standards at a concentration of 0.5% in the final sample for untargeted metabolomics. To permeabilize the cells and facilitate the release of intracellular metabolites, the resuspended mixtures were subjected to a freeze-thaw cycle. This process involved freezing the mixture in liquid nitrogen followed by thawing on ice, which was repeated three times. Subsequently, the mixture was maintained on ice water for 15 min and vigorously resuspended using a vortex mixer. The obtained samples as well as the extracellular supernatant samples were then centrifuged with 300 μL of each sample at 14,000 x *g* and 4 °C for 10 min. A total of 50 μL of the particle-free supernatant were then extracted via addition of

200 μ L methanol (including internal standard). Samples were thoroughly vortexed and incubated for 20 min at -20 °C. After centrifugation for 10 min at 15,000 $\times$ g and 4 °C with a 5415R microcentrifuge (Eppendorf, Hamburg, Germany), supernatants were transferred and dried under a stream of nitrogen (Organomation Microvap, MA, USA). Dried samples were reconstituted in 50 μ L of 80% methanol (v:v), vortexed, and centrifuged (see conditions described above). Ultimately, samples were transferred to silanized glass vials and directly injected into the analytical system.

LC-MS/MS analysis was performed on a Vanquish UHPLC system coupled to an Orbitrap Exploris 240 high-resolution mass spectrometer (Thermo Fisher Scientific, MA, USA) in negative and positive ESI (electrospray ionization) mode. Chromatographic separation was carried out on an Atlantis Premier BEH Z-HILIC column (Waters, MA, USA; 2.1 mm $\times$ 100 mm, 1.7 μ m) at a flow rate of 0.25 mL/min. The mobile phase consisted of water:acetonitrile (9:1, v/v; mobile phase A) and acetonitrile:water (9:1, v/v; mobile phase B), which were modified with a total buffer concentration of 10 mM ammonium acetate, when analysing in negative mode, and 10 mM ammonium formate, when analysing in positive mode. The aqueous portion of each mobile phase was pH-adjusted (negative mode: pH 9.0 via addition of ammonium hydroxide; positive mode: pH 3.0 via addition of formic acid). The following gradient (20 min total run time including re-equilibration) was applied (time [min]/%B): 0/95, 2/95, 14.5/60, 16/60, 16.5/95, 20/95. Column temperature was maintained at 40 °C, the autosampler was set to 4 °C and sample injection volume was 5 μ L. Analytes were recorded via a full scan with a mass resolving power of 120,000 over a mass range from 60 – 900 m/z (scan time: 100 ms, RF lens: 70%). To obtain MS/MS fragment spectra, data-dependent acquisition was carried out (resolving power: 15,000; scan time: 22 ms; stepped collision energies [%]: 30/50/70; cycle time: 900 ms). Ion source parameters were set to the following values: spray voltage: 4100 V (positive mode) / -3500 V (negative

mode), sheath gas: 30 psi, auxiliary gas: 5 psi, sweep gas: 0 psi, ion transfer tube temperature: 350 °C, vaporizer temperature: 300 °C.

All experimental samples were measured in a randomized manner, with cell media extracts and cell extracts being analysed in individual sequences. Pooled quality control (QC) samples were prepared by mixing equal aliquots from each processed sample type (individual QC sample for cell media extracts and cell extracts). Multiple QCs were injected at the beginning of the analysis in order to equilibrate the analytical system. A QC sample was analysed after every 5th experimental sample to monitor instrument performance throughout the sequence. For determination of background signals and subsequent background subtraction, an additional processed blank sample was recorded. Data was processed using MS-DIAL 4.9¹⁶⁵ and raw peak intensity data was exported and normalized via total ion count for relative metabolite quantification¹⁶⁶. Level 1 feature identification was based on an in-house library for metabolomics (EMBL-MCF 2.0, Heidelberg, Germany) using accurate mass, isotope pattern, MS/MS fragmentation, and retention time information with a minimum matching score of 80%. Total Ion Current (TIC)-normalized data from positive and negative ion modes were merged, and duplicate metabolite findings were removed by retaining the metabolite with the higher absolute signal intensity.

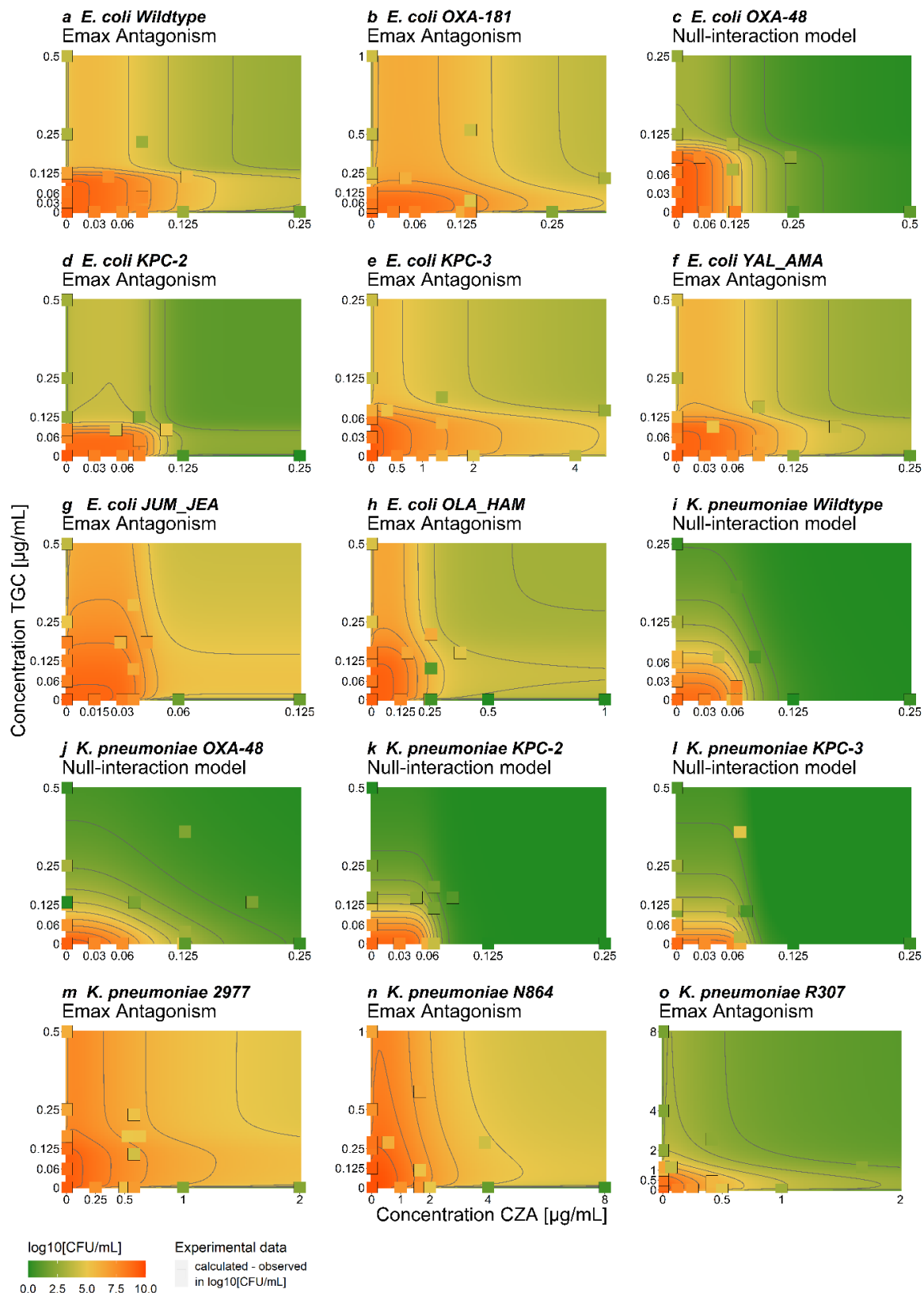


Figure S3. Heatmaps with response surface areas for the drug-drug interaction of ceftazidime/avibactam and tigecycline calculated by the GPDI model. Combined effects are represented by the color gradient indicating no effect (red) to total eradication of bacteria (green). Each line represents same effects in changing concentrations of both antibiotics. CZA, ceftazidime/avibactam; TGC, tigecycline

References

- [1] The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, version 14.0 2024.
- [2] Akaike H. A New Look at the Statistical Model Identification. *IEEE Transactions on Automatic Control* 1974;19:716–23. <https://doi.org/10.1109/TAC.1974.1100705>.
- [3] Wicha SG, Chen C, Clewe O, Simonsson USH. A general pharmacodynamic interaction model identifies perpetrators and victims in drug interactions. *Nature Communications* 2017;8:1–11. <https://doi.org/10.1038/s41467-017-01929-y>.
- [4] Han ML, Zhu Y, Creek DJ, Lin YW, Anderson D, Shen HH, et al. Alterations of Metabolic and Lipid Profiles in Polymyxin-Resistant *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy* 2018;62:1–14. <https://doi.org/10.1128/AAC.02656-17>.
- [5] Tsugawa H, Cajka T, Kind T, Ma Y, Higgins B, Ikeda K, et al. MS-DIAL: data-independent MS/MS deconvolution for comprehensive metabolome analysis. *Nat Methods* 2015;12:523–6. <https://doi.org/10.1038/nmeth.3393>.
- [6] Drotleff B, Lämmerhofer M. Guidelines for Selection of Internal Standard-Based Normalization Strategies in Untargeted Lipidomic Profiling by LC-HR-MS/MS. *Anal Chem* 2019;91:9836–43. <https://doi.org/10.1021/acs.analchem.9b01505>.

9.4 Supplementary data Publication IV

Clinical Microbiology and Infection Electronic Supplementary Material

Meropenem and fosfomycin against *K. pneumoniae*: Towards a combination breakpoint using a pharmacometric approach

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Text S1: Methods: Bacterial strains, experimental setup and data preparation

Origin of bacterial strains

Bacterial strains were analyzed at Medical and Molecular Microbiology, University of Fribourg, Switzerland or the BCARE, UK. Detailed information regarding their geographic origin and isolation is given in **Tab. S1**.

Table S2 Characteristics of *Klebsiella pneumoniae* strains, their geographic origins, and isolation sources.

Strain	Origin	Isolation	Experiment <i>in vitro</i> system
<i>K. pneumoniae</i> 2977	Liebefeld, Switzerland	Urine	Hollow Fibre
<i>K. pneumoniae</i> N2859	Bellinzona, Switzerland	-	Hollow Fibre
<i>K. pneumoniae</i> N864	Lausanne, Switzerland	Rectal swab	Hollow Fibre
<i>K. pneumoniae</i> R307	Brazil	Blood culture	Hollow Fibre
<i>K. pneumoniae</i> C1.184	Kuwait	Blood culture	Open flow
<i>K. pneumoniae</i> C1.151	France	Blood culture	Open flow
<i>K. pneumoniae</i> C1.121	United Kingdom	Blood culture	Open flow
<i>K. pneumoniae</i> C1.182	Kuwait	Blood culture	Open flow
<i>K. pneumoniae</i> C1.173	France	Blood culture	Open flow
<i>K. pneumoniae</i> C1.164	Italy	Blood culture	Open flow
<i>K. pneumoniae</i> 113321	Kuwait	Blood culture	Open flow
<i>K. pneumoniae</i> KLEB3	United Kingdom	Blood culture	Open flow

Antimicrobial susceptibility testing

The minimal inhibitory concentration (MIC) of meropenem and fosfomycin was determined as triplicates using the broth microdilution and agar dilution method, respectively, according to the EUCAST recommendation (1).

Experimental setup: Open flow system

A one compartment open flow dilutional *in vitro* model (**Fig. S1**) was used as described previously (2). A FerMac 310 fermentation system (Electrolab, Tewkesbury, UK) *in vitro* pharmacokinetic (PK) model, as previously described was used to simulate fAUC/MIC 0 to 500 of free (unbound) fosfomycin for each strain. Fosfomycin alone was administered every 8 h over 48 h with a simulated half-life of 2.5 h. Subsequently, exposure-ranging experiments with fosfomycin were performed in the presence of meropenem simulated at free drug concentrations associated with a range of meropenem exposures based on the strains' meropenem MICs. For *K. pneumoniae* strains with meropenem MICs of <32 mg/L, meropenem was simulated to produce half of the

24 h $ft > MIC$ value associated with a bacteriostatic effect. For strains with $MIC \geq 32$ mg/L a dose of 2 g 8-hourly meropenem was simulated to produce a C_{max} of 100 mg/L. The half-life of meropenem was 1 h. Owing to the differences in half-life between fosfomycin (2.5 h) and meropenem (1.0 h), the model was supplemented with fosfomycin for combination experiments throughout each dosing period via a separate dosing chamber to achieve the required concentration–time profile. The temperature was maintained at 36.5 °C and the broth was agitated by a rotary stirrer. Freshly prepared cation-adjusted Mueller-Hinton-broth II (ca-MHB, 100%, ThermoFisher, Basingstoke, UK), supplemented with glucose-6-phosphate (G6P) (25 mg/L) was used in all experiments. Nutrient agar plates (ThermoFisher, Basingstoke, UK), again supplemented with G6P (25 mg/L), were used to recover bacterial strains from the model. Aliquots of 1 mL each were taken from the central compartment in dose-ranging studies at 0, 1, 2, 3, 4, 5, 6, 7, 12, 24 and 48 h; 100 μ L was subsequently plated on nutrient agar plates using a spiral plater (Don Whitley Scientific) for determination of total bacterial counts.

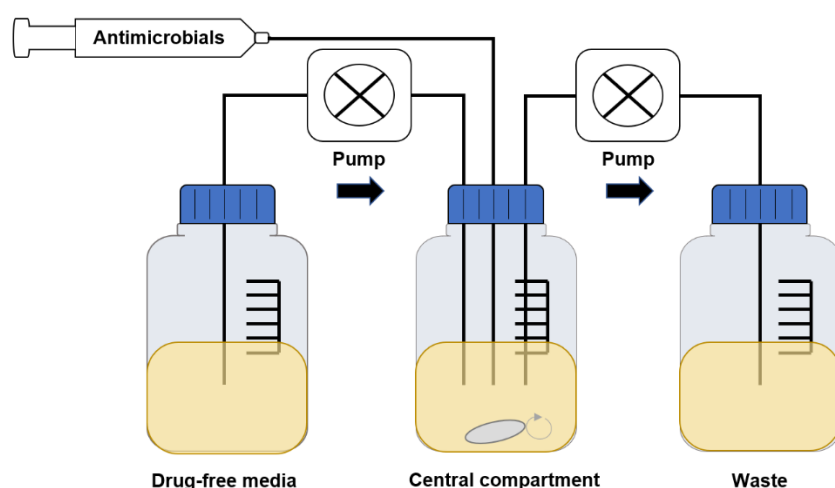


Figure S1 Schematic setup of an Open Flow Infection Model for the *in vitro* testing of single antibiotics or combinations of antibiotics illustrating the key components, including central compartment, broth and waste reservoirs, peristaltic pumps transporting broth and syringe pump for drug infusions. Constant influx of fresh and drug-free broth enables appropriate growth conditions for bacteria over a period of two days and the manual infusion allows for mimicking human concentration-time profiles of antibiotics.

Experimental setup: Hollow Fibre Infection Model (HFIM)

The HFIM setup (**Fig. S2**) consisted of a central compartment connected to a cellulosic hollow-fibre cartridge (catalogue no. C8008, FiberCell® Systems Inc., USA) via tubing, pre-programmed peristaltic pumps (ISMATEC®, ISM 4412 Reglo ICC Pump; Masterflex® Drive MFLEX 115/230; Darmstadt, Germany) and syringe pumps (Cole-Parmer Syringe Pump SKU 7490554), as described previously (3). The required infusion volumes, concentrations and pump rates to achieve the target concentration-time profiles were calculated using R. HFIM experiments were

performed by inoculating and pre-incubating the bacterial isolate (approx. 10^6 cfu/mL) in freshly prepared ca-MHB II (NutriSelect® Plus, Sigma-Aldrich, USA) for 2 h at 37 °C ambient air. Following pre-incubation, meropenem, mimicking 2 g q8h, and/or fosfomycin, mimicking 8 g q8h, were administered over 72 h. Killing kinetics were observed after collecting samples at 0, 0.75, 2, 4, 6, 8, 8.75, 24, 24.75, 28, 32, 48, 52, 56 and 72 h from the extra-capillary space of the cartridge and determination of the bacterial count.

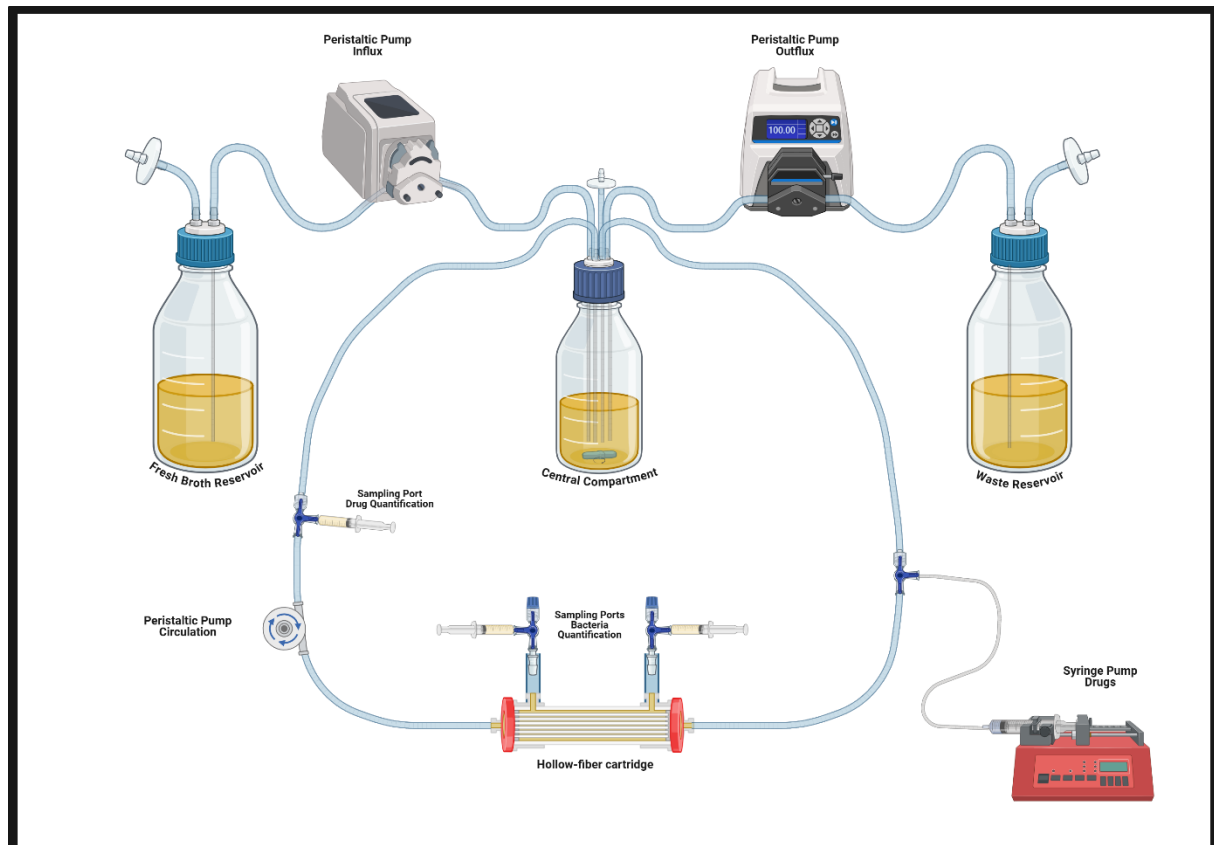


Figure S12 Schematic setup of a Hollow Fibre Infection Model for the *in vitro* testing of single antibiotics or combinations of antibiotics illustrating the key components, including central compartment, broth and waste reservoirs, peristaltic pumps transporting broth, syringe pump for drug infusions, sampling ports, and the hollow fibre cartridge containing bacteria entrapped in the extra capillary space. Constant influx of fresh and drug-free broth enables optimal growth conditions for bacteria over a period of multiple days and the programmable syringe pump allows for mimicking human concentration-time profiles of antibiotics. Created in BioRender. Farooq, A. (2025) <https://BioRender.com/e28m303>.

Table S2 Overview table summarising exposures of experimentally tested strains in both *in vitro* systems.

Strain	IVM	SCENARIO	MIC	MIC	C _{min}	C _{max}	C _{min}	C _{max}	t _{1/2}	t _{1/2} FOF [h]
			MEM [mg/L]	FOF [mg/L]	MEM [mg/L]	MEM [mg/L]	FOF [mg/L]	FOF [mg/L]	MEM [h]	
KP 2977	Hollow-Fibre	Mono MEM	32	32	0.951	41.19	-	-	3.6	3.6
KP 2977	Hollow-Fibre	Mono FOF	32	32	-	-	78.74	312.2	3.6	3.6
KP 2977	Hollow-Fibre	Combo MEM-FOF	32	32	6.211	24.63	78.74	312.2	3.6	3.6
KP N2859	Hollow-Fibre	Mono MEM	64	4	1.393	41.89	-	-	3.6	3.6
KP N2859	Hollow-Fibre	Mono FOF	64	4	-	-	78.74	312.2	3.6	3.6
KP N2859	Hollow-Fibre	Combo MEM-FOF	64	4	6.802	51.25	78.74	312.2	3.6	3.6
KP N864	Hollow-Fibre	Mono MEM	32	16	0	36.08	-	-	3.6	3.6
KP N864	Hollow-Fibre	Mono FOF	32	16	-	-	78.74	312.2	3.6	3.6
KP N864	Hollow-Fibre	Combo MEM-FOF	32	16	7.239	19.945	78.74	312.2	3.6	3.6
KP R307	Hollow-Fibre	Mono MEM	128	64	3.219	43.62	-	-	3.6	3.6
KP R307	Hollow-Fibre	Mono FOF	128	64	-	-	78.74	312.2	3.6	3.6
KP R307	Hollow-Fibre	Combo MEM-FOF	128	64	1.586	35.30	78.74	312.2	3.6	3.6
KP C1.184	Open Flow	Mono MEM	32	32	0.226	2056	-	-	1	2.5
KP C1.184	Open Flow	Mono FOF	32	32	-	-	201.5	1848	1	2.5
KP C1.184	Open Flow	Combo MEM-FOF	32	32	0.402	102.8	201.5	1248	1	2.5
KP C1.151	Open Flow	Mono MEM	64	16	0.452	1850	-	-	1	2.5
KP C1.151	Open Flow	Mono FOF	64	16	-	-	100.8	923.9	1	2.5
KP C1.151	Open Flow	Combo MEM-FOF	64	16	0.603	154.2	100.8	624.0	1	2.5
KP C1.121	Open Flow	Mono MEM	0.06	256	0.000	16.06	-	-	1	2.5
KP C1.121	Open Flow	Mono FOF	0.06	256	-	-	968.2	8877	1	2.5
KP C1.121	Open Flow	Combo MEM-FOF	0.06	256	0.000	0.096	242.0	1499	1	2.5
KP C1.182	Open Flow	Mono FOF	>1024	8	-	-	50.38	462.0	1	2.5
KP C1.182	Open Flow	Combo MEM-FOF	>1024	8	0.251	64.25	50.38	312.0	1	2.5
KP C1.173	Open Flow	Mono FOF	>1024	16	-	-	100.8	923.9	1	2.5

KP C1.173	Open Flow	Combo MEM-FOF	>1024	16	0.251	64.25	100.8	624.0	1	2.5
KP C1.164	Open Flow	Mono MEM	1	1024	0.004	124.5	-	-	1	2.5
KP C1.164	Open Flow	Mono FOF	1	1024	-	-	387.0	3549	1	2.5
KP C1.164	Open Flow	Combo MEM-FOF	1	1024	0.022	5.622	129.0	798.9	1	2.5
KP 113321	Open Flow	Combo MEM-FOF	256	512	0.251	64.25	129.0	798.7	1	2.5
KP KLEB3	Open Flow	Combo MEM-FOF	128	256	0.804	205.6	242.0	1499	1	2.5

MEM, meropenem; FOF, fosfomycin ; IVM, *in vitro* model

Experimental data preparation

Mono and combination experimental data of 4 clinical *K. pneumoniae* isolates from the HFIM and 8 clinical *K. pneumoniae* isolates from the open flow system were collected and transformed into a data format readable for NONMEM® 7.5 using R (4.2.1).

Text S2: Methods: Pharmacometric modelling and simulation studies

Pharmacometric modelling – Population PK models

Previously published population PK models for meropenem and fosfomycin were recoded in R (4.2.1) using mrgsolve and coupled to the PD model developed from the *in vitro* experiments described. For meropenem, the population PK model of Li *et al.* was applied (4), while for fosfomycin three different population PK models (Parker *et al.*, Merino-Bohórquez *et al.*, and Pfausler *et al.*) were applied (5–7), and later evaluated during simulation studies. **Fig. S3** exemplifies resulting population PK profiles for n = 1000 Monte Carlo simulations of MEM 2 g q8h when applying the Li *et al.* model, and FOF 8 g q8h when applying the Parker *et al.* model.

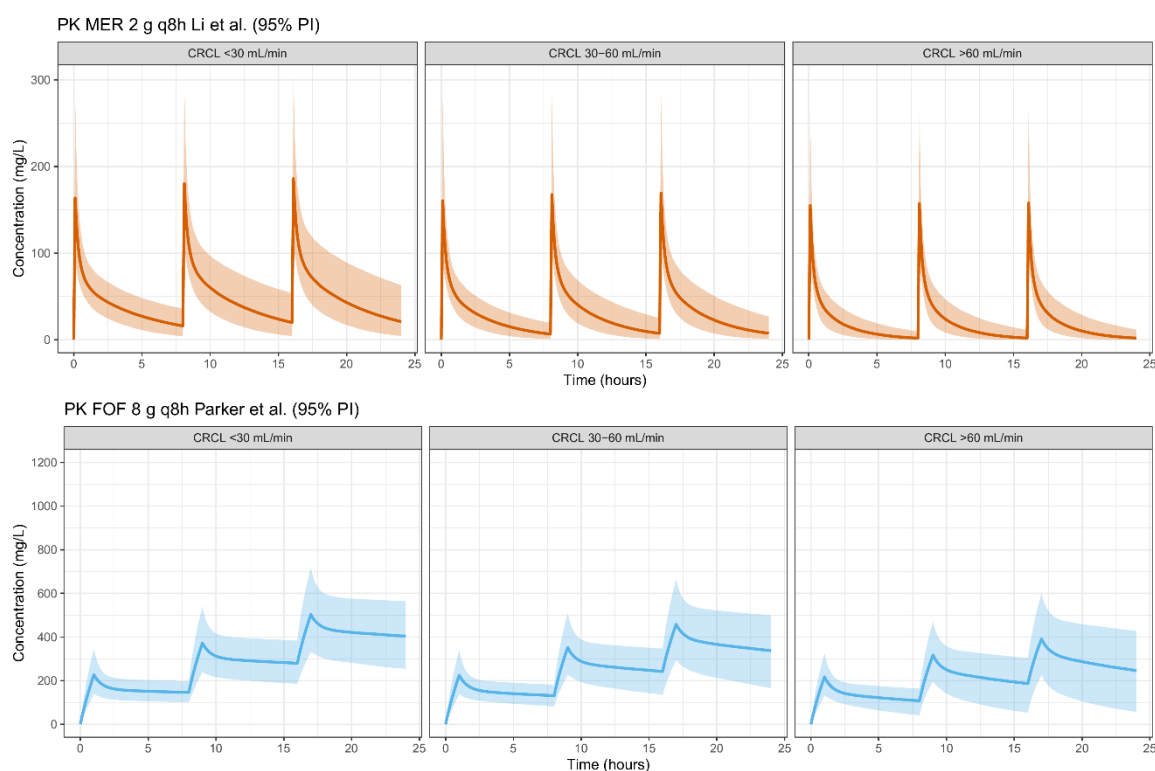


Figure S3 Simulated population pharmacokinetic profiles of meropenem and fosfomycin for three different Creatinine Clearance (CRCL) categories. Solid lines, median predictions; shaded areas, 95% prediction intervals.

Pharmacometric modelling – Pharmacodynamic model

Quantitative analyses were performed using first-order-conditional-estimation with interaction (FOCE-I) for maximum-likelihood estimations and executed using NONMEM® 7.5 (Icon Development Solutions, USA) and PsN 5.3.1 (Uppsala University, Uppsala, Sweden).

The concentration–time profiles used for pharmacodynamic modelling were reconstructed directly from the experimentally implemented dosing schedules and system-specific elimination rates, which were designed to mimic human PK and were validated in each study through measured antibiotic concentrations.

The pharmacometric model consisted of two bacterial compartments representing subpopulations with higher and lower susceptibility (S and R in the model), respectively, accounting for the biphasic killing patterns in the experimental data.

In vitro model specific (HFIM or open flow) parameter sets were used wherever biologically plausible and respective meropenem and fosfomycin MIC values served as covariates on effect- and interaction-EC50s. Most of the observed differences between strains were already captured through the strain-specific MIC covariates implemented on all EC50 parameters, which provided a mechanistic and well-supported explanation for variability in drug response. Including additional random-effect terms, such as inter-strain variability on growth kinetics, led to imprecise estimates and excessively wide prediction intervals, indicating overfitting given the limited number of strains per system. To account for robust and experimentally verified differences between the two dynamic *in vitro* systems, system-specific inocula and bacterial capacities were implemented instead. HFIM and Open Flow experiments differ systematically in starting bacterial density, achievable maximum bacterial load, and dilution characteristics, and incorporating these system-specific parameters improved the description of bacterial growth and regrowth across experiments.

The resulting differential equations for growth kinetics of susceptible bacteria B_S and less susceptible bacteria B_R without drug exposure are

$$\frac{dB_S}{dt} = k_{g,S} \times \left(1 - \frac{B_S + B_R}{B_{MAX}}\right) \times B_S, \quad (\text{Eq. S1})$$

$$\frac{dB_R}{dt} = k_{g,R} \times \left(1 - \frac{B_S + B_R}{B_{MAX}}\right) \times B_R, \quad (\text{Eq. S2})$$

$$B = B_S + B_R, \quad (\text{Eq. S3})$$

with k_g growth rate constants and B_{MAX} representing maximum bacterial capacity.

Antimicrobial effects of meropenem and fosfomycin were described using the sigmoidal maximum effect (Emax) model.

$$E(c) = \frac{E_{max} \times c^H}{EC_{50}^H + c^H} \quad (\text{Eq. S4})$$

Combined effects were quantified using the General Pharmacodynamic Interaction (GPDI) model (8,9). The GPDI model which quantifies a potential PD interaction as shifts of the potency (EC50) or efficacy (Emax). The GPDI model was implemented using Bliss Independence as null-interaction model with E_A and E_B representing the effect of drug A and B:

$$E_{comb} = E_A + E_B - E_A \times E_B, \quad (\text{Eq. S5})$$

The GPDI model is given by Eq. S6 - S13 for two drugs as interaction partners with an interaction on the PD parameters Emax or EC50. The GPDI term is a function of the perpetrator drug concentration, which affects a PD parameter θ (either EC50 or Emax) of the victim drug with INT as maximum possible fractional shift of the PD parameter θ , $EC_{50_{INT}}$ as potency of the perpetrator and H_{INT} representing the sigmoidicity of the interaction (Eq. S6). The fractional shift of the victim's PD parameter caused by the perpetrator drug allowed the directional quantification of the PD interaction, i.e., either synergy, antagonism or additivity. Whenever $-1 < INT < 0$ applies to Emax based PD interactions, antagonism is described, and whenever $-1 < INT < 0$ applies to EC50 based PD interactions, synergy is described (vice versa for $INT > 0$). In the case of bidirectional PD interactions, a drug could be perpetrator and victim at the same time. Whenever synergism or antagonism were not significant (confidence interval of INT not including 0), the interaction was described as null-interaction model by Bliss Independence with E_A and E_B representing the effect of drug A and B (Eq. 10). Since Bliss Independence was derived from a probabilistic approach, the Bliss Independence term was calculated using the single drug effects, which were normalized to the maximum drug effect possible with a single drug, and afterwards scaled back to the effect scale (Eq. S11 - S13). After comparison of all possible interactions between both drugs and on each of the effect parameters, the interaction terms that produced the lowest AIC were selected for the final model.

$$\theta \times \left(1 + \frac{\text{INT} \times C^{\text{H}_{\text{INT}}}}{\text{EC50}_{\text{INT}}^{\text{H}_{\text{INT}}} + C^{\text{H}_{\text{INT}}}} \right) \quad (\text{Eq. S6})$$

$$E_A = \frac{\text{Emax}_A \times \left(1 + \frac{\text{INT}_{AB} \times C_B^{\text{H}_{\text{INT},AB}}}{\text{EC50}_{\text{INT},AB}^{\text{H}_{\text{INT},AB}} + C_B^{\text{H}_{\text{INT},AB}}} \right) \times C_A^{\text{H}_A}}{\left(\text{EC50}_A^{\text{H}_A} \right)^{\text{H}_A} + C_A^{\text{H}_A}} \quad (\text{Eq. S7})$$

$$E_B = \frac{\text{Emax}_B \times \left(1 + \frac{\text{INT}_{BA} \times C_A^{\text{H}_{\text{INT},BA}}}{\text{EC50}_{\text{INT},BA}^{\text{H}_{\text{INT},BA}} + C_B^{\text{H}_{\text{INT},AB}}} \right) \times C_B^{\text{H}_B}}{\left(\text{EC50}_B^{\text{H}_B} \right)^{\text{H}_B} + C_B^{\text{H}_B}} \quad (\text{Eq. S8})$$

$$E_A = \frac{\text{Emax}_A \times C_A^{\text{H}_A}}{\left(\text{EC50}_A \times \left(1 + \frac{\text{INT}_{AB} \times C_B^{\text{H}_{\text{INT},AB}}}{\text{EC50}_{\text{INT},AB}^{\text{H}_{\text{INT},AB}} + C_B^{\text{H}_{\text{INT},AB}}} \right) \right)^{\text{H}_A} + C_A^{\text{H}_A}} \quad (\text{Eq. S9})$$

$$E_B = \frac{\text{Emax}_B \times C_B^{\text{H}_B}}{\left(\text{EC50}_B \times \left(1 + \frac{\text{INT}_{BA} \times C_B^{\text{H}_{\text{INT},BA}}}{\text{EC50}_{\text{INT},BA}^{\text{H}_{\text{INT},BA}} + C_B^{\text{H}_{\text{INT},BA}}} \right) \right)^{\text{H}_B} + C_B^{\text{H}_B}} \quad (\text{Eq. S10})$$

$$E = E_A + E_B - E_A \times E_B \quad (\text{Eq. S11})$$

$$\text{Max}_E = \max (E_{\max,A}, E_{\max,B}) \quad (\text{Eq. S12})$$

$$E_{\text{comb}} = \left(\frac{E_A}{\text{Max}_E} + \frac{E_B}{\text{Max}_E} - \frac{E_A}{\text{Max}_E} \cdot \frac{E_B}{\text{Max}_E} \right) \cdot \text{Max}_E \quad (\text{Eq. S13})$$

Therefore, the resulting differential equations for the subpopulations of susceptible bacteria B_s and resistant bacteria B_R under drug exposure are

$$\frac{dB_s}{dt} = k_{g,s} \times \left(1 - \frac{B_s + B_R}{B_{\text{MAX}}} \right) \times B_s - E_{\text{comb},s} \times B_s, \quad (\text{Eq. S14})$$

$$\frac{dB_R}{dt} = k_{g,R} \times \left(1 - \frac{B_s + B_R}{B_{\text{MAX}}} \right) \times B_R - E_{\text{comb},R} \times B_R. \quad (\text{Eq. S15})$$

Strain-specific MIC_i values were used as covariates on typical values (TV) of drug EC50s and GPDI interaction EC50s. First MICs were normalized to median MIC_{med} of meropenem or fosfomycin,

and then exponents (COV) were estimated to assess the strengths of covariate relationships for each of the EC50 of interest:

$$EC50_i = EC50_{TV} \cdot \left(\frac{MIC_i}{MIC_{med}} \right)^{COV}. \quad (\text{Eq. S15})$$

Simulations studies

Aforementioned population PK models for meropenem and fosfomycin were coupled with the developed PD model from the *in vitro* experiments described above in R (4.2.1), utilizing the mrgsolve package. To robustly characterize the expected range of clinical responses across different patient scenarios, we performed Monte Carlo simulations for each single drug and their combination. Specifically, we executed $n = 1000$ Monte Carlo simulations for each *in vitro* system, for each defined PD target (bacteriostasis, 1-log killing, and 2-log killing), and across nine distinct combination dosing regimens. To comprehensively cover the clinically relevant spectrum of bacterial susceptibility, we simulated virtual bacterial strains with MICs ranging from 0.125 to 1024 mg/L for both agents. For each virtual strain and dosing regimen, the probability of target attainment (PTA) was calculated based on the proportion of simulations achieving the predefined PD endpoint, evaluated using the predicted bacterial load 24 h after treatment initiation. The PTA for a given MIC was calculated as follows:

$$PTA_{MIC_{MEM}, MIC_{FOF}} = \frac{\text{Number of simulations attaining PD target for } MIC_{MEM}, MIC_{FOF}}{n}, \quad (\text{Eq. S16})$$

where $n = 1000$ denotes the total number of Monte Carlo replicates per scenario.

To assess the therapeutic success across a clinically relevant population, the cumulative fractional response (CFR) was determined. For this purpose, MIC distributions of 36 NDM-producing and/or OXA-48-producing *K. pneumoniae* isolates were collected from the literature, by combining data from two studies to ensure a sufficiently large and representative population for the CFR calculation (10,11). A distribution of the selected bacterial population with relative frequency is given in **Fig. S4**.

The CFR was calculated as follows:

$$CFR = \sum_{i=1}^N PTA_{MIC_{MEM}, MIC_{FOF}, i} \cdot w_i, \quad (\text{Eq. S17})$$

where N is the total number of unique strains in the population, $PTA_{MIC_{MEM}, MIC_{FOF}, i}$ is the PTA for the i -th strain at its specific meropenem and fosfomycin MICs, and w_i is the relative frequency of that MIC pair in the population. For assessing the combined effects, this approach is optimal,

as it accounts for the MIC values of both antibiotics and incorporates their joint distributions, providing a representative estimate of efficacy across real-world bacterial populations.

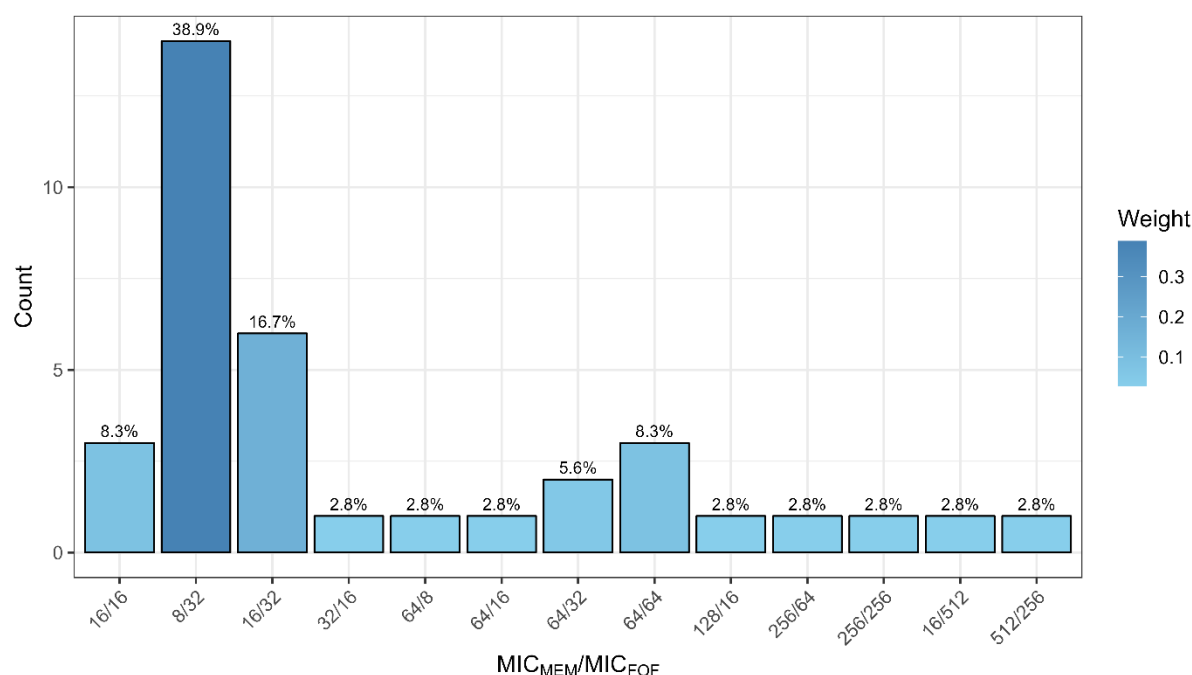


Figure S4 Distribution of *K. pneumoniae* strains by combined meropenem and fosfomycin MIC values. Color shades indicate the relative frequency of each MIC pair in the population and values above bars indicate the relative frequency in percent. MEM, meropenem; FOF, fosfomycin.

Text S3: Results: Pharmacometric model

Table S3 Parameter estimates for final pharmacokinetic/pharmacodynamic model of meropenem and fosfomycin against twelve tested *K. pneumoniae*. Confidence intervals (95% CI) were calculated from standard errors. (S): bacteria of susceptible compartment; (R): bacteria of less-susceptible compartment; MEM, meropenem, FOF, fosfomycin; COV: covariate effect exponents.

Parameter description	Final estimate (RSE%; 95% CI)
Bacterial system parameters	
Growth rate constant of S [h^{-1}]	1.19 (10.1; 0.95 – 1.43)
Growth rate constant of R [h^{-1}]	1.11 (5.2; 1.00 – 1.22)
Inoculum of S – Openflow [$\log_{10}$ cfu/mL]	5.64 (1.4; 5.49 – 5.79)
Inoculum of R – Openflow [$\log_{10}$ cfu/mL]	0.888 (14.8; 0.63 – 1.15)
Inoculum of S – HFIM [$\log_{10}$ cfu/mL]	6.78 (5.6; 6.38 – 7.18)
Inoculum of R – HFIM [$\log_{10}$ cfu/mL]	4.22 (3.0; 3.76 – 4.68)
Maximum bacterial capacity – Openflow [$\log_{10}$ cfu/mL]	8.04 (1.4; 7.82 – 8.26)
Maximum bacterial capacity – HFIM [$\log_{10}$ cfu/mL]	9.96 (2.1; 9.55 – 10.37)
Pharmacokinetic system parameters	
MEM elimination rate constant – Open Flow [h^{-1}]	0.693 (–, fixed)

MEM elimination rate constant – HFIM [h ⁻¹]	0.19 (–, fixed)
FOF elimination rate constant – Open Flow [h ⁻¹]	0.277 (–, fixed)
FOF elimination rate constant – HFIM [h ⁻¹]	0.19 (–, fixed)

Single drug effect parameters

For typical strain with $MIC_{MEM} = 64 \text{ mg/L}$ and $MIC_{FOF} = 128 \text{ mg/L}$

Meropenem

E _{max} of MEM on S [h ⁻¹]	3.5 (5.4; 3.13 – 3.87)
EC ₅₀ of MEM on S [mg/L]	194 (17.8; 126.32 – 261.68)
Hill coefficient of MEM on S [-]	20 (–, fixed)
E _{max} of MEM on R [h ⁻¹]	0.955 (6.2; 0.84 – 1.07)
EC ₅₀ of MEM on R [mg/L]	9.81 (12.2; 7.46 – 12.16)
Hill coefficient of MEM on R [-]	20 (–, fixed)

Fosfomycin

E _{max} of FOF on S [h ⁻¹]	4.01 (4.1; 3.69 – 4.33)
EC ₅₀ of FOF on S [mg/L]	267 (14.0; 193.74 – 340.26)
Hill coefficient of FOF on S [-]	1.17 (11.6; 0.90 – 1.44)
E _{max} of FOF on R – Openflow [h ⁻¹]	0.769 (6.6; 0.67 – 0.87)
E _{max} of FOF on R – HFIM [h ⁻¹]	0.982 (6.4; 0.86 – 1.11)
EC ₅₀ of FOF on R [mg/L]	354 (11.8; 272.13 – 435.87)
Hill coefficient of FOF R [-]	20 (–, fixed)

MIC covariate effects

COV Effect of MEM MIC on EC ₅₀ for R [-]	1.35 (2.8; 1.28 – 1.42)
COV Effect of MEM MIC on EC ₅₀ for R [-]	1.07 (8.6; 0.89 – 1.25)
COV Effect of FOF MIC on EC ₅₀ for S [-]	1.3 (2.8; 1.23 – 1.37)
COV Effect of FOF MIC on EC ₅₀ for R [-]	0.542 (8.7; 0.45 – 0.63)

Pharmacodynamic interaction parameters

Synergy: MEM → FOF: MEM affecting EC₅₀ of FOF on S

INT: Maximum-Interaction as EC ₅₀ reduction [%]	-99.99 (2.5; -99.999 – -95.0)
EC ₅₀ of interaction [mg/L]	0.128 (13.8; 0.09 – 0.16)
Hill coefficient of interaction [-]	2.59 (9.3; 2.12 – 3.06)

Synergy: MEM → FOF: MEM affecting EC₅₀ of FOF on R

INT: Maximum-Interaction as EC ₅₀ reduction [%]	-98.1 (3.8; -99.9 – -91.0)
EC ₅₀ of interaction [mg/L]	0.00673 (5.8; 0.006 – 0.007)
Hill coefficient of interaction [-]	20 (–, fixed)

Synergy: FOF → MEM: FOF affecting E_{max} of MEM on R

INT: Maximum-Interaction as E _{max} increase [%]	0.249 (14.4; 0.18 – 0.32)
EC ₅₀ of interaction [mg/L]	241 46.1; (23.24 – 458.76)
Hill coefficient of interaction [-]	2.74 (43.1; 0.43 – 5.05)

Residual error model

Additive residual error [log ₁₀ cfu/mL]	1.26 (1.6; 1.22 – 1.29)
--	-------------------------

Final NONMEM control stream model run216.3

```
;; 1. Based on: run216.2
```

```
;; 2. Description: Modeling of IVM/HFIM MEM/FOF in twelve KP
```

```
;; x1. Author: farooq
```

```
;; 3. Label:
```

```
$PROB Modelling of IVM MEM/FOF in twelve KP
```

```
$INPUT
```

ID	; experiment ID
TIME	; h
DV	; lnCFU/mL
EVID	; event identifier
MDV	; MDV
AMT	; amount of dose in mg
TIMEMIC_MEM	; time over Mlc in %
AUCMIC_FOF	; AUC over MIC
CMT	; 1-4
FLAG_SCENARIOS	; 1-4
MIC_MEM	; mg/L
MIC_FOF	; mg/L
FLAG_IVM	; 1 = Macgowan IVM, 2 = Wicha conventional HFIM

```
$DATA NONMEM_twelvestrains_v2.csv IGNORE=@
```

```
ACCEPT = (FLAG_SCENARIOS.EQ.1) ;growth
```

```
ACCEPT = (FLAG_SCENARIOS.EQ.2) ;MEM
```

```
ACCEPT = (FLAG_SCENARIOS.EQ.3) ;FOF
```

```
ACCEPT = (FLAG_SCENARIOS.EQ.4) ;MEM-FOF
```

```
$SUBROUTINE ADVAN13 TOL=10
```

```
$MODEL
```

```
COMP (DRUG_A)
```

```
COMP (DRUG_B)
```

```
COMP (OBS_S)
```

```
COMP (R)
```

```
$PK
```

```
;Elimination constants for MEM and FOF resulting from IVM experiments
```

```
; Remove decay of drugs in control scenarios
```

```
IF (FLAG_SCENARIOS.EQ.1.OR.FLAG_SCENARIOS.EQ.3) THEN
```

```
    KE_MEM = 0
```

```
ELSE
```

```
    KE_MEM = THETA(1) ;derived from 1 h halftime
```

```
    IF(FLAG_IVM.EQ.2) KE_MEM = THETA(35) ;half-life of 3.6 h in HFIM
```

```
ENDIF
```

```
IF (FLAG_SCENARIOS.EQ.1.OR.FLAG_SCENARIOS.EQ.2) THEN
```

```
    KE_FOF = 0
```

```
ELSE
```

```
    KE_FOF = THETA(2) ;derived from 2.5 h halftime
```

```
    IF(FLAG_IVM.EQ.2) KE_FOF = THETA(36) ;half-life of 3.6 h in HFIM
```

```
ENDIF
```

```
; account for elimination in openflow system compared to HFIM
```

```
IF ( FLAG_SCENARIOS.EQ.2.OR.FLAG_SCENARIOS.EQ.4.AND.FLAG_IVM.EQ.1) THEN ;case of MEM or COMBO
```

```
    KE_SR_OPENFLOW = THETA(37)
```

```
ELSE
```

```
    KE_SR_OPENFLOW = 0
```

```
ENDIF
```

```
IF ( FLAG_SCENARIOS.EQ.3.AND.FLAG_IVM.EQ.1) THEN ;case of FOF
```

```
    KE_SR_OPENFLOW = THETA(38)
```

```
ELSE
```

```
    KE_SR_OPENFLOW = 0
```

```
ENDIF
```

```

;KE_MEM = THETA(1) ;derived from 1 h halftime
;KE_FOF = THETA(2) ;derived from 2.5 h halftime

;Bacterial system with bacterium-specific parameters

KGS = THETA(3) ; 1/h growth constant both
populations

KGR = THETA(4)

IF (FLAG_IVM.EQ.1) THEN
  INOC_AB_S = 10**(THETA(5)+ ETA(6)) ; CFU/mL start inoculum susceptible population OPENFLOW
ELSE
  INOC_AB_S = 10**(THETA(41) + ETA(6)) ; CFU/mL start inoculum susceptible population HFIM
ENDIF

IF (FLAG_IVM.EQ.1) THEN
  INOC_AB_R = 10**(THETA(6)+ ETA(2)) ; CFU/mL start inoculum resistant population OPENFLOW
ELSE
  INOC_AB_R = 10**(THETA(40) + ETA(2)) ; CFU/mL start inoculum resistant population HFIM
ENDIF

; Define the maximum bacterial capacity in CFU/mL based on the IVM system
IF (FLAG_IVM.EQ.1) THEN
  BMAX = 10**THETA(7) ;IVM MacGowan
ELSE
  BMAX = 10**THETA(8) ;HFIM Wicha
ENDIF

;Drug-specific parameters
;Effect model drug A
EMAX_A_S = THETA(9) ; 1/h maximal effect
TVEC50_A_S = THETA(10) ; mg/L

```


H_A_S = THETA(11) ; Hill-coefficient

EC50_A_S = TVEC50_A_S * (MIC_MEM/64)**THETA(12) ;MIC_MEM as covariate

EMAX_A_R = THETA(13) ; 1/h maximal effect

TV_EC50_A_R = THETA(14) ; mg/L

H_A_R = THETA(15) ; Hill-coefficient

EC50_A_R = TV_EC50_A_R * (MIC_MEM/64)**THETA(16) *exp(ETA(1)) ;MIC_MEM as covariate

;Effect model drug B

EMAX_B_S = THETA(17) ; 1/h maximal effect

TV_EC50_B_S = THETA(18) ; mg/L

H_B_S = THETA(19) ; Hill-coefficient

EC50_B_S = TV_EC50_B_S * (MIC_FOF/128)**THETA(20) ;MIC_FOF as covariate

IF (FLAG_IVM.EQ.1) THEN

EMAX_B_R = THETA(21) *exp(ETA(4)) ; 1/h maximal effect MacGowan

ELSE

EMAX_B_R = THETA(39) *exp(ETA(4)) ; 1/h maximal effect Wicha

ENDIF

TV_EC50_B_R = THETA(22) ; mg/L

H_B_R = THETA(23) ; Hill-coefficient

EC50_B_R = TV_EC50_B_R * (MIC_FOF/128)**THETA(24) *exp(ETA(3)) ;MIC_FOF as covariate

;Combined effects

;EC50 Synergy MEM -> FOF on S

EC50_INT_BA_S = exp(THETA(25)) - 1

EC50_EC50INT_BA_S = THETA(26) * (MIC_MEM/64) ;MIC scaling on interaction EC50

EC50_H_INT_BA_S = THETA(27)

;EC50 Synergy MEM -> FOF on R

EC50_INT_BA_R = exp(THETA(28) + ETA(5)) - 1

EC50_EC50INT_BA_R = THETA(29) * (MIC_MEM/64) ;MIC scaling on interaction EC50

EC50_H_INT_BA_R = THETA(30)

```
;EMAX Synergy FOF -> MEM
```

```
EMAX_INT_AB_R = THETA(31)
```

```
EMAX_EC50INT_AB_R = THETA(32) * (MIC_FOF/128) ;MIC scaling on interaction EC50
```

```
EMAX_H_INT_AB_R = THETA(33)
```

```
A_0(1) = 0.0001 ; Initial condition of ODE MEM should be greater than zero for Control computation
```

```
A_0(2) = 0.0001 ; Initial condition of ODE FOF should be greater than zero for Control computation
```

```
A_0(3) = INOC_AB_S ; Initial condition of ODE
```

```
A_0(4) = INOC_AB_R ; Initial condition of ODE
```

```
$DES
```

```
C_A = A(1)
```

```
C_B = A(2)
```

```
B_AB_S = A(3)
```

```
B_AB_R = A(4)
```

```
CA = C_A ; concentration as covariate drug A
```

```
CB = C_B ; concentration as covariate drug B
```

```
SPOP = LOG(A(3))
```

```
RPOP = LOG(A(4))
```

```
;Slope model
```

```
;E_A_S = SLOPE_A_S * (CA**H_A_S)
```

```
;E_A_R = SLOPE_A_R * (CA**H_A_R)
```

```
;Sigmoidal Emax model
```

$$E_A_S = (EMAX_A_S * (CA^{**}H_A_S)) / ((EC50_A_S^{**}H_A_S) + (CA^{**}H_A_S))$$

$$E_A_R = (EMAX_A_R * (CA^{**}H_A_R)) / ((EC50_A_R^{**}H_A_R) + (CA^{**}H_A_R))$$

$$E_B_S = (EMAX_B_S * (CB^{**}H_B_S)) / ((EC50_B_S^{**}H_B_S) + (CB^{**}H_B_S))$$

$$E_B_R = (EMAX_B_R * (CB^{**}H_B_R)) / ((EC50_B_R^{**}H_B_R) + (CB^{**}H_B_R))$$

;Combined effects (INT EMAX on R)

;Interaction on resistant population

;A affected by B

$$GPDI_AB_R_EMAX = EMAX_A_R * (1 + ((EMAX_INT_AB_R * (CB^{**}EMAX_H_INT_AB_R)) / ((EMAX_EC50_INT_AB_R^{**}EMAX_H_INT_AB_R) + (CB^{**}EMAX_H_INT_AB_R))))$$

$$E_A_R = (GPDI_AB_R_EMAX * (CA^{**}H_A_R)) / ((EC50_A_R^{**}H_A_R) + (CA^{**}H_A_R))$$

;Combined effects (INT EC50 on R)

;Interaction on susceptible population

;B affected by A

$$GPDI_BA_S_EC50 = EC50_B_S * (1 + ((EC50_INT_BA_S * (CA^{**}EC50_H_INT_BA_S)) / ((EC50_EC50_INT_BA_S^{**}EC50_H_INT_BA_S) + (CA^{**}EC50_H_INT_BA_S))))$$

$$E_B_S = (EMAX_B_S * (CB^{**}H_B_S)) / ((GPDI_BA_S_EC50^{**}H_B_S) + (CB^{**}H_B_S))$$

;Interaction on resistant population

;B affected by A

$$GPDI_BA_R_EC50 = EC50_B_R * (1 + ((EC50_INT_BA_R * (CA^{**}EC50_H_INT_BA_R)) / ((EC50_EC50_INT_BA_R^{**}EC50_H_INT_BA_R) + (CA^{**}EC50_H_INT_BA_R))))$$

$$E_B_R = (EMAX_B_R * (CB^{**}H_B_R)) / ((GPDI_BA_R_EC50^{**}H_B_R) + (CB^{**}H_B_R))$$

;Bliss Independence

$E_AB_S_MAX = \max(EMAX_A_S, EMAX_B_S)$

$E_AB_R_MAX = \max(EMAX_A_R, EMAX_B_R, GPDI_AB_R_EMAX) ;$

$E_AB_S = ((E_A_S/E_AB_S_MAX) + (E_B_S/E_AB_S_MAX) - ((E_A_S/E_AB_S_MAX) * (E_B_S/E_AB_S_MAX))) * E_AB_S_MAX$
;Effect scaled with EMAX

$E_AB_R = ((E_A_R/E_AB_R_MAX) + (E_B_R/E_AB_R_MAX) - ((E_A_R/E_AB_R_MAX) * (E_B_R/E_AB_R_MAX))) * E_AB_R_MAX$
;Effect scaled with EMAX

;Avoiding nuMEMical errors due to very low MEM concentrations in HFIM due to KPC degradation

IF (A(1) < 1E-6) THEN

SWITCH_A = 0

ELSE

SWITCH_A = 1

ENDIF

IF (A(2) < 1E-6) THEN

SWITCH_B = 0

ELSE

SWITCH_B = 1

ENDIF

;Bacterial system under drug exposure

$DADT(1) = -KE_MEM * A(1) * SWITCH_A$

$DADT(2) = -KE_FOF * A(2) * SWITCH_B$

$DADT(3) = B_AB_S * KGS * (1 - ((B_AB_S + B_AB_R) / BMAX)) - E_AB_S * B_AB_S - KE_SR_OPENFLOW * B_AB_S$

$DADT(4) = B_AB_R * KGR * (1 - ((B_AB_S + B_AB_R) / BMAX)) - E_AB_R * B_AB_R - KE_SR_OPENFLOW * B_AB_R$

\$ERROR

$ODECFU = A(3) + A(4)$

$CFU = A(3) + A(4)$

IF (CFU.LT.1) CFU = 1 ;set CFU<1 to 1 to comply with setting LOGCFU to zero in dataset -> dont limit kill to 1 CFU

$LOG10CFU = LOG10(CFU)$

$IPRED = LOG(CFU)$; total bacterial concentration

$W1 = SQRT(THETA(34)**2)$; ADDITIVE ERROR

RESV = EPS(1)

IRES = DV-IPRED

W=W1

IWRES = IRES/W

Y= IPRED+W*RESV

\$THETA

(0.693) FIX ;1_KE_MEM

(0.277) FIX ;2_KE_FOF

(0, 1.19) ;3_KGS

(0, 1.11) ;4_KGR

(0.1, 5.64) ;5_INOC_AB_S_OPENFLOW

(0.889) ;6_INOC_AB_R_OPENFLOW

(0, 8.04) ;7_BMAX_IVM

(0, 9.96) ;8_BMAX_HFIM

(0, 3.5) ;9_EMAX_A_S

(0, 194) ;10_TV_EC50_A_S

(20) FIX ;11_H_A_S

(-1, 1.35) ;12_COV_MIC_MEM_on_EC50_MEM_R

(0, 0.955) ;13_EMAX_A_R

(0, 9.81) ;14_TV_EC50_A_R

(0, 20) FIX ;15_H_A_R

(-1, 1.07) ;16_COV_MIC_MEM_on_EC50_MEM_R

(0, 4.01) ;17_EMAX_B_S

(0, 267) ;18_TV_EC50_B_S

(0, 1.17) ;19_H_B_S

(-1, 1.3) ;20_COV_MIC_FOF_on_EC50_FOF_S

(0, 0.769) ;21_EMAX_B_R_OPENFLOW

(0, 354) ;22_TV_EC50_B_R

(0, 20) FIX ;23_H_B_R

(-1, 0.542) ;24_COV_MIC_FOF_on_EC50_FOF_R

(-20, -12.5) ;25_EC50_INT_BA_S

(0, 0.128) ;26_EC50_EC50INT_BA_S

(0, 2.59) ;27_EC50 H_INT_BA_S

(-20, -3.98) ;28_EC50_INT_BA_R
 (0, 0.00673) ;29_EC50_EC50INT_BA_R
 (0, 20) FIX ;30_EC50_H_INT_BA_R
 (0, 0.249) ;31_EMAX_INT_AB_R
 (0, 242) ;32_EMAX_EC50INT_AB_R
 (0, 2.74) ;33_EMAX_H_INT_AB_R
 (0, 2.89) ;34_Add.Err.
 (0.19) FIX ;35_KE_MEM_HFIM (half-life of 3.6 h calculated from Farooq et al JAC 2025)
 (0.19) FIX ;36_KE_FOF_HFIM (half-life of 3.6 h calculated from Farooq et al JAC 2025)
 (0) FIX ;37_KE_OPENFLOW_MEM_u_COMBO (assuming that FOF is supplemented in combo)
 (0) FIX ;38_KE_OPENFLOW_FOF
 (0, 0.982) ;39_EMAX_B_R_HFIM
 (4.22) ;40_INOC_AB_R_HFIM
 (0, 6.78) ;41_INOC_AB_S_HFIM

\$OMEGA

0 FIX ;IIV_EC50_A_R
 0 FIX ;IIV_INOC_AB_R
 0 FIX ;IIV_EC50_B_R
 0 FIX ;IIV_EMAX_B_R
 0 FIX ;EC50_INT_BA_R
 0 FIX ;IIV_INOC_AB_S

\$\$SIGMA

1 FIX

\$EST METHOD=1 INTER SLOW MAXEVAL=9999 NOABORT NSIG=2 PRINT=5

\$COV PRINT=E MATRIX=R

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\$TABLE ID INOC_AB_S EC50_A_R EC50_B_R INOC_AB_S INOC_AB_R BMAX EMAX_B_R EC50_INT_BA_R KGS KGR
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References

1. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, version 14.0 [Internet]. 2024. Available from: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_14.0_Breakpoint_Tables.pdf
2. MacGowan AP, Griffin P, Attwood MLG, Daum AM, Avison MB, Noel AR. The pharmacodynamics of fosfomycin in combination with meropenem against *Klebsiella pneumoniae* studied in an in vitro model of infection. *Journal of Antimicrobial Chemotherapy*. 2025 Apr 1;80(4):967–75.
3. Cadwell JJS. The hollow fiber infection model: Principles and practice. *Clin Microbiol* [Internet]. 2018 [cited 2023 Jan 16];07. Available from: <https://www.omicsonline.org/conference-proceedings/clinical-microbiology-2018-scientifictracks.digital>
4. Li C, Kuti JL, Nightingale CH, Nicolau DP. Population Pharmacokinetic Analysis and Dosing Regimen Optimization of Meropenem in Adult Patients. *The Journal of Clinical Pharmacology*. 2006;46(10):1171–8.
5. Parker SL, Frantzeskaki F, Wallis SC, Diakaki C, Giamarellou H, Koulenti D, et al. Population Pharmacokinetics of Fosfomycin in Critically Ill Patients. *Antimicrobial Agents and Chemotherapy*. 2015 Sep 18;59(10):6471–6.
6. Merino-Bohórquez V, Docobo-Pérez F, Sojo J, Morales I, Lupión C, Martín D, et al. Population pharmacokinetics and pharmacodynamics of fosfomycin in non-critically ill patients with bacteremic urinary infection caused by multidrug-resistant *Escherichia coli*. *Clin Microbiol Infect*. 2018 Nov;24(11):1177–83.
7. Pfausler B, Spiss H, Dittrich P, Zeitlinger M, Schmutzhard E, Joukhadar C. Concentrations of fosfomycin in the cerebrospinal fluid of neurointensive care patients with ventriculostomy-associated ventriculitis. *Journal of Antimicrobial Chemotherapy*. 2004 May;53(5):848–52.
8. Wicha SG, Chen C, Clewe O, Simonsson USH. A general pharmacodynamic interaction model identifies perpetrators and victims in drug interactions. *Nature Communications*. 2017;8(1):1–11.
9. Bliss CI. The Toxicity of Poisons Applied Jointly. *Annals of Applied Biology*. 1939;26(3):585–615.
10. Erturk Sengel B, Altinkanat Gelmez G, Soyletir G, Korten V. In vitro synergistic activity of fosfomycin in combination with meropenem, amikacin and colistin against OXA-48 and/or NDM-producing *Klebsiella pneumoniae*. *Journal of Chemotherapy*. 2020 Jul 3;32(5):237–43.
11. Della Rocca MT, Di Caprio G, Colucci F, Merola F, Panetta V, Cordua E, et al. Fosfomycin-meropenem synergistic combination against NDM carbapenemase-producing *Klebsiella pneumoniae* strains. *New Microbiol*. 2023 Sep;46(3):264–70.

Clinical Microbiology and Infection
Electronic Supplementary Material- Heatmaps

Meropenem and fosfomycin against *K. pneumoniae*: Towards a combination breakpoint using a pharmacometric approach

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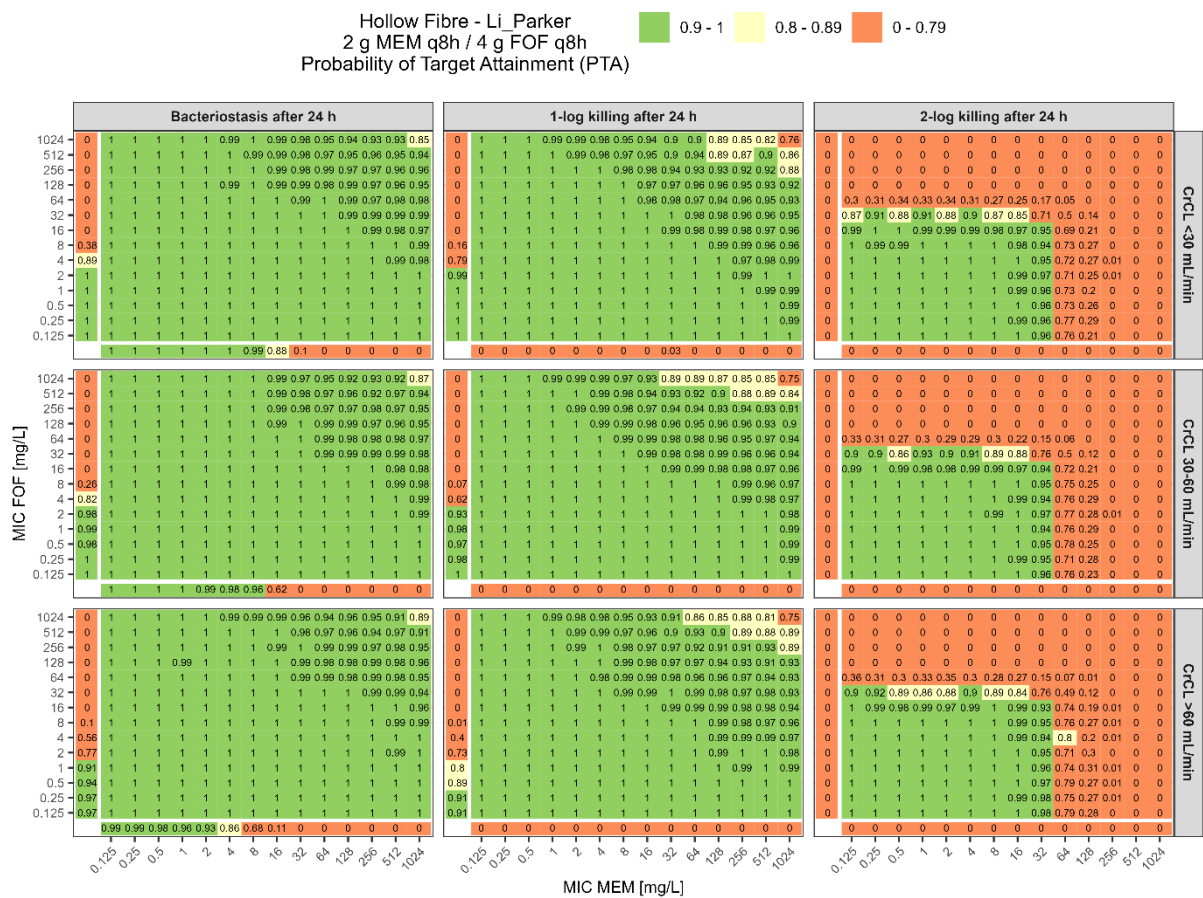
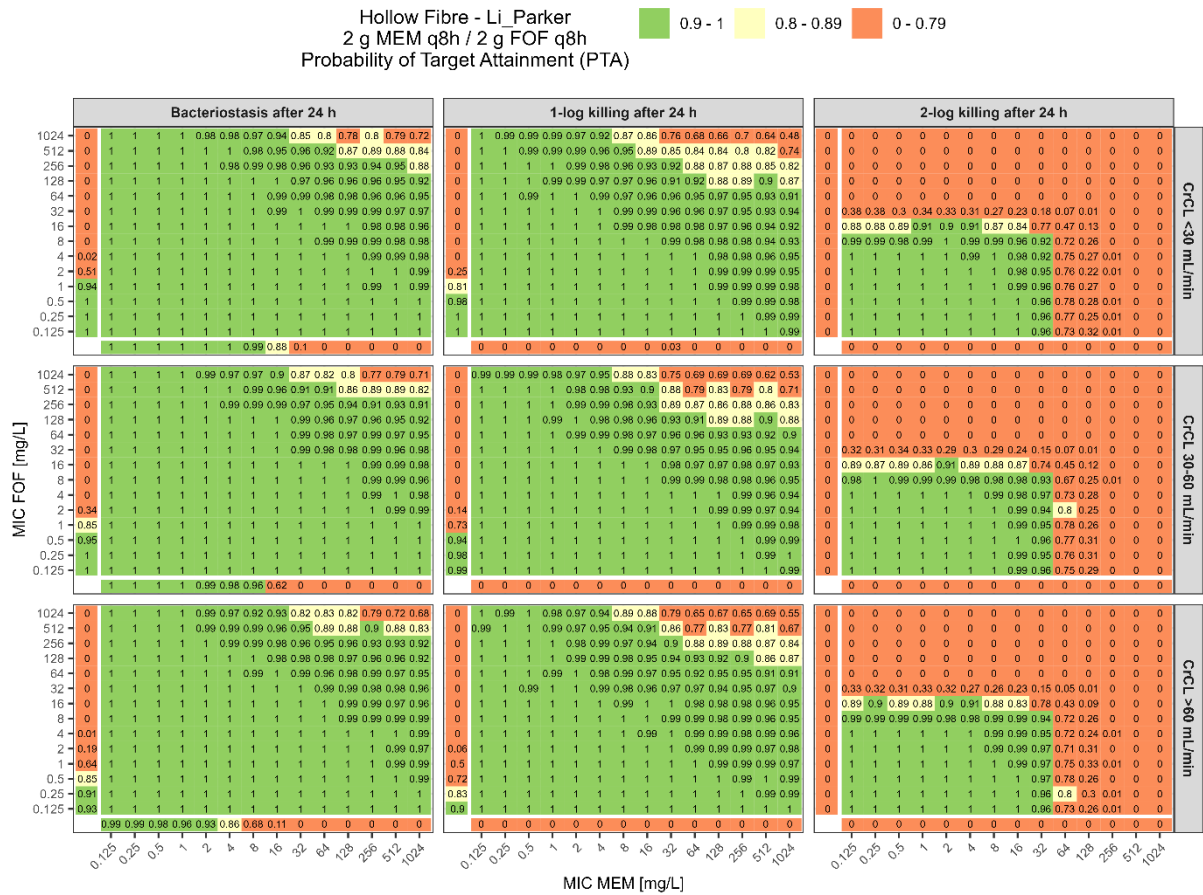
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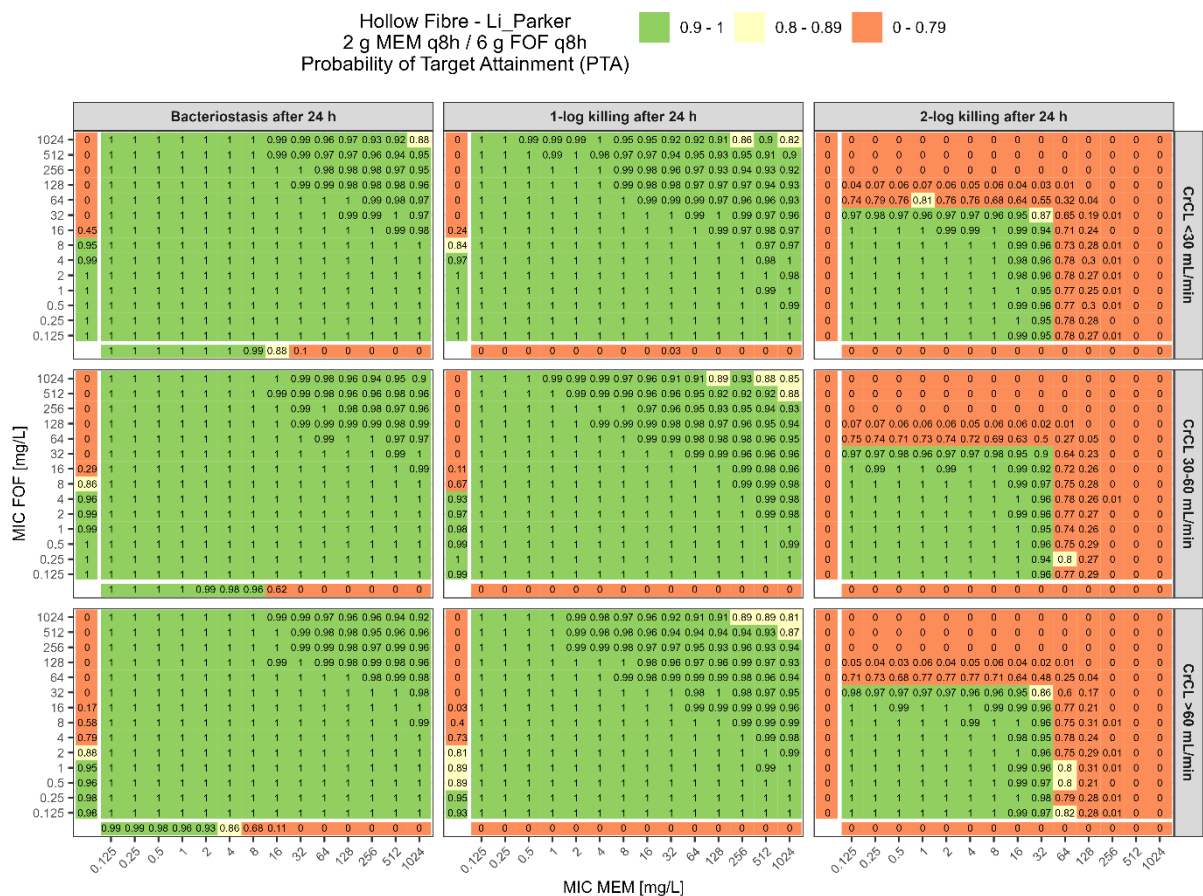
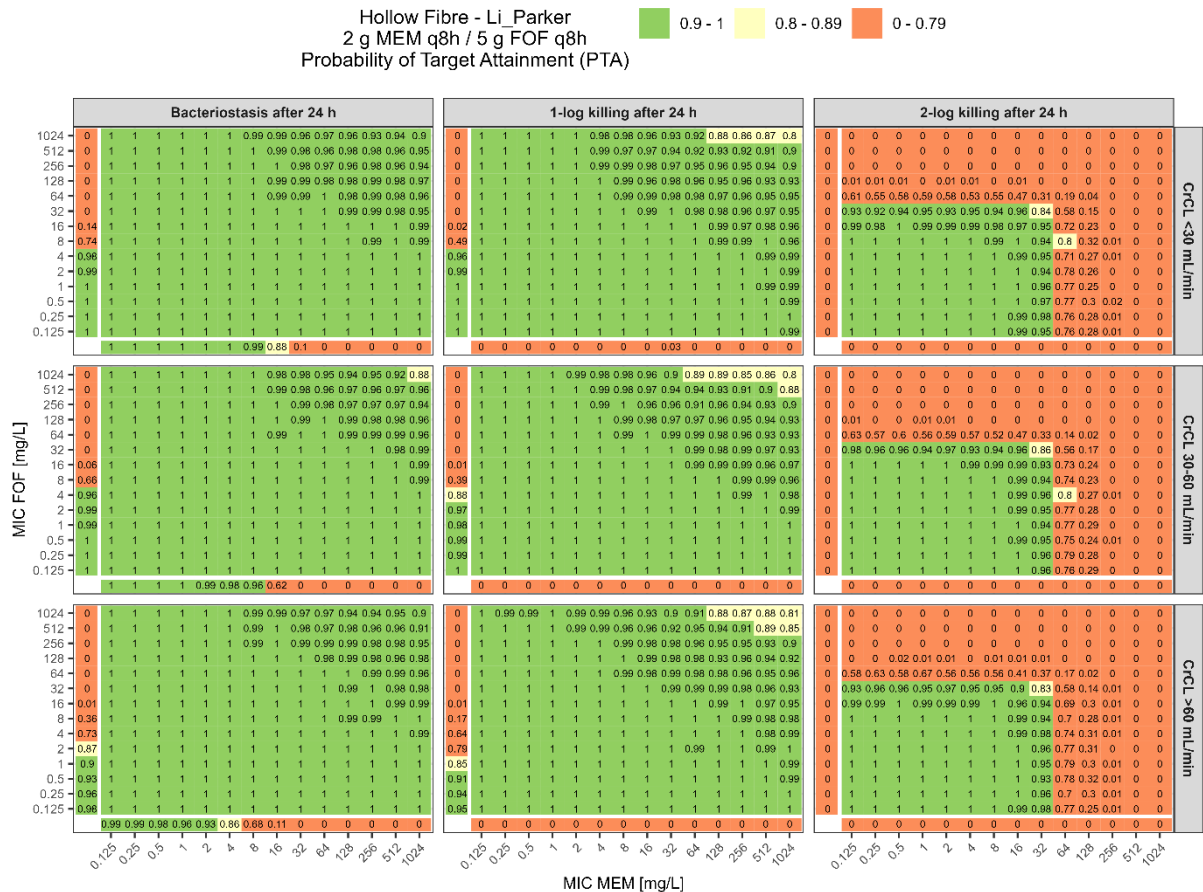
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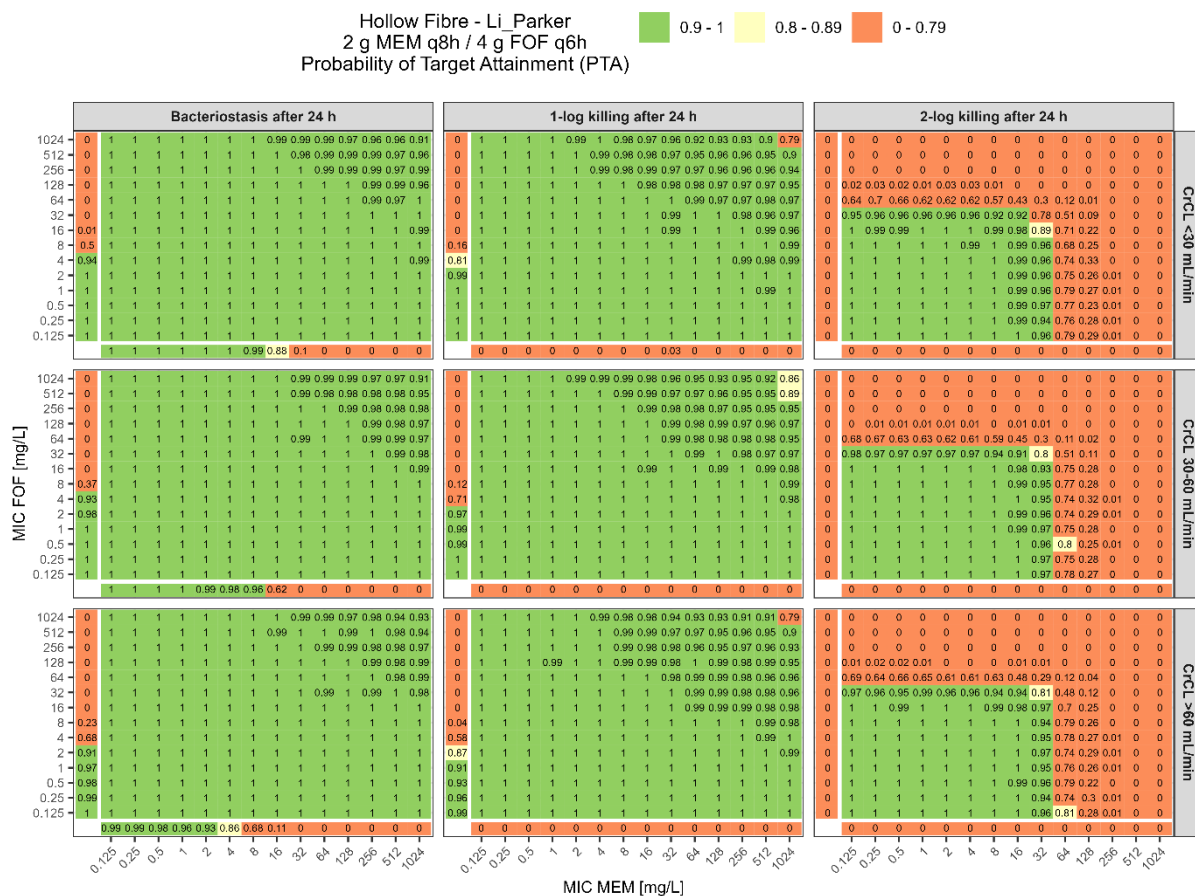
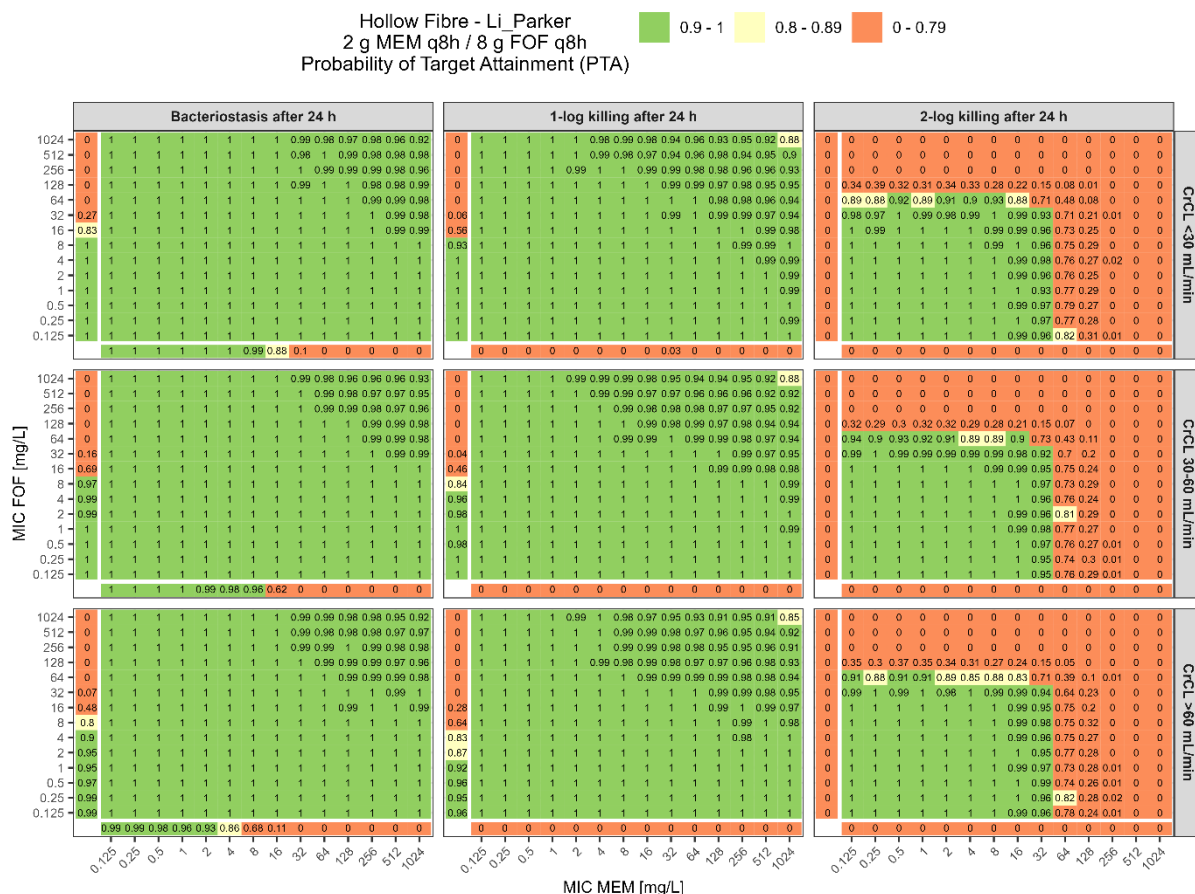
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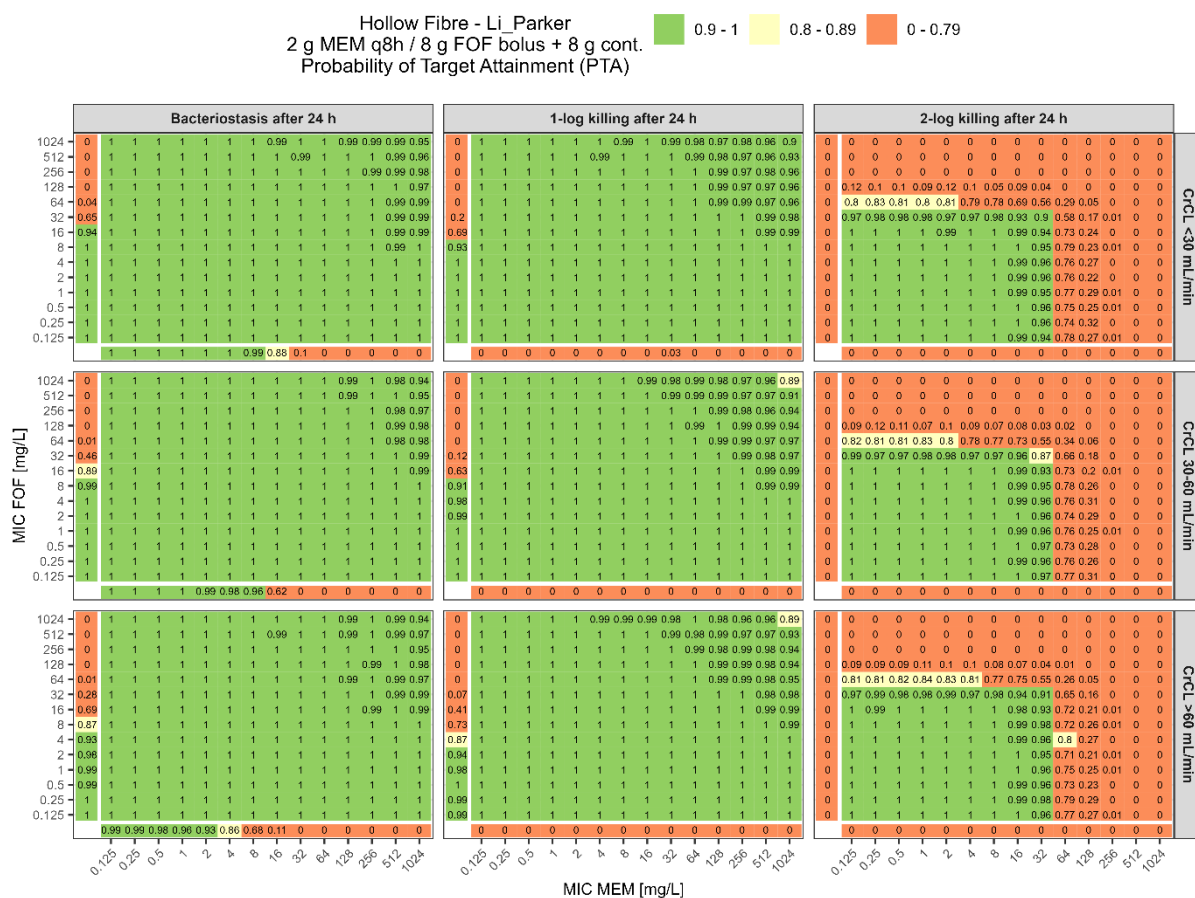
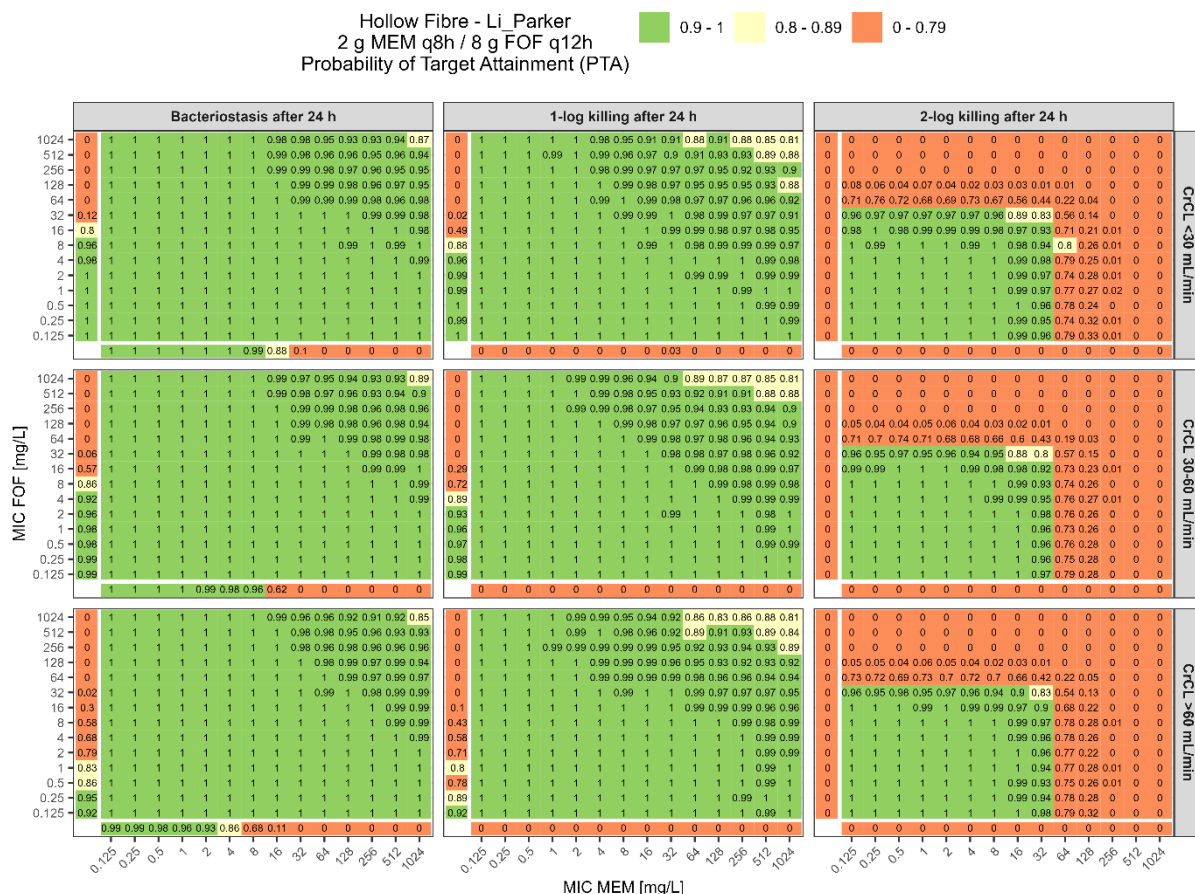
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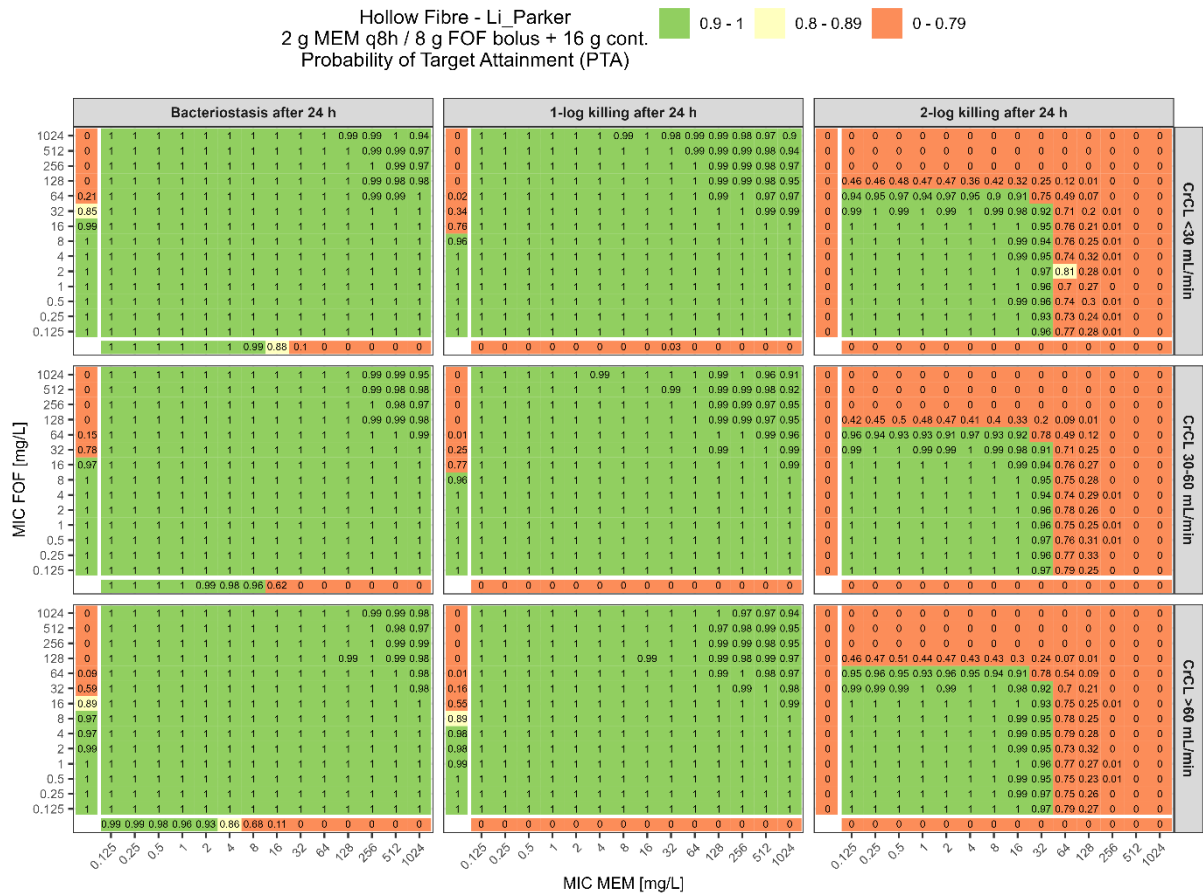
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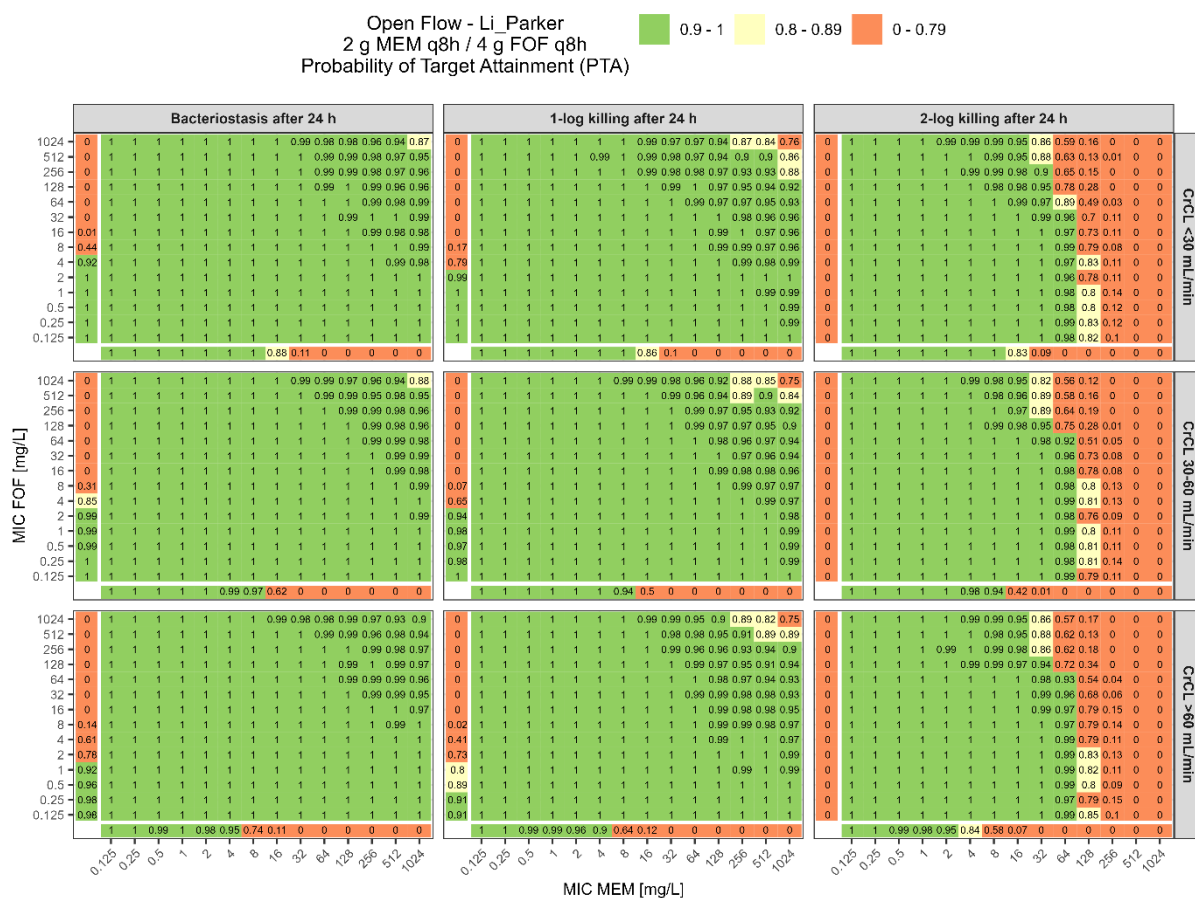
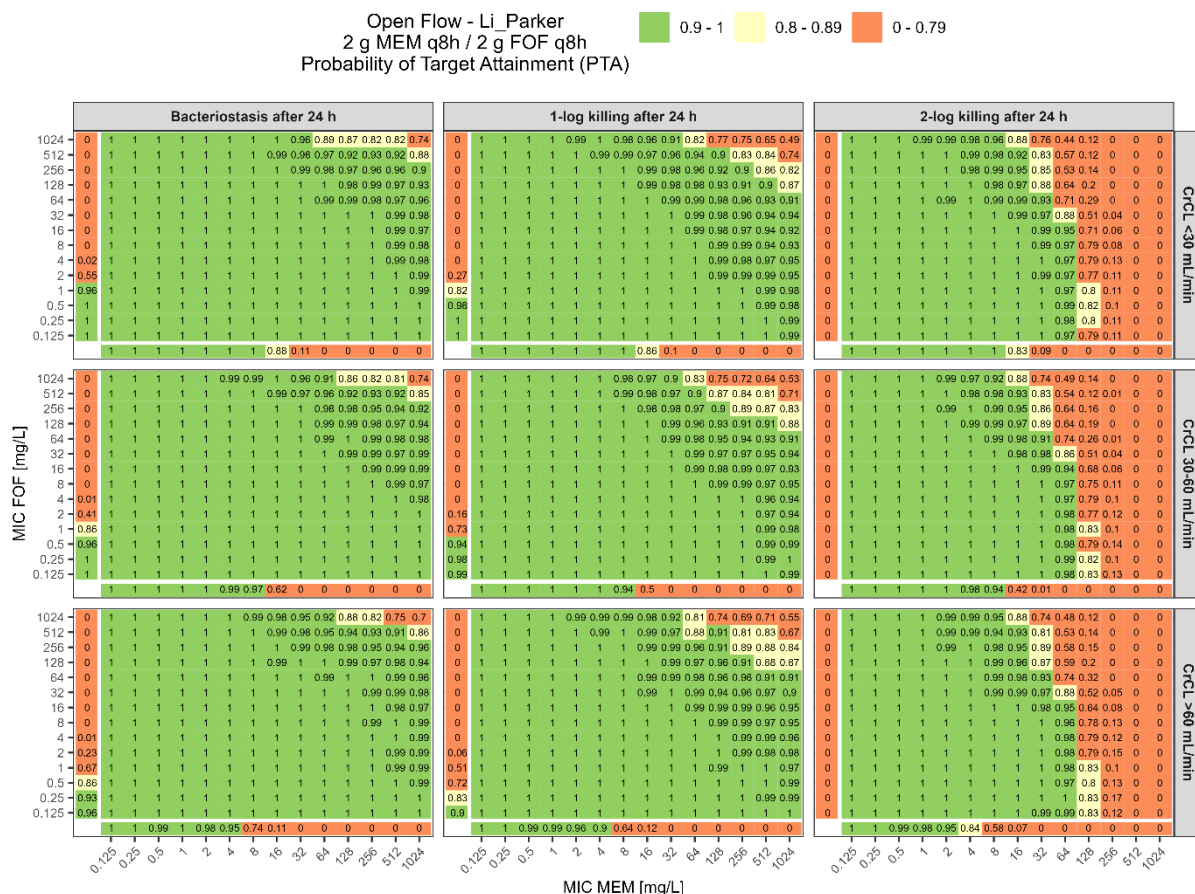


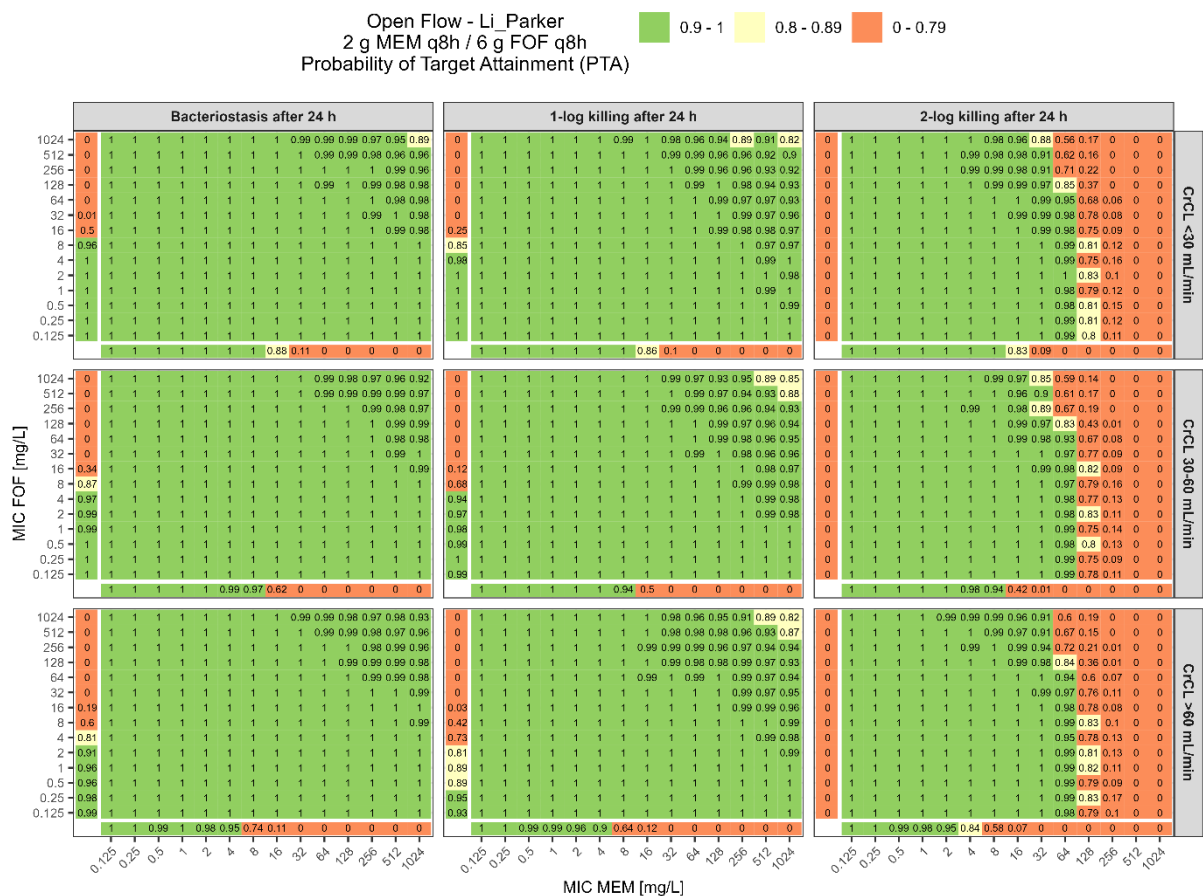
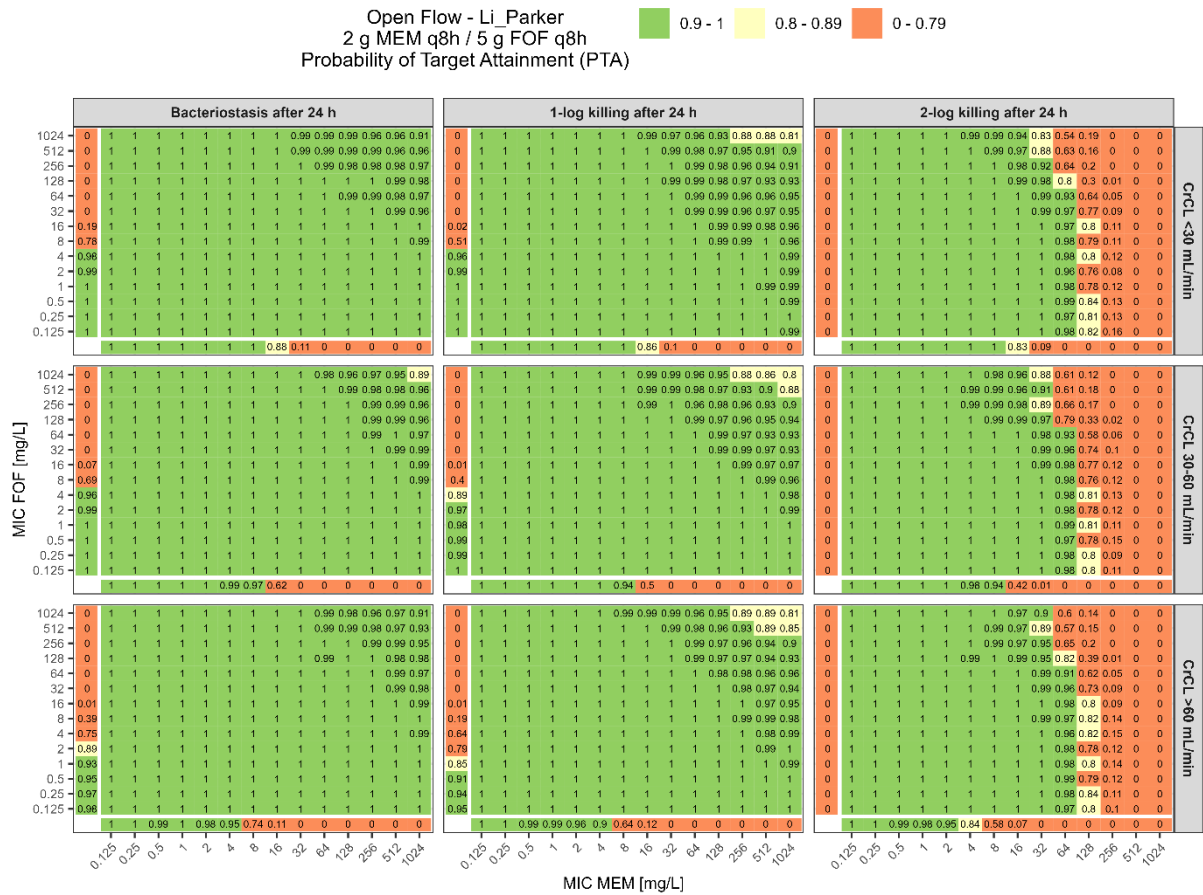


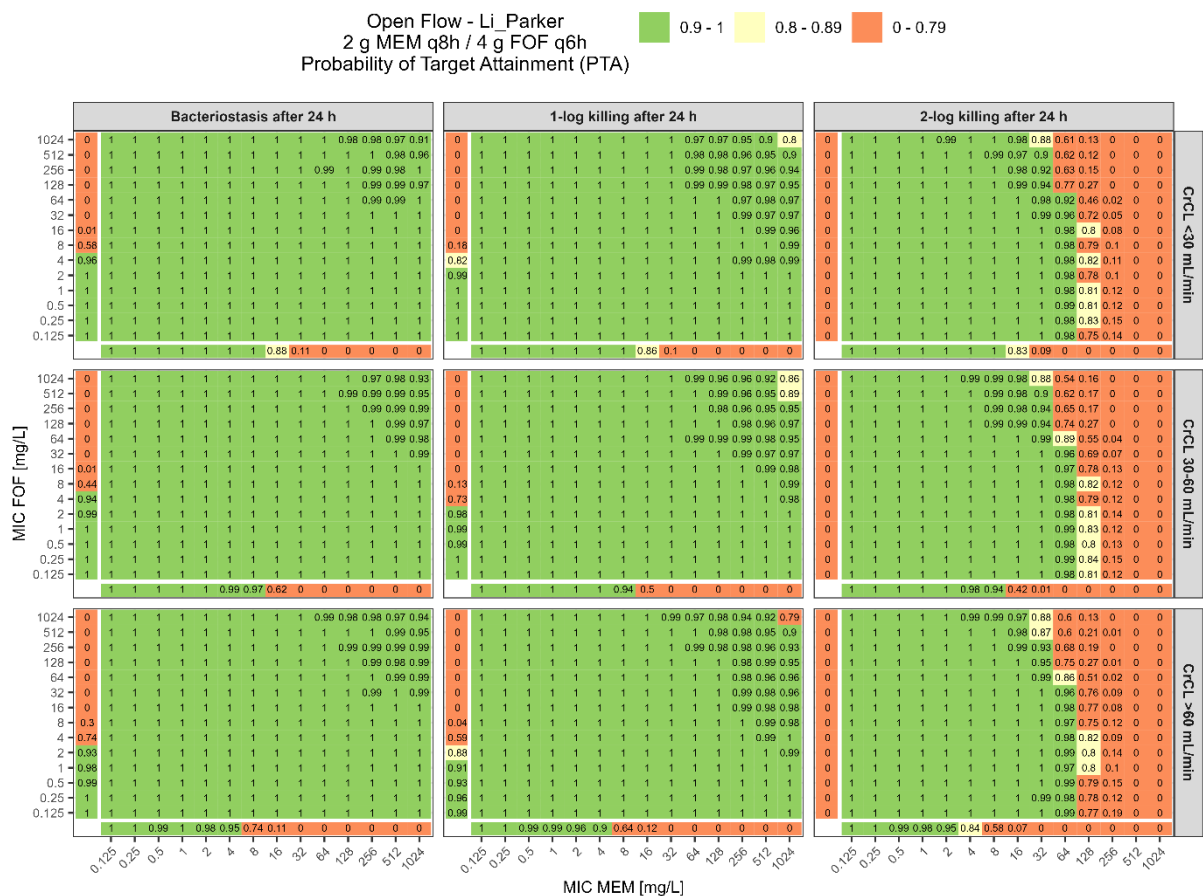
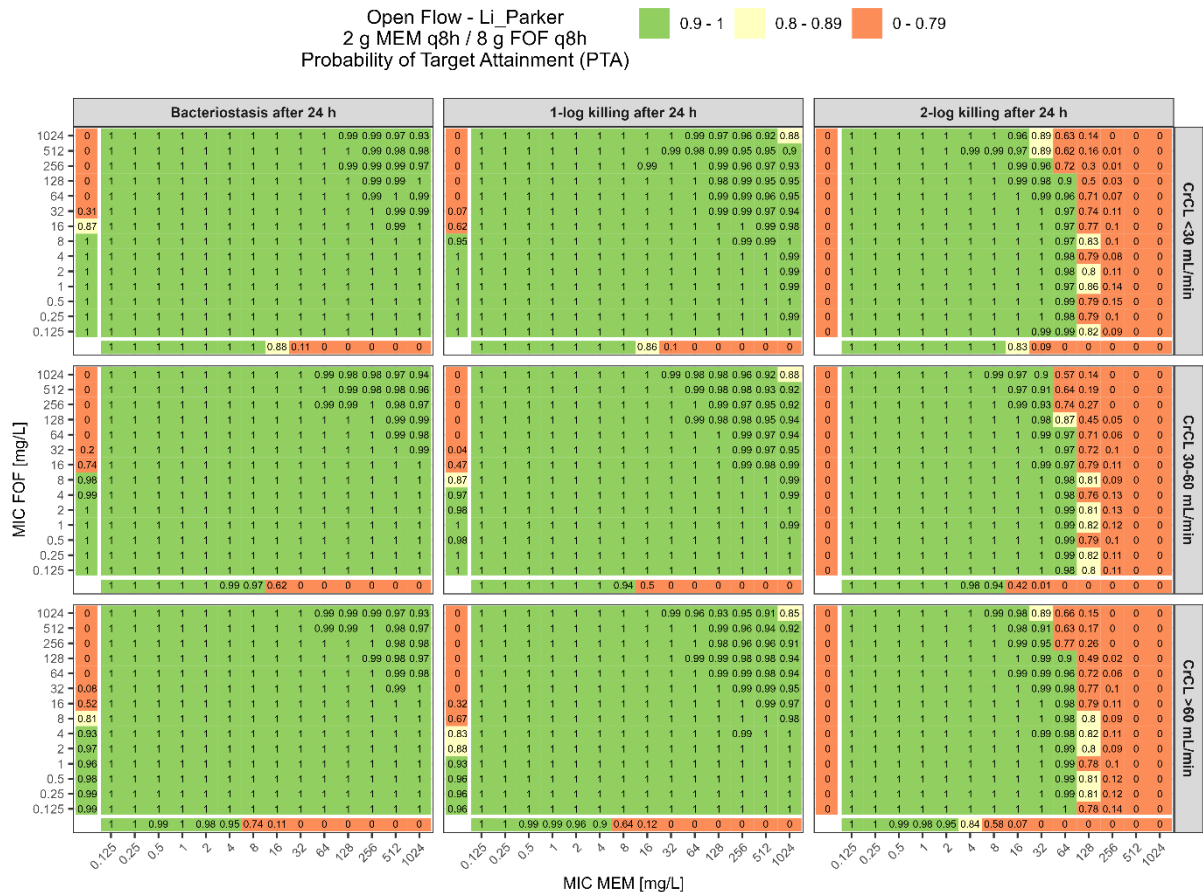


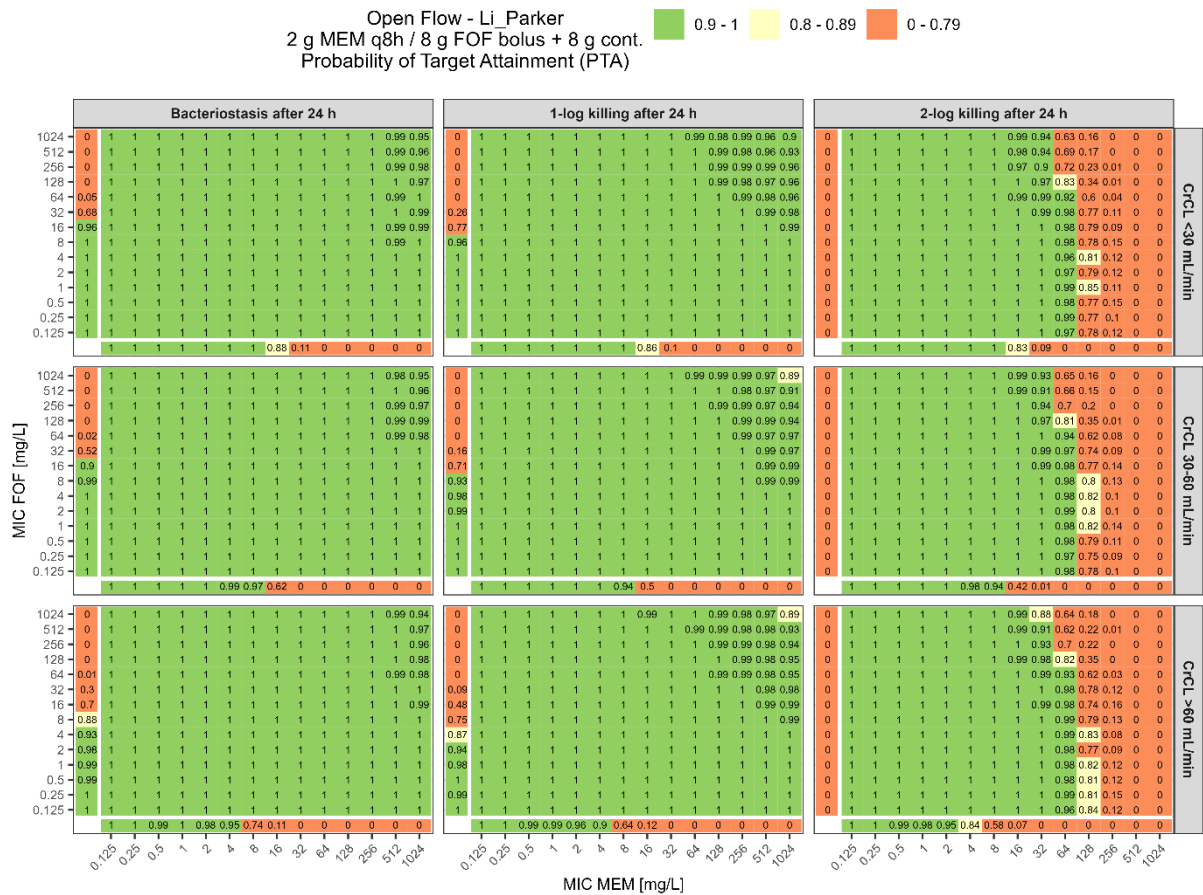
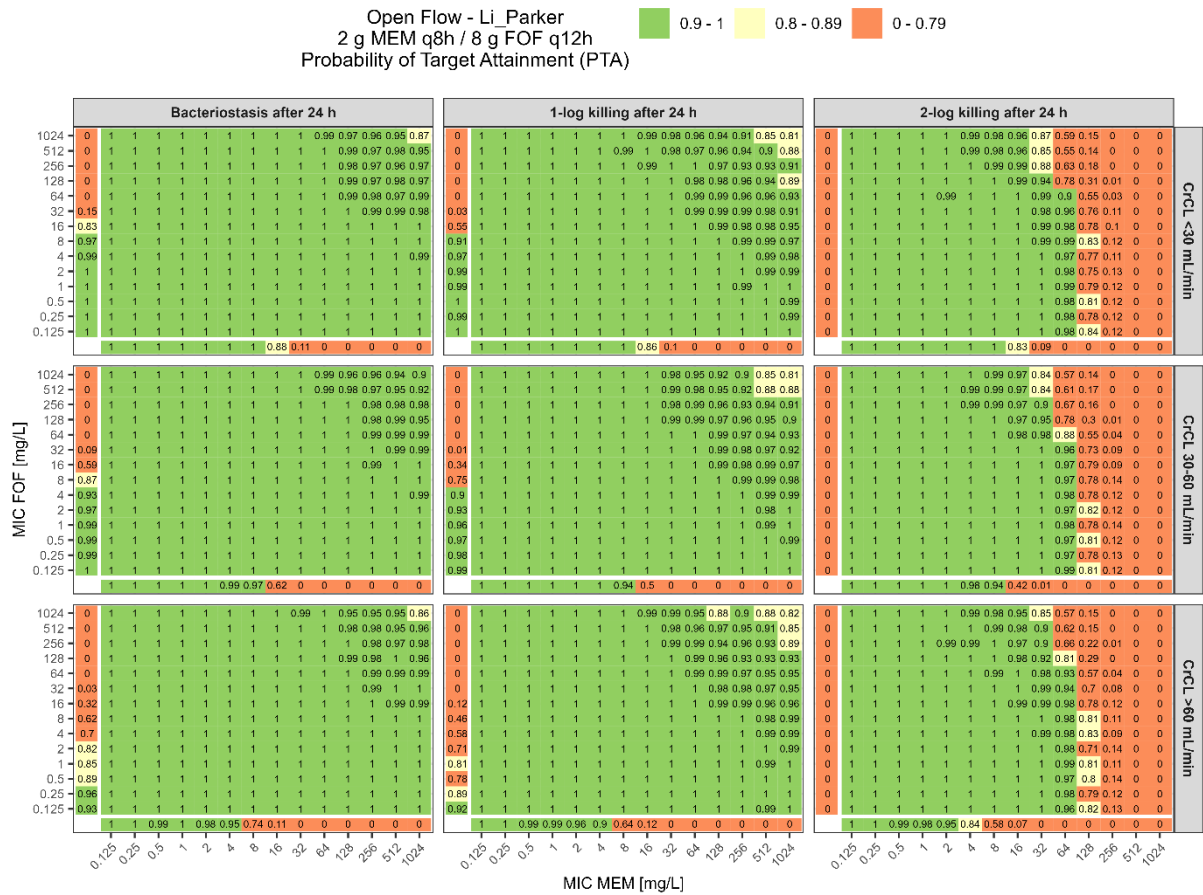






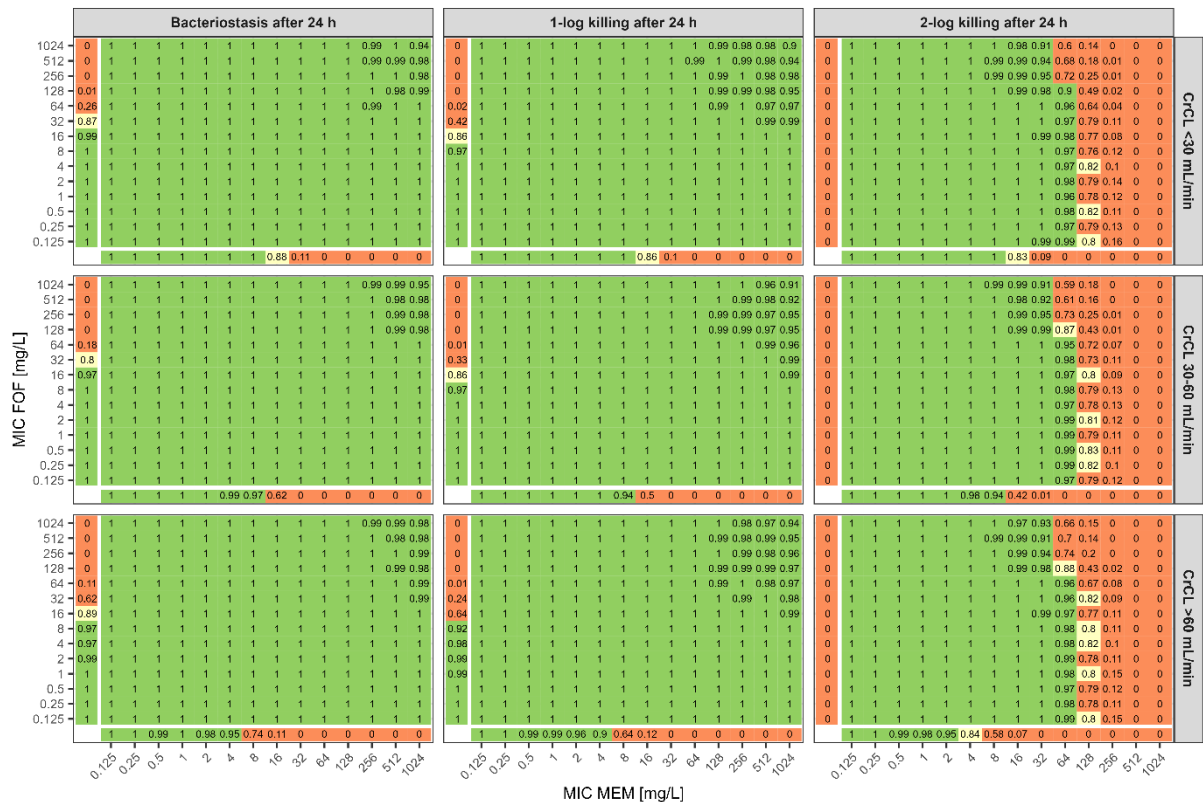


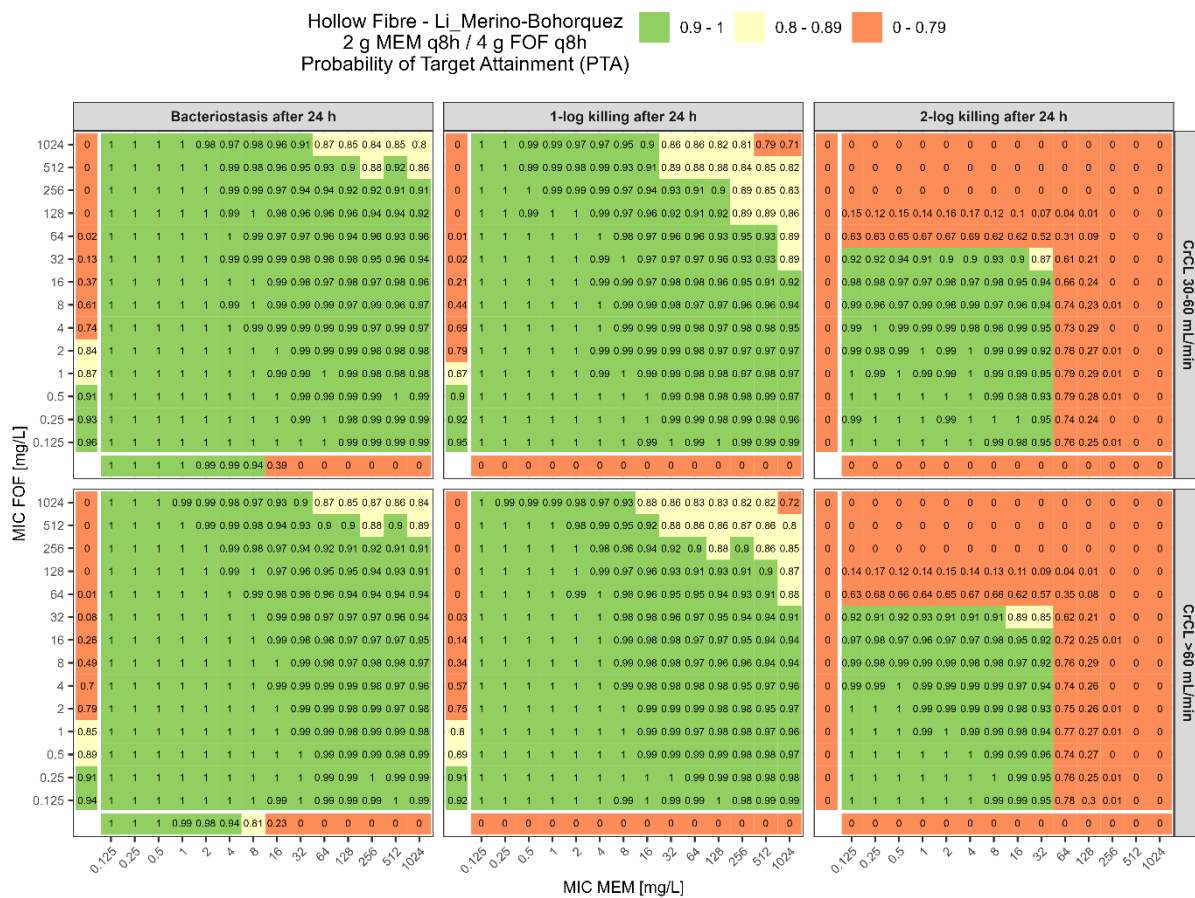
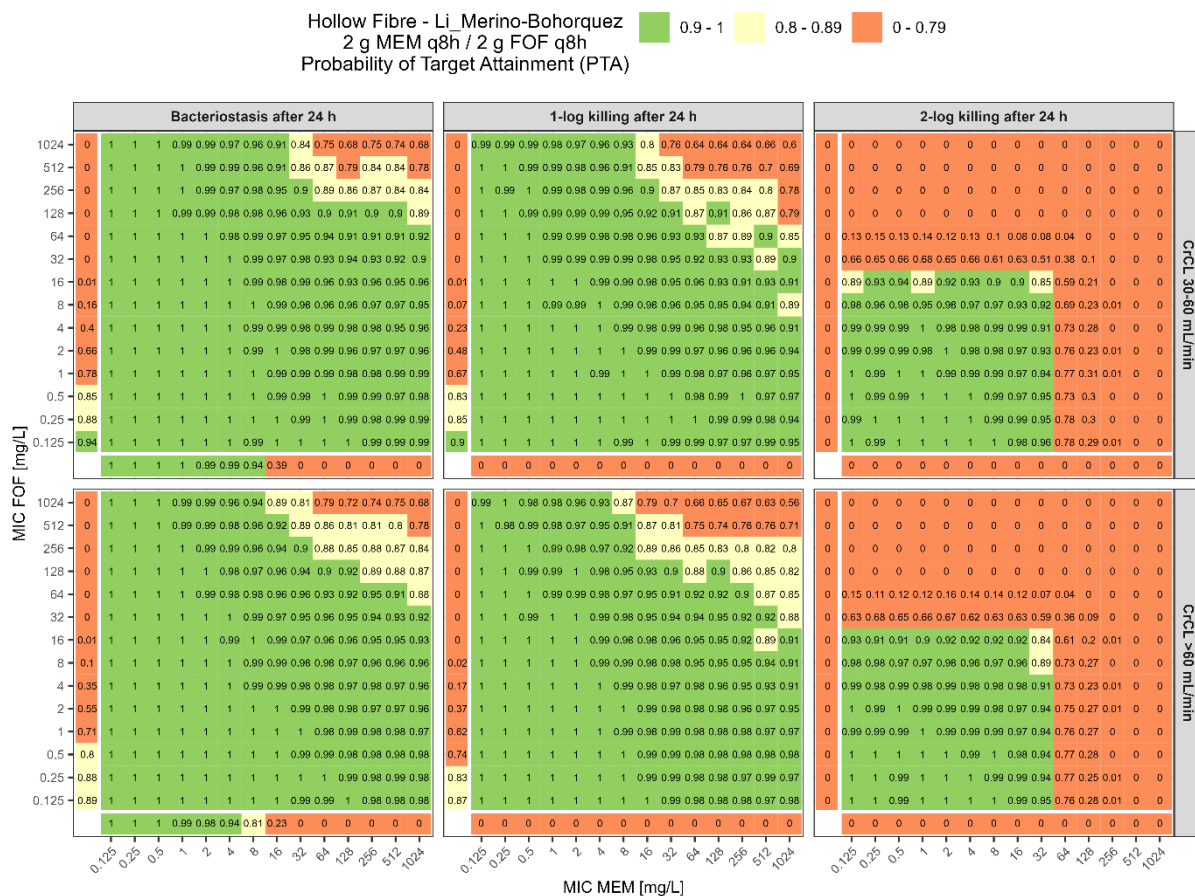


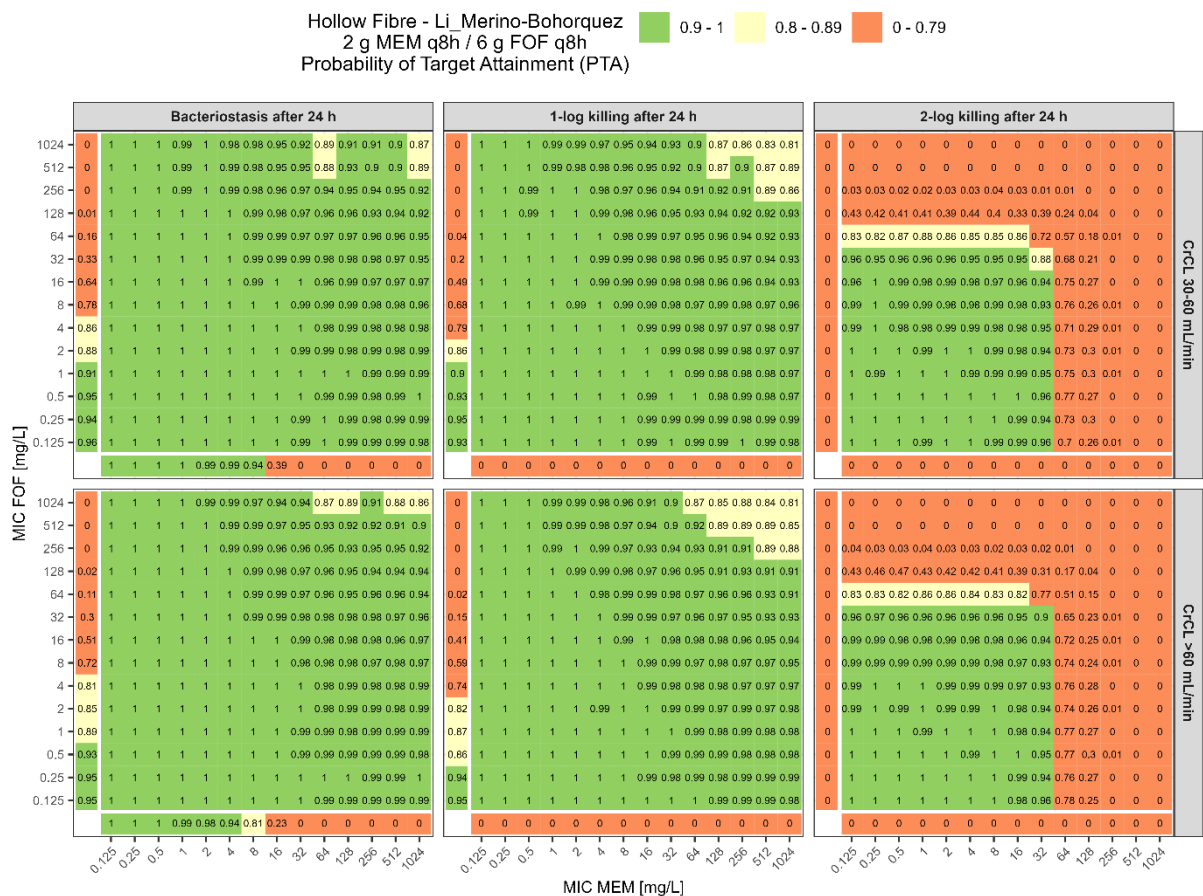
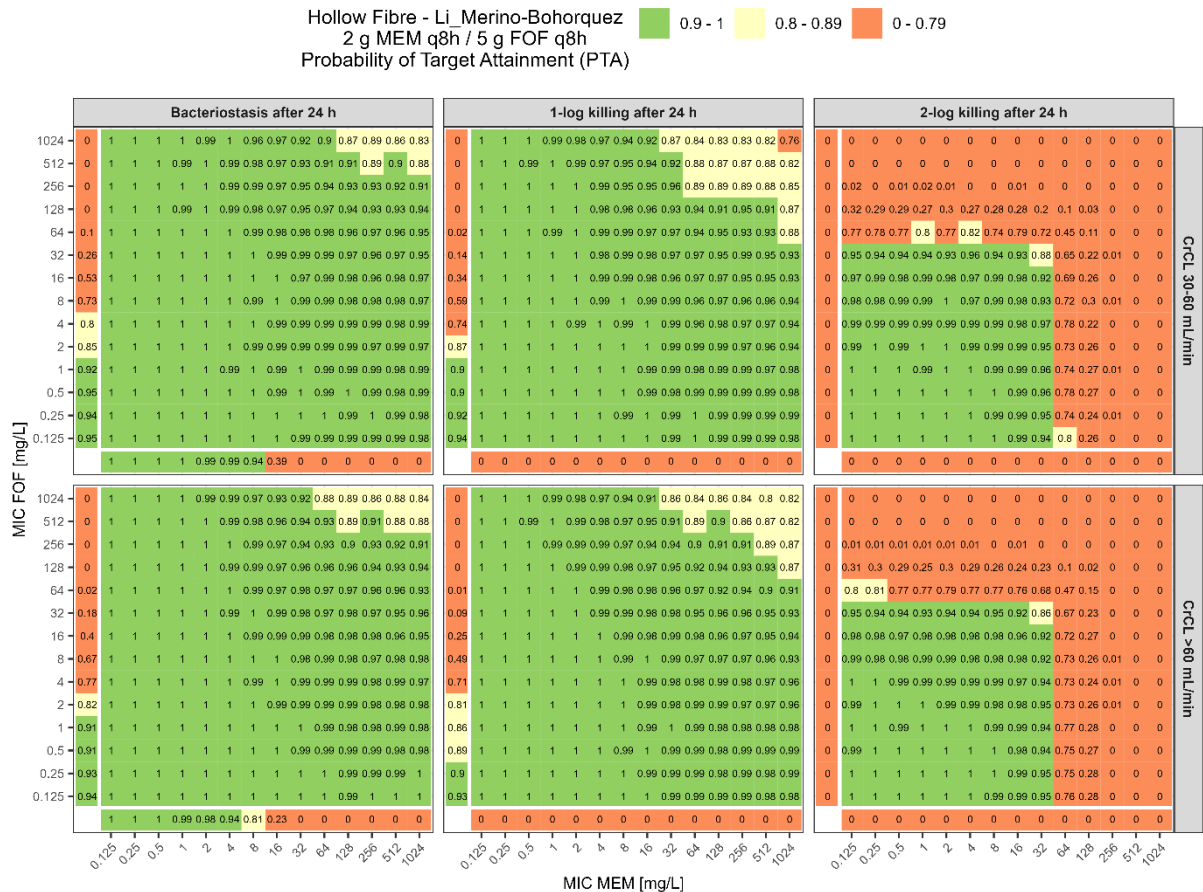


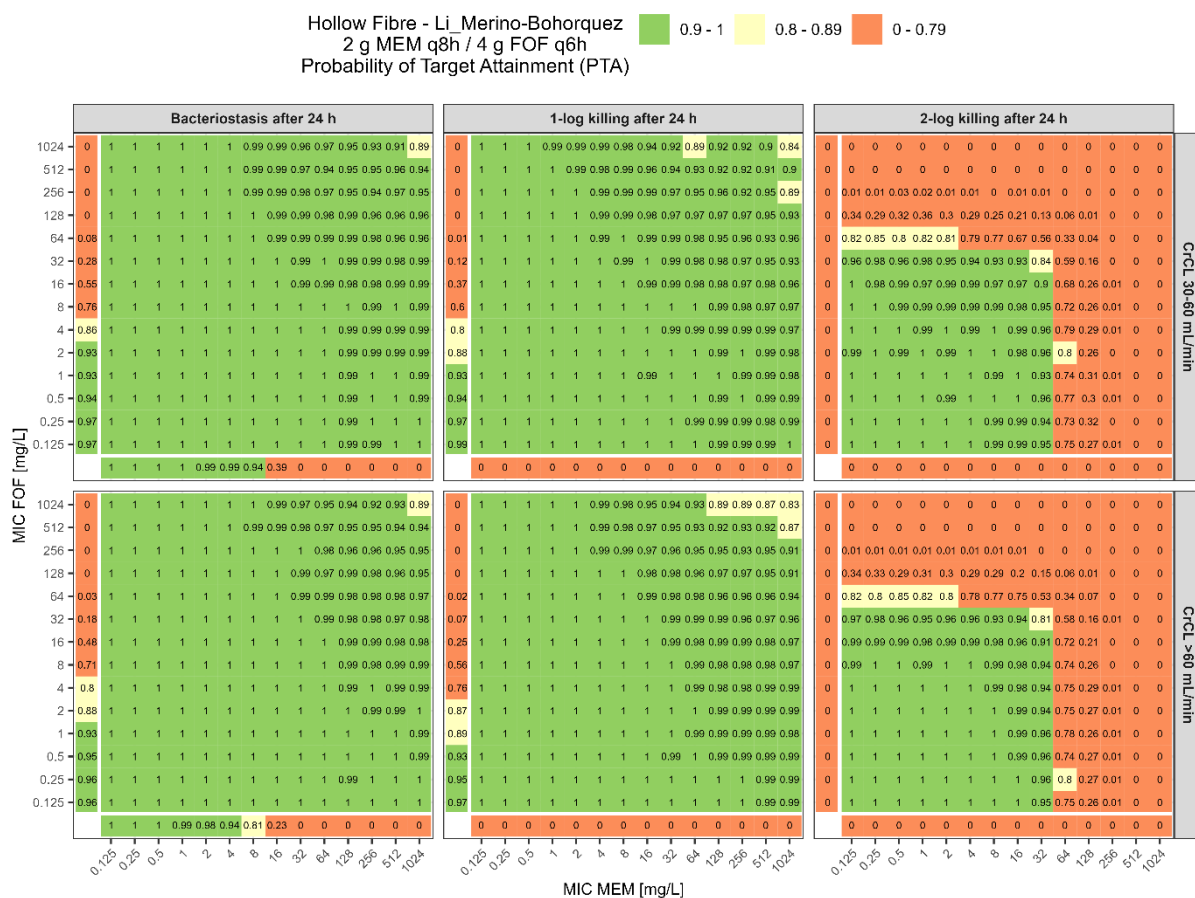
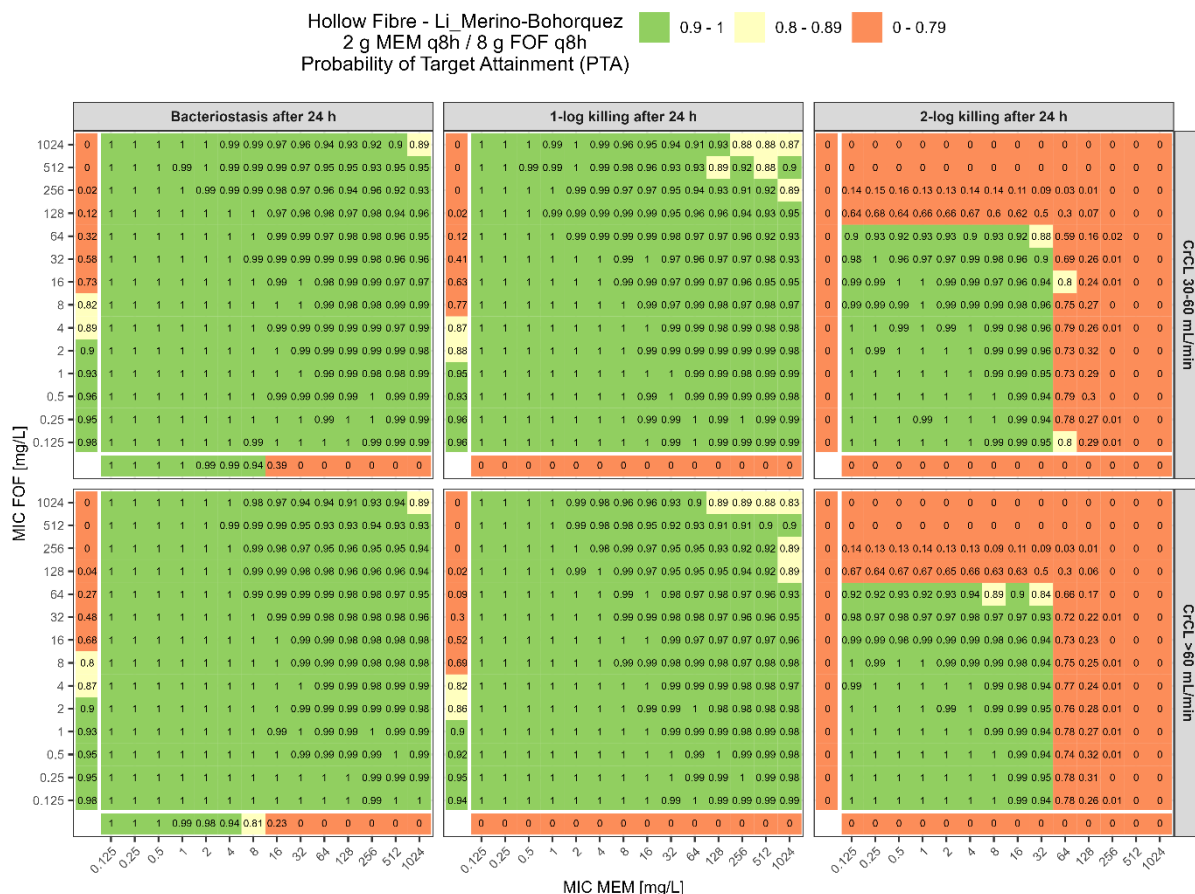
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Probability of Target Attainment (PTA)

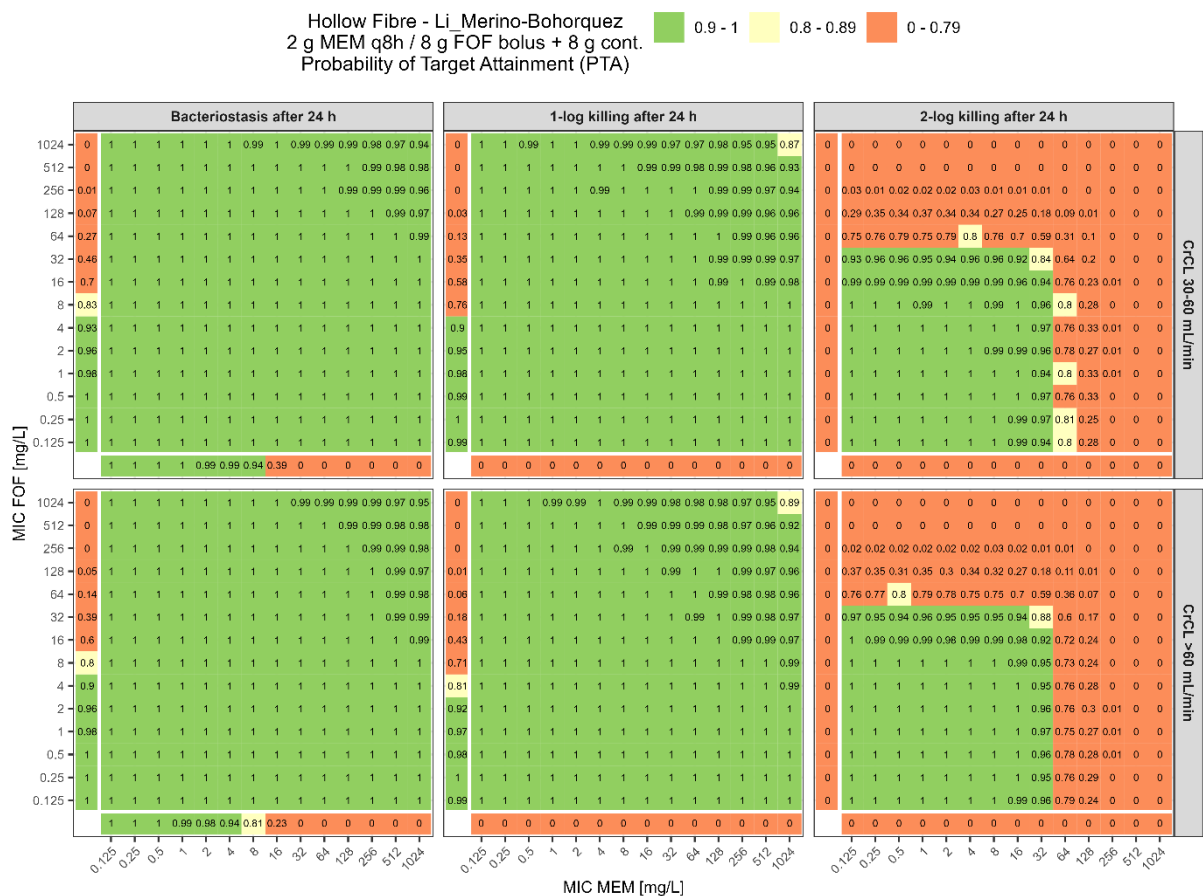
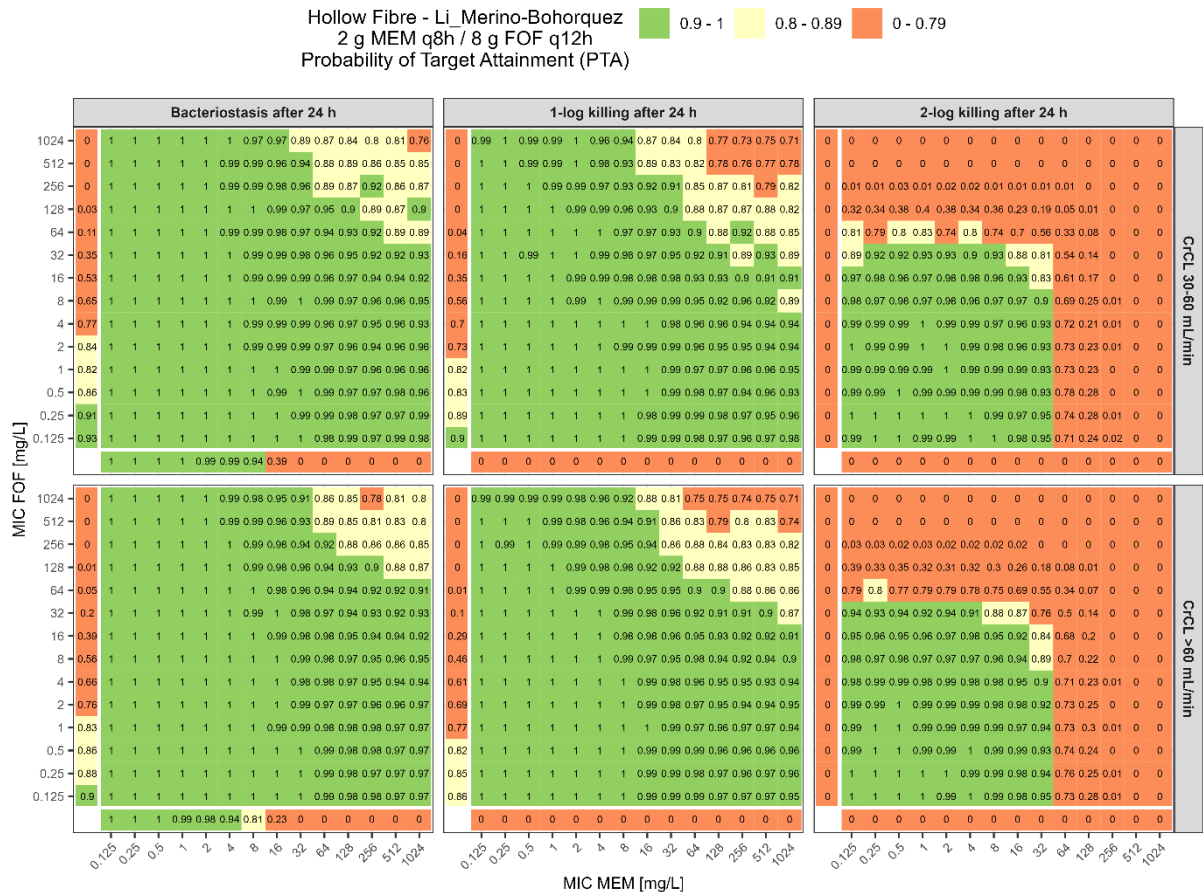
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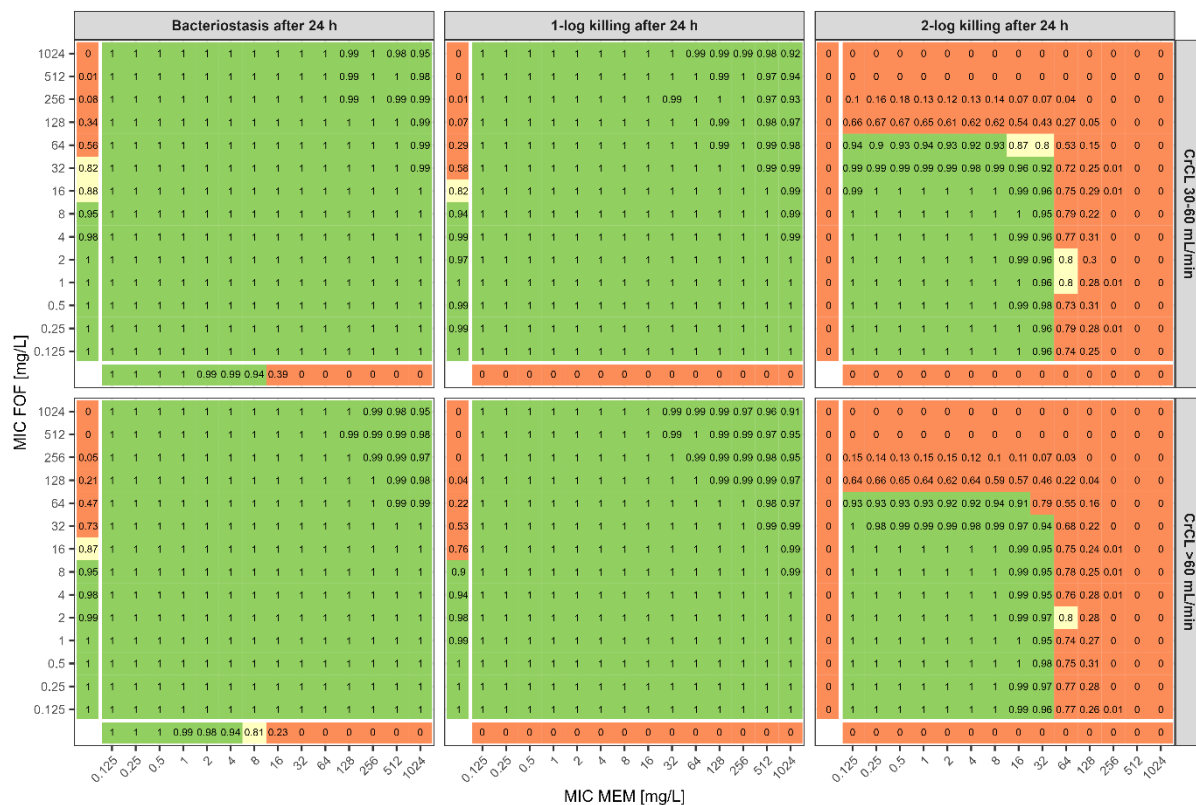


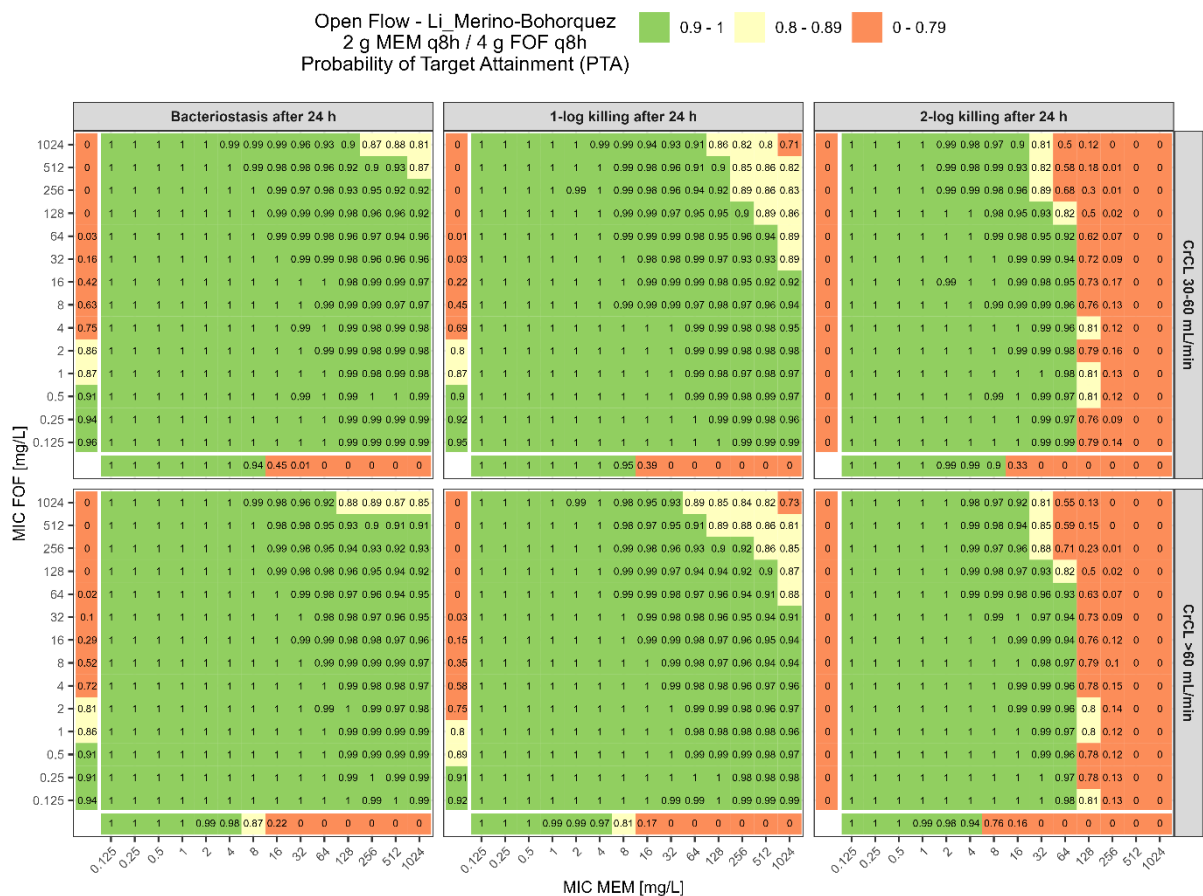
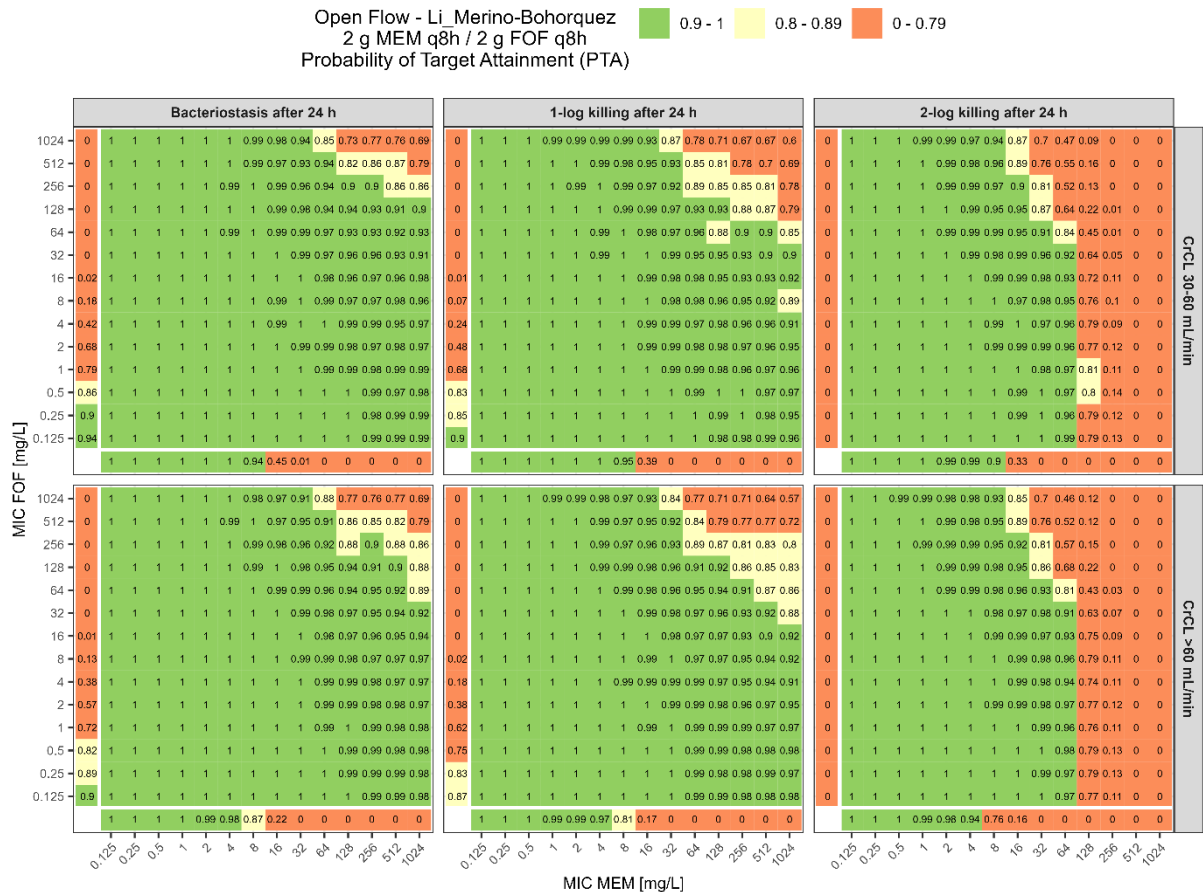


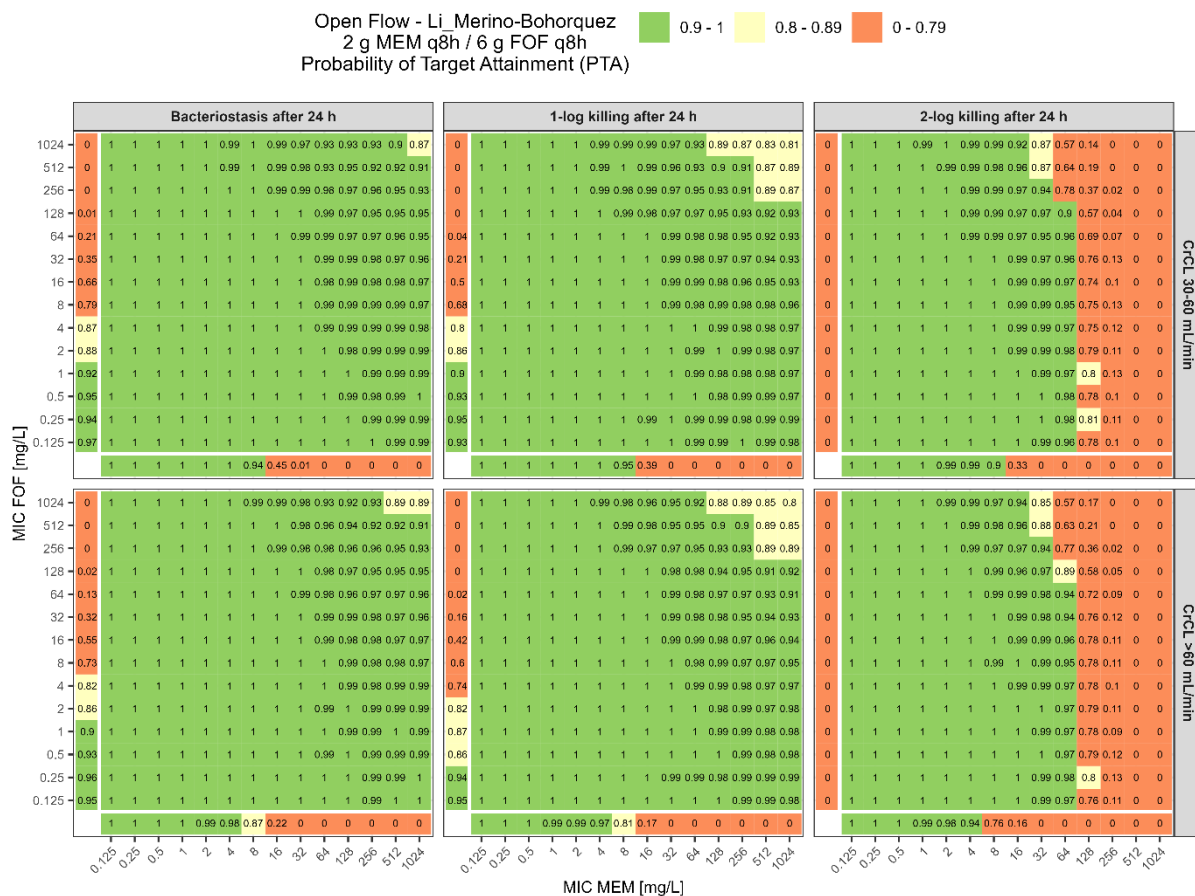
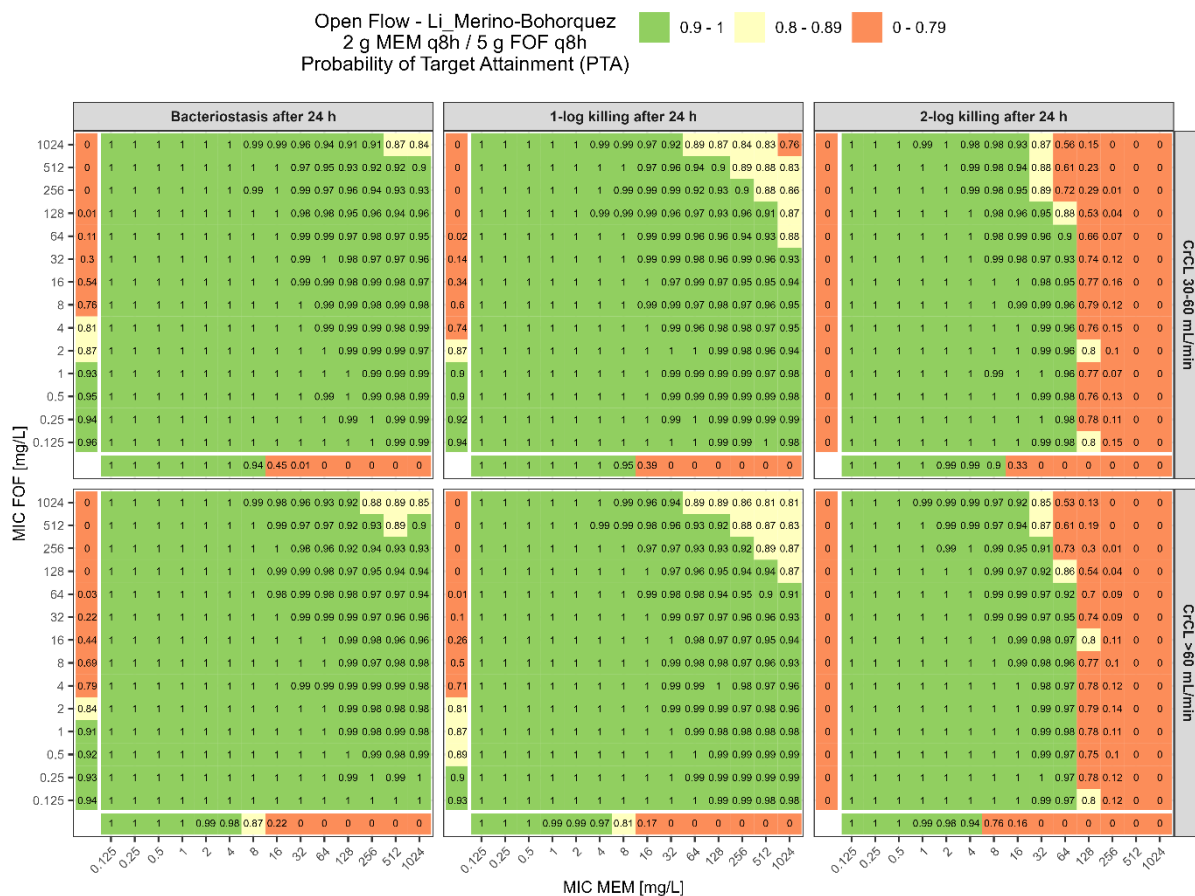


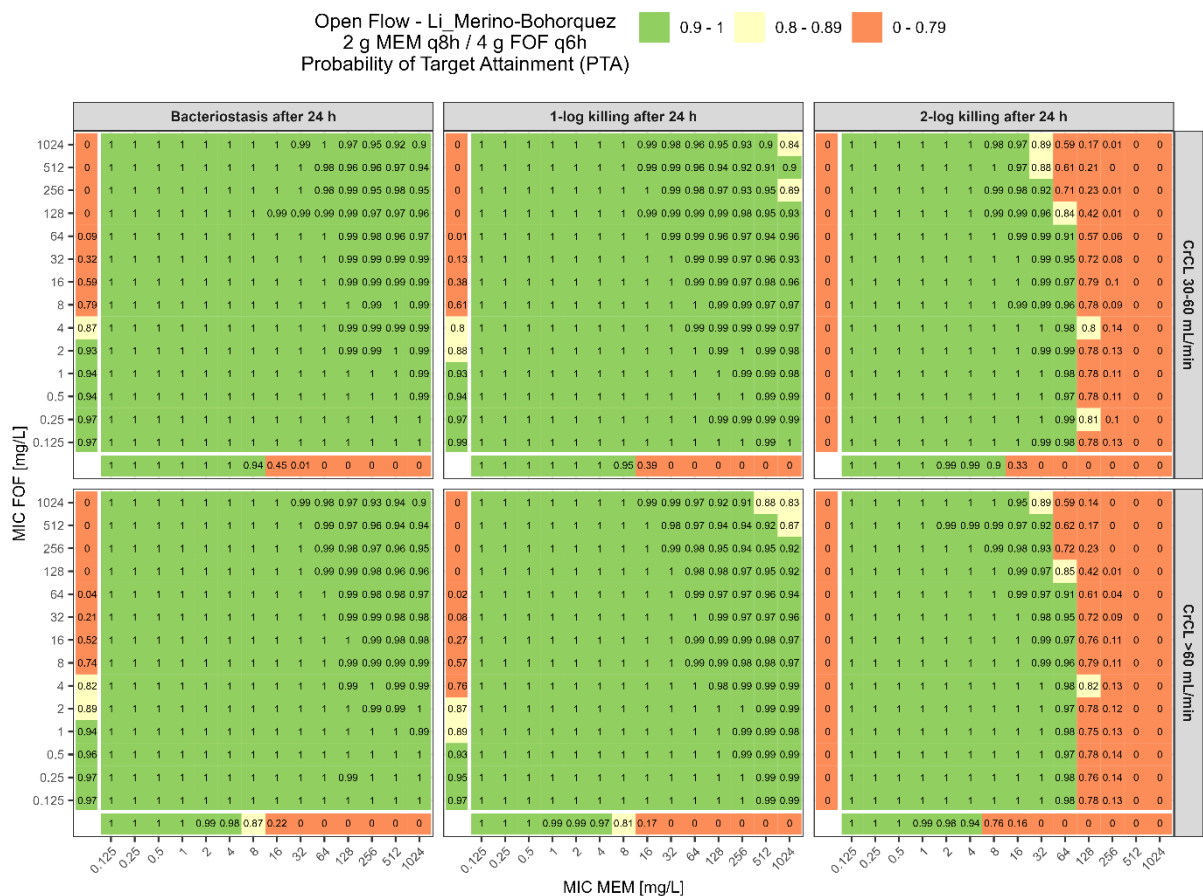
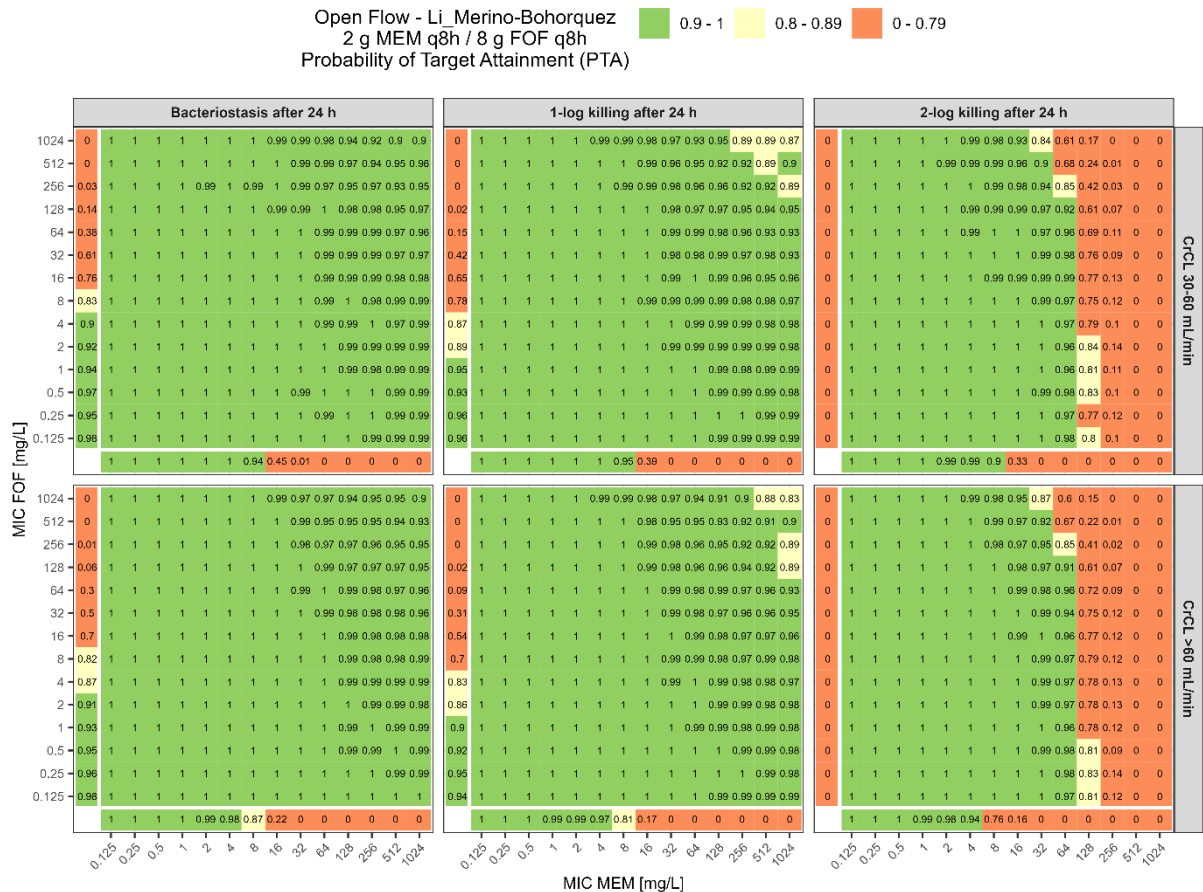
Hollow Fibre - Li Merino-Bohorquez
2 g MEM q8h / 8 g FOF bolus + 16 g cont.
Probability of Target Attainment (PTA)

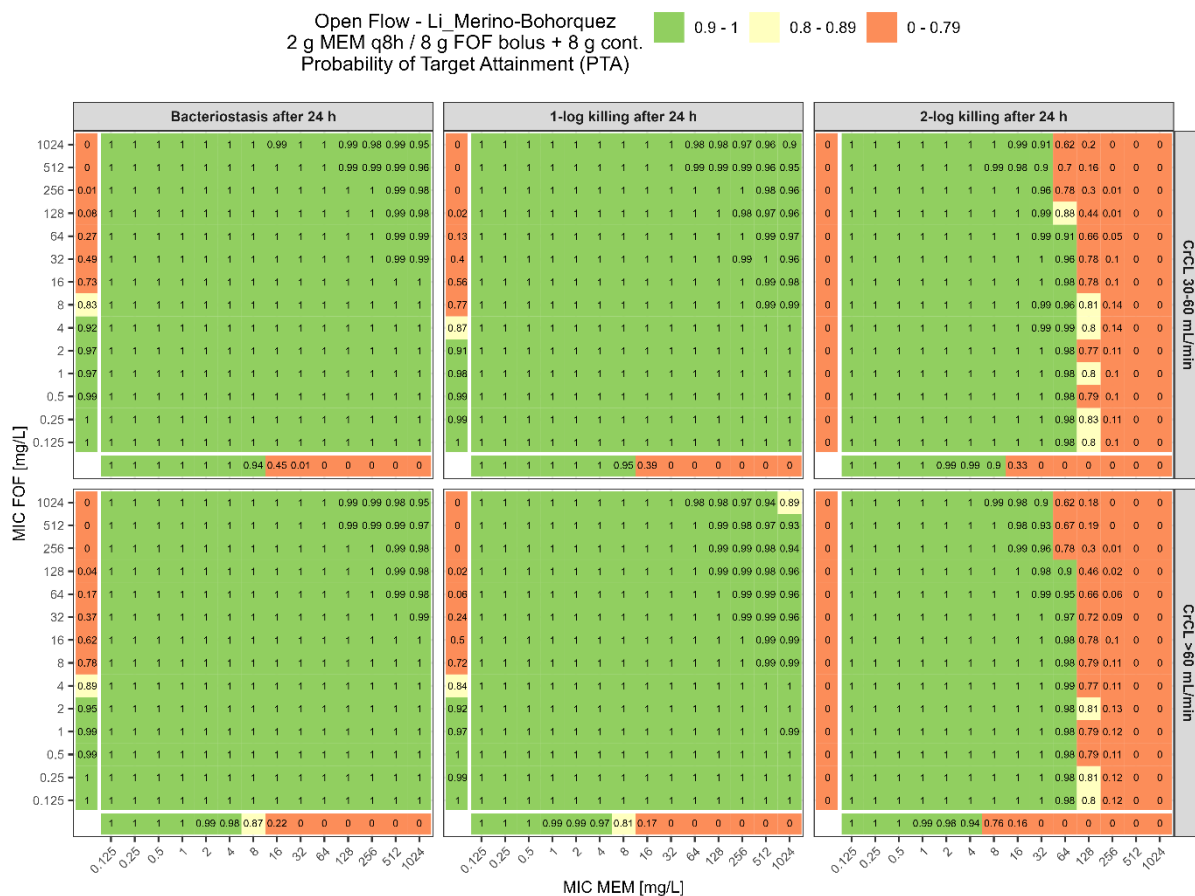
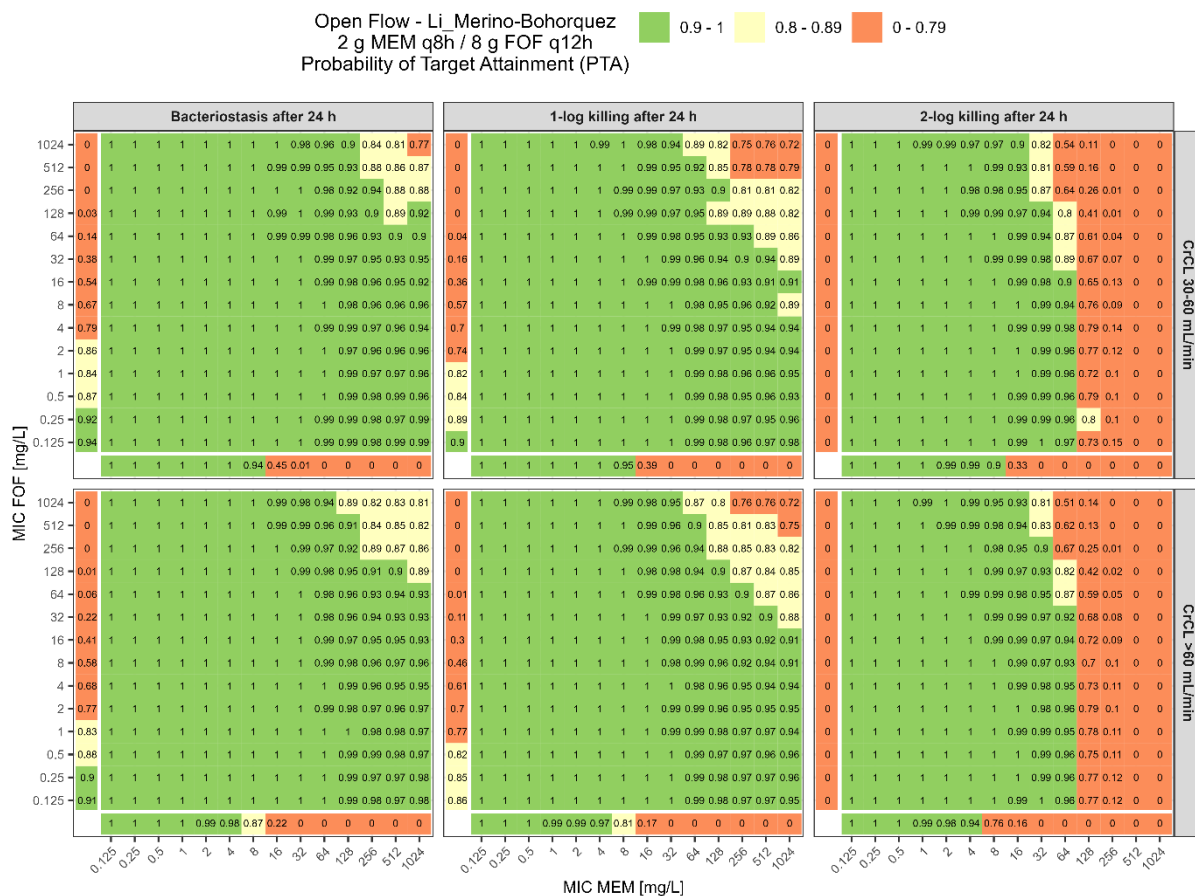
0.9 - 1 0.8 - 0.89 0 - 0.79





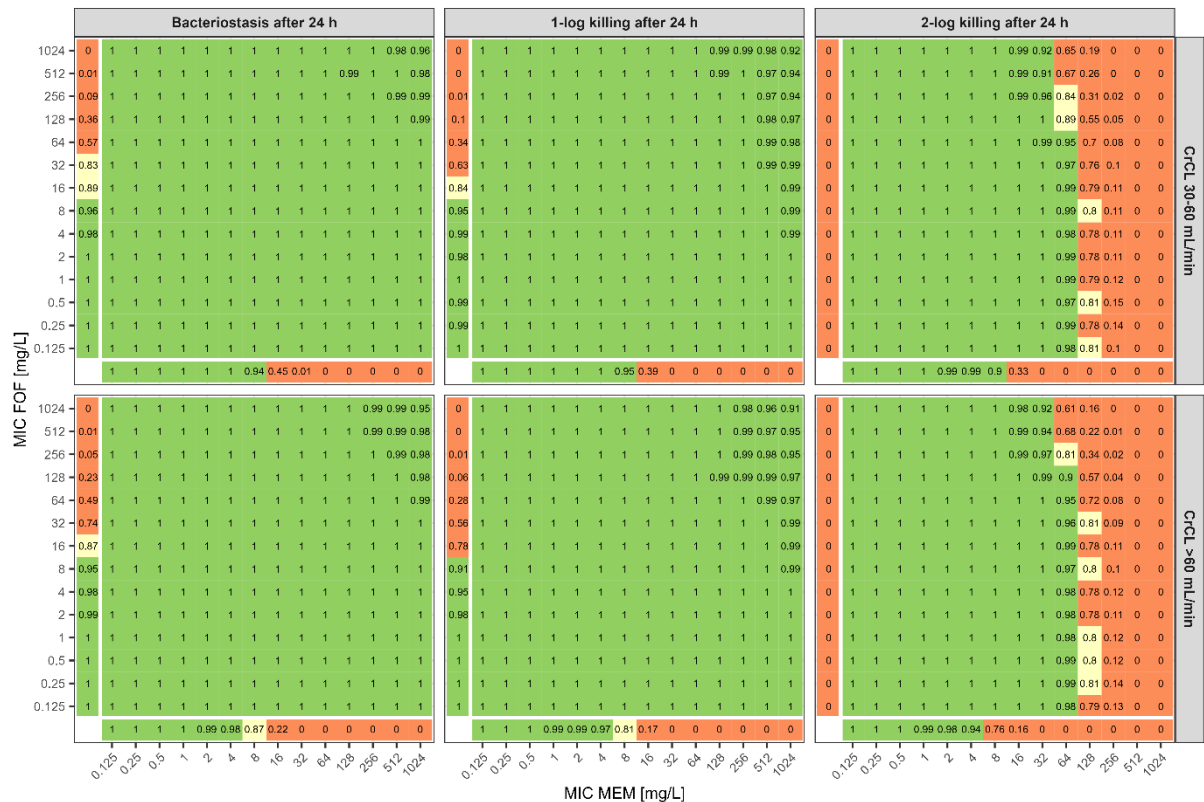


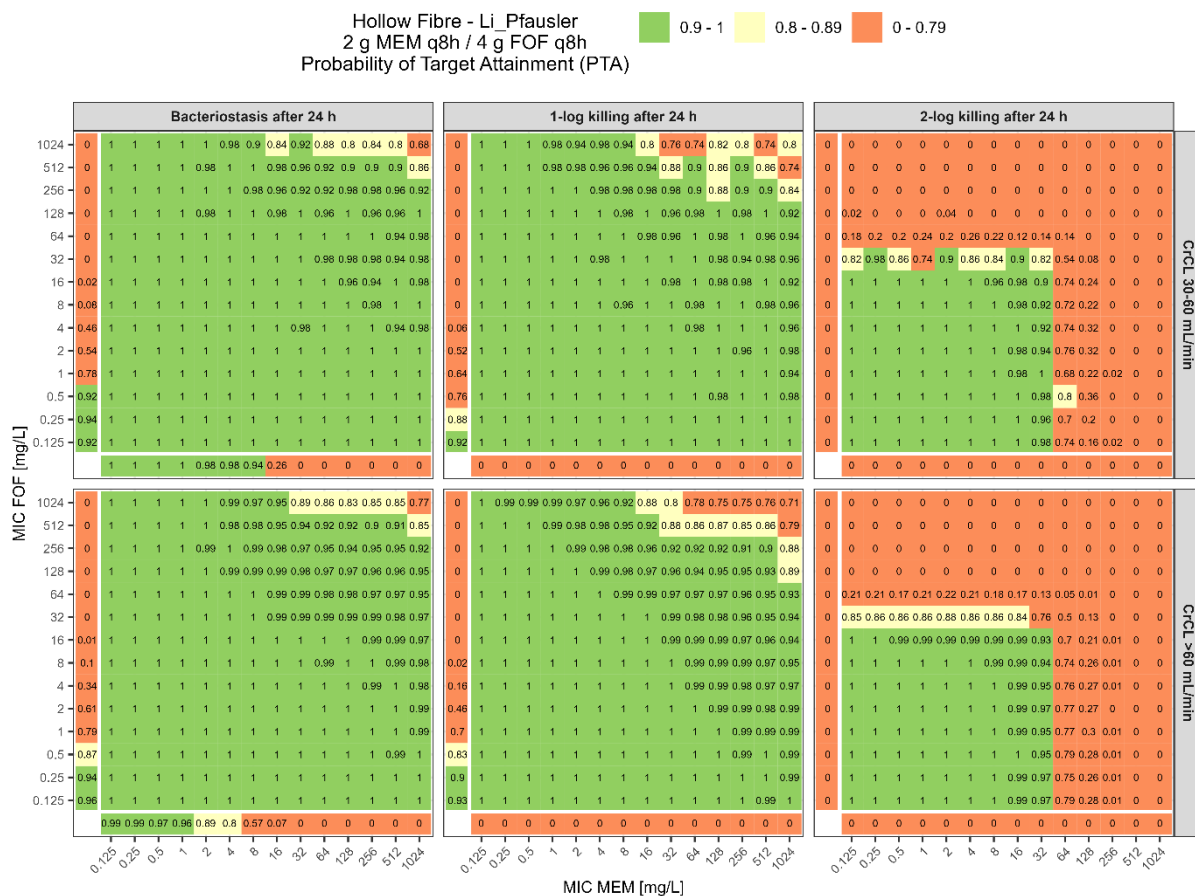
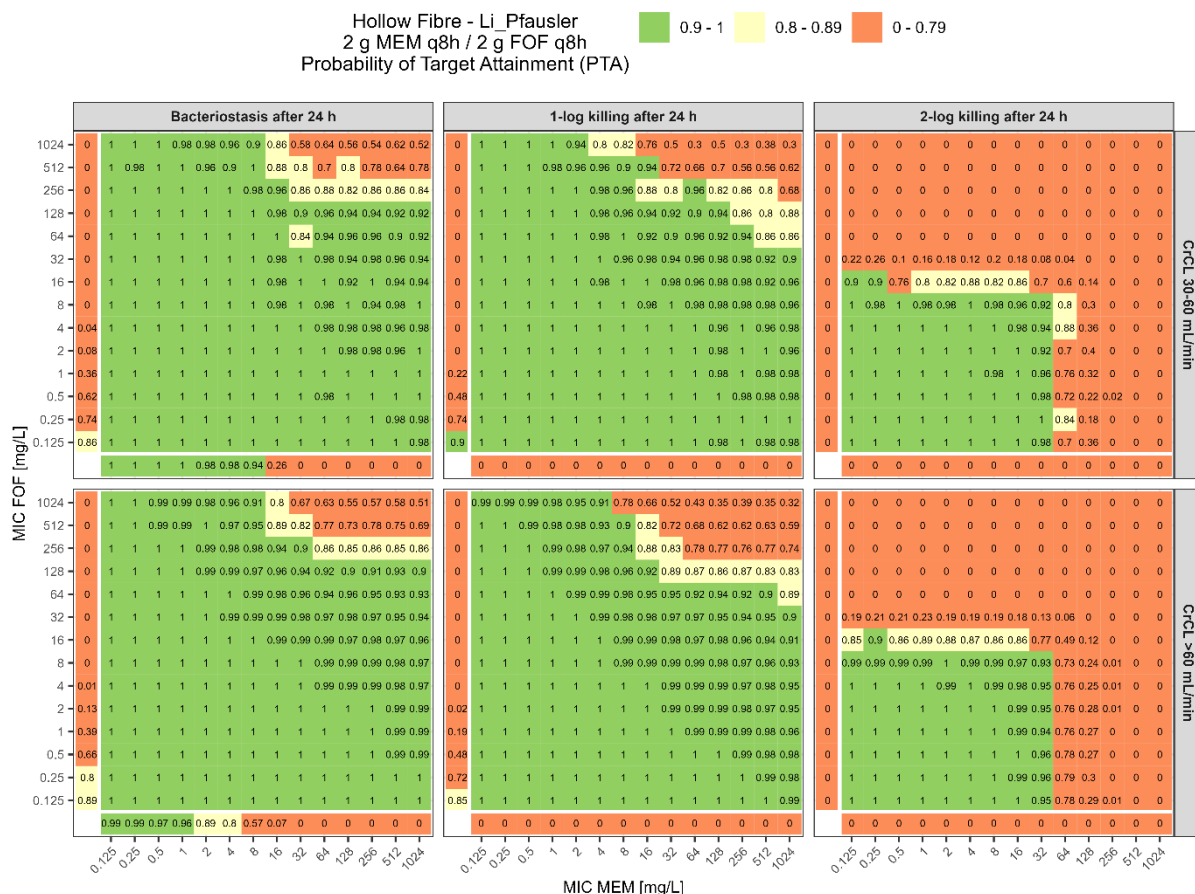


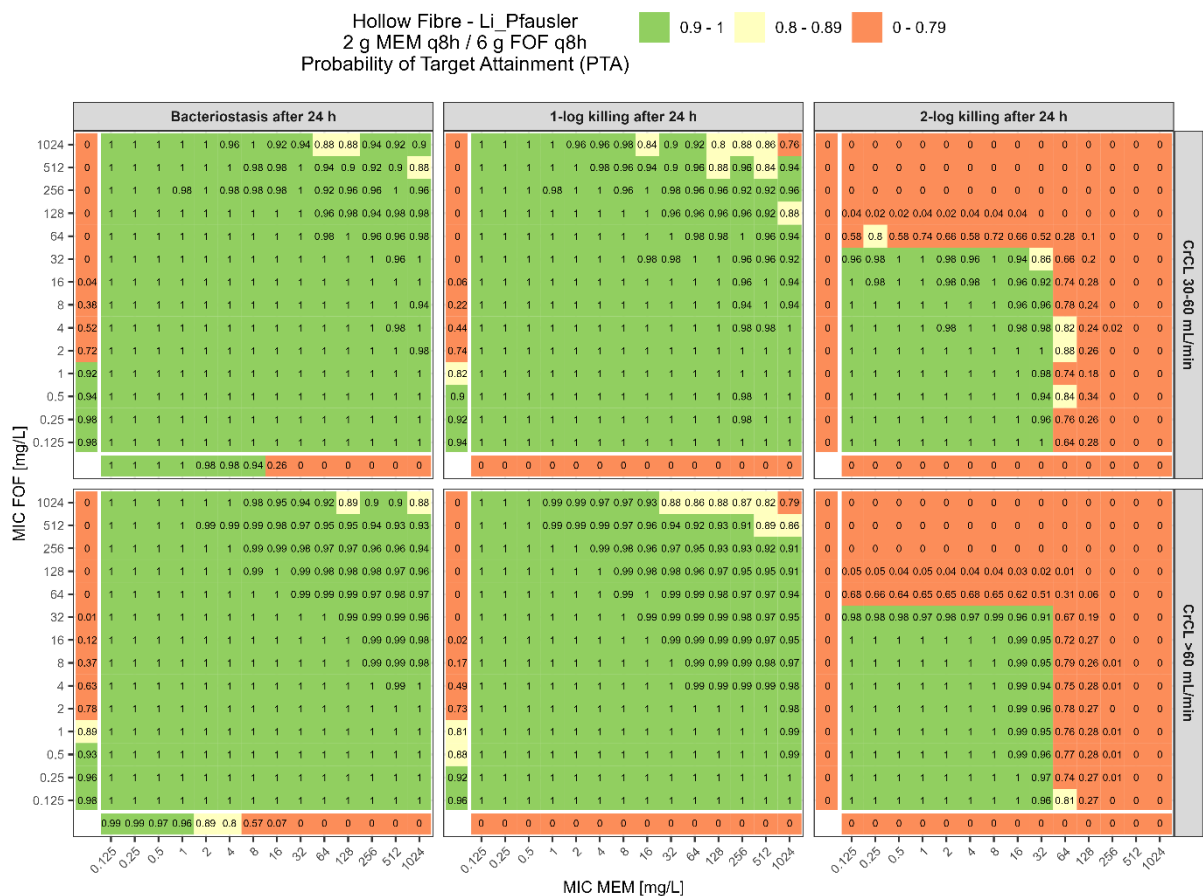
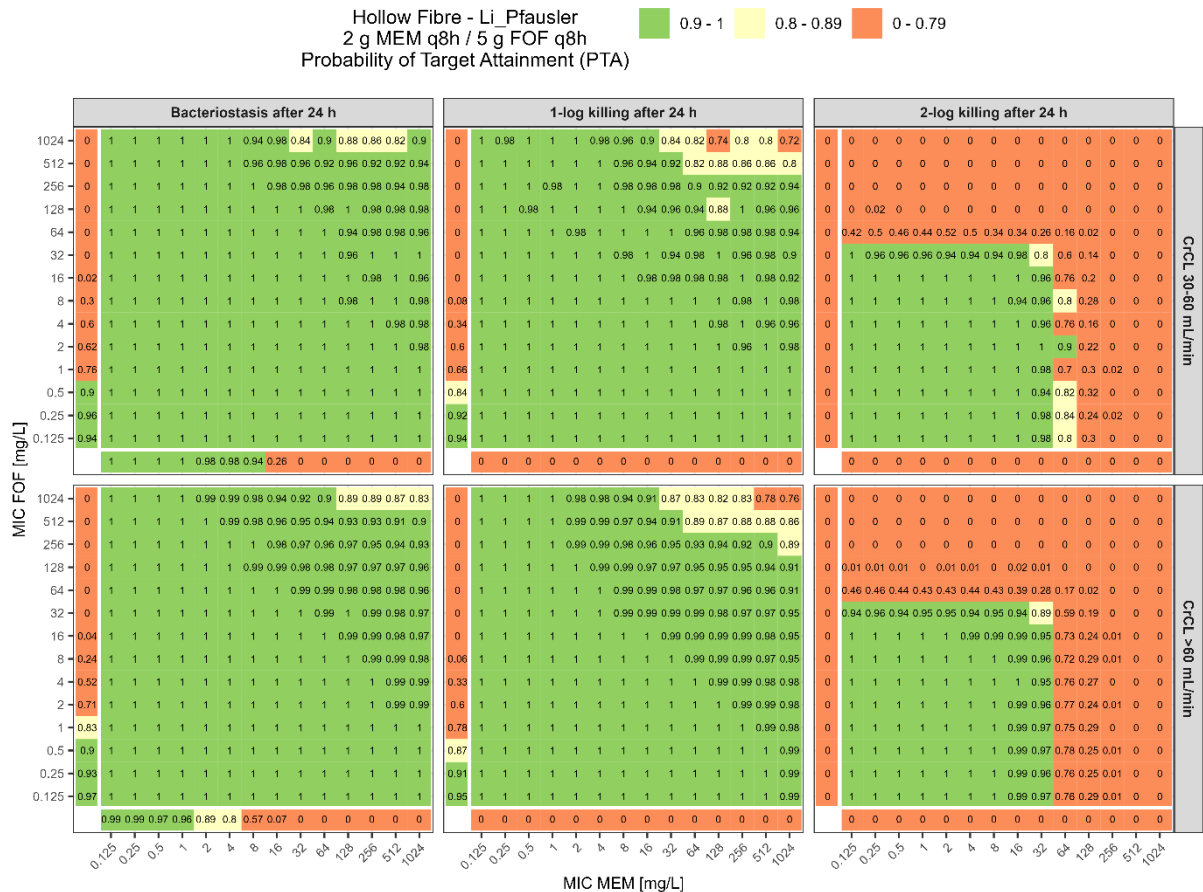


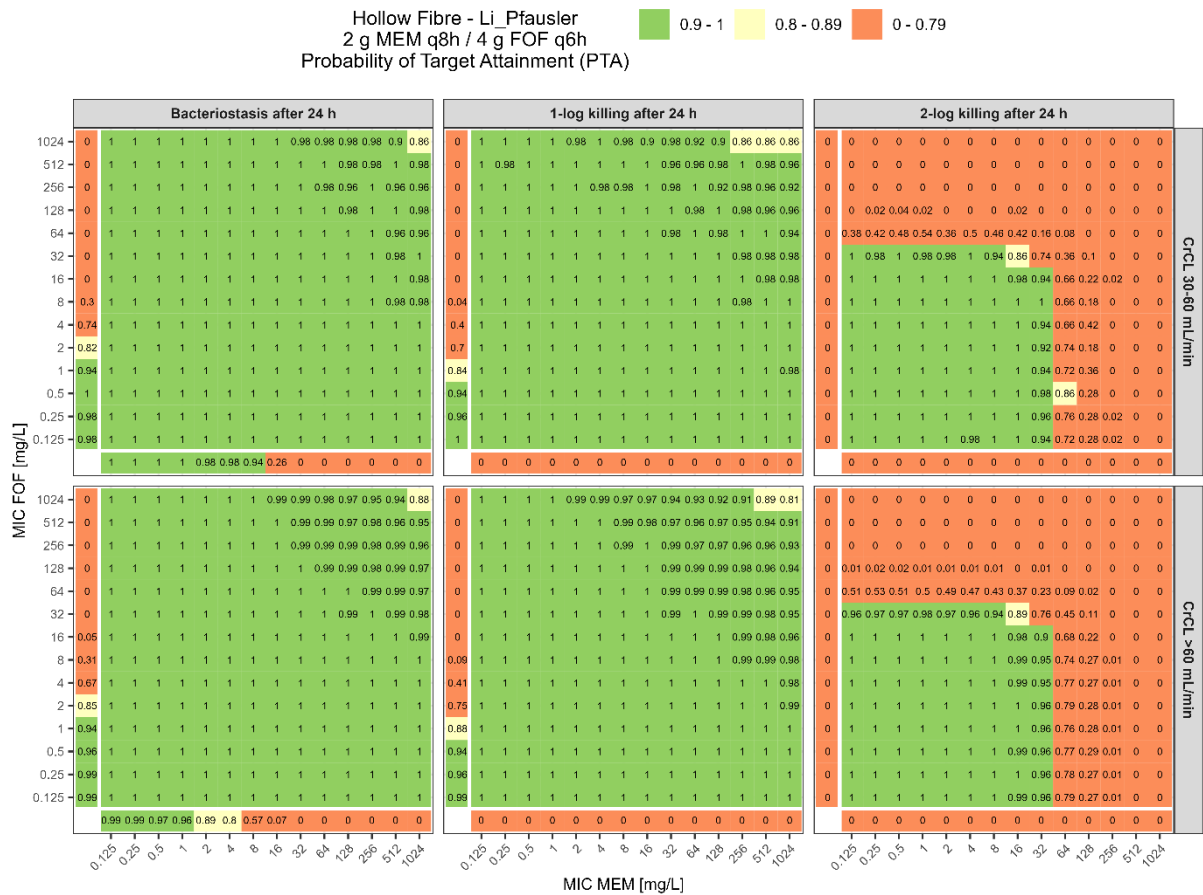
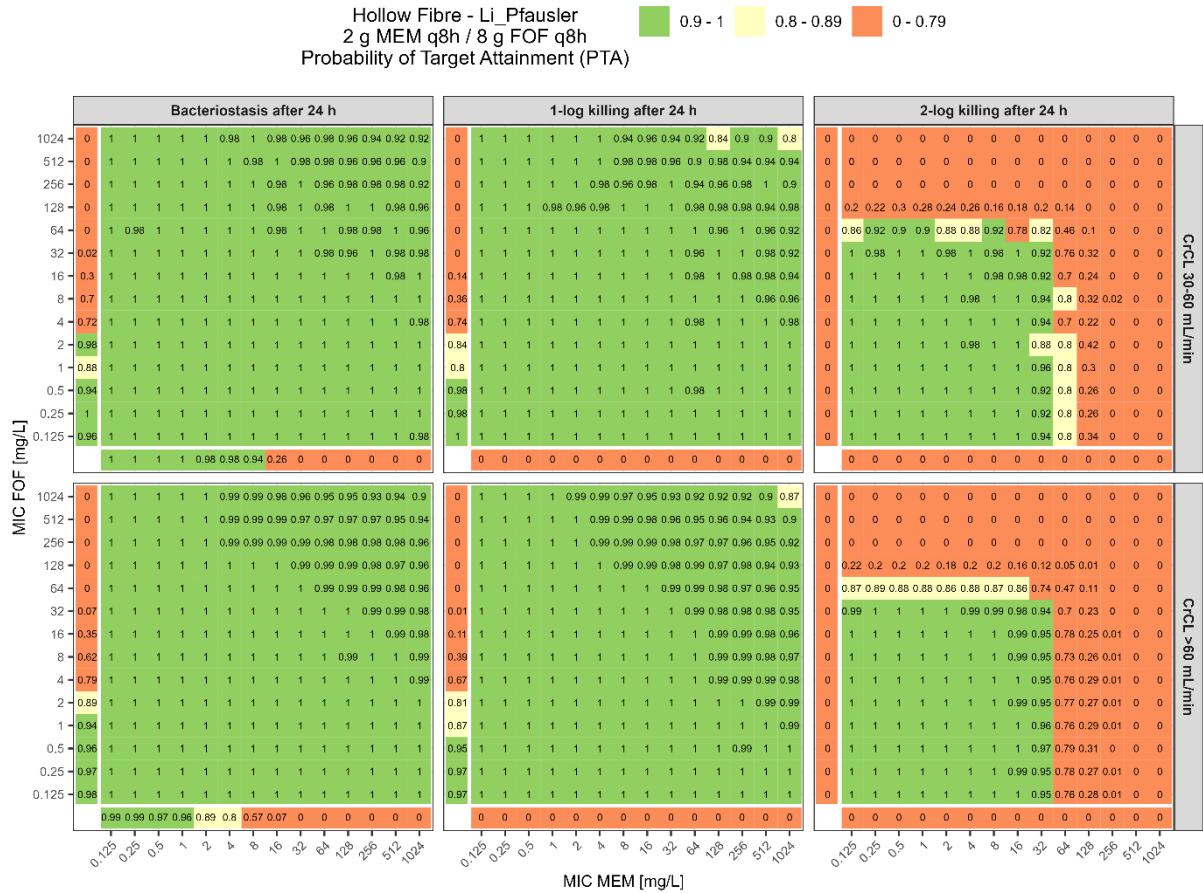
Open Flow - Lj_Merino-Bohorquez
2 g MEM q8h / 8 g FOF bolus + 16 g cont.
Probability of Target Attainment (PTA)

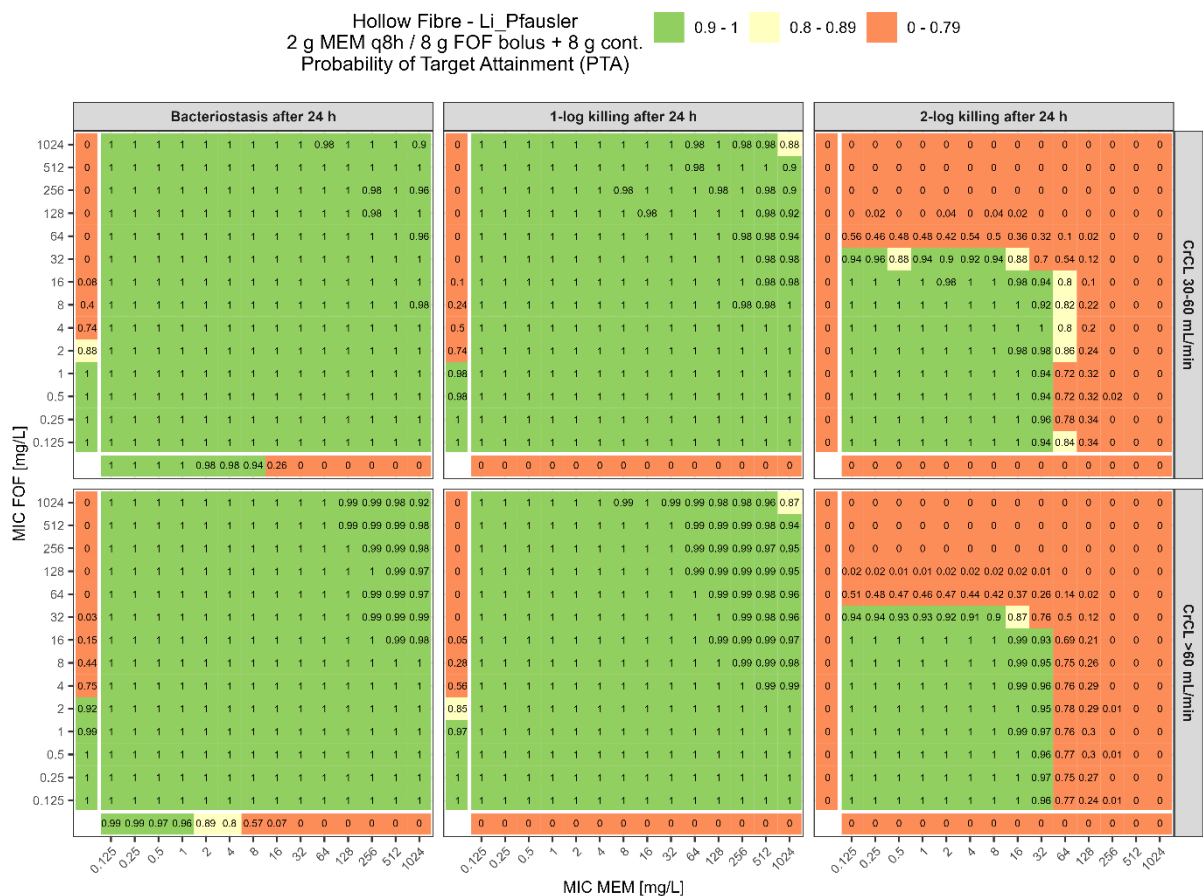
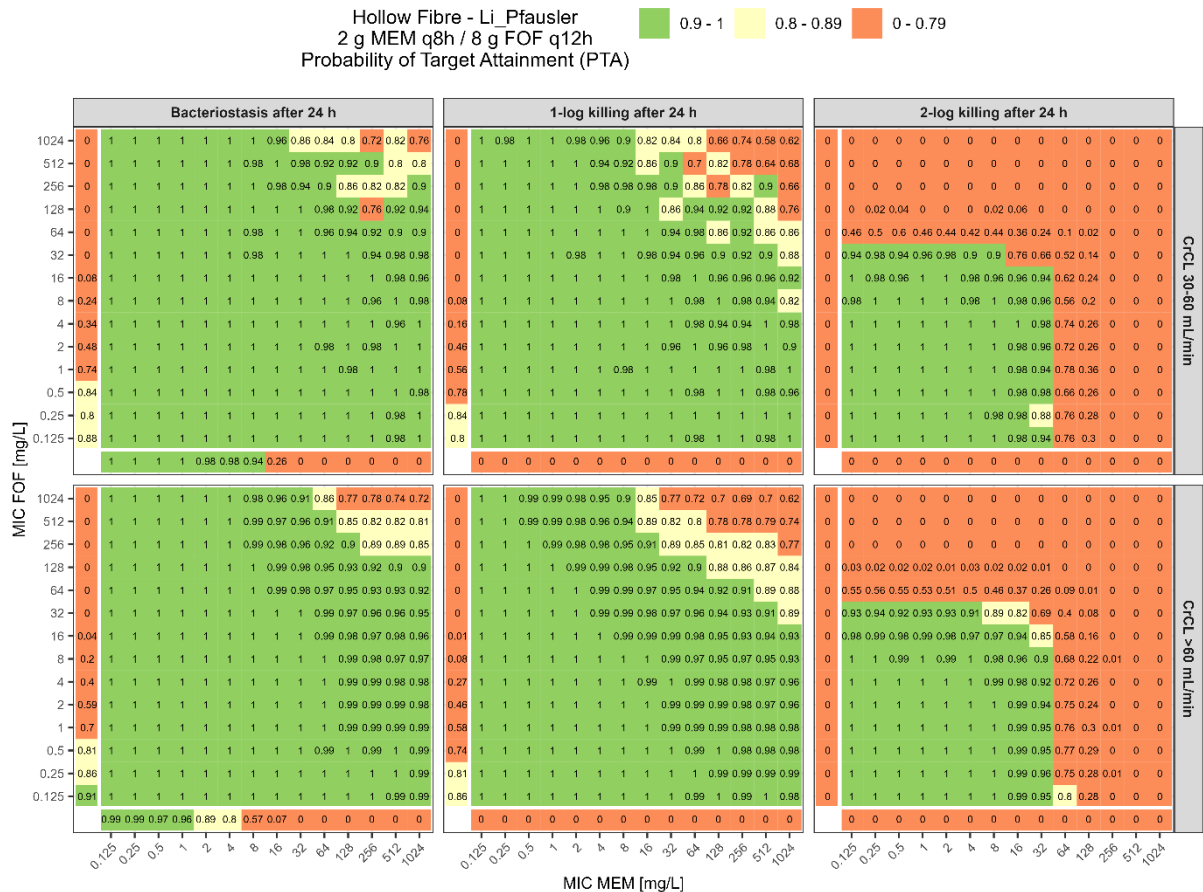
0.9 - 1 0.8 - 0.89 0 - 0.79





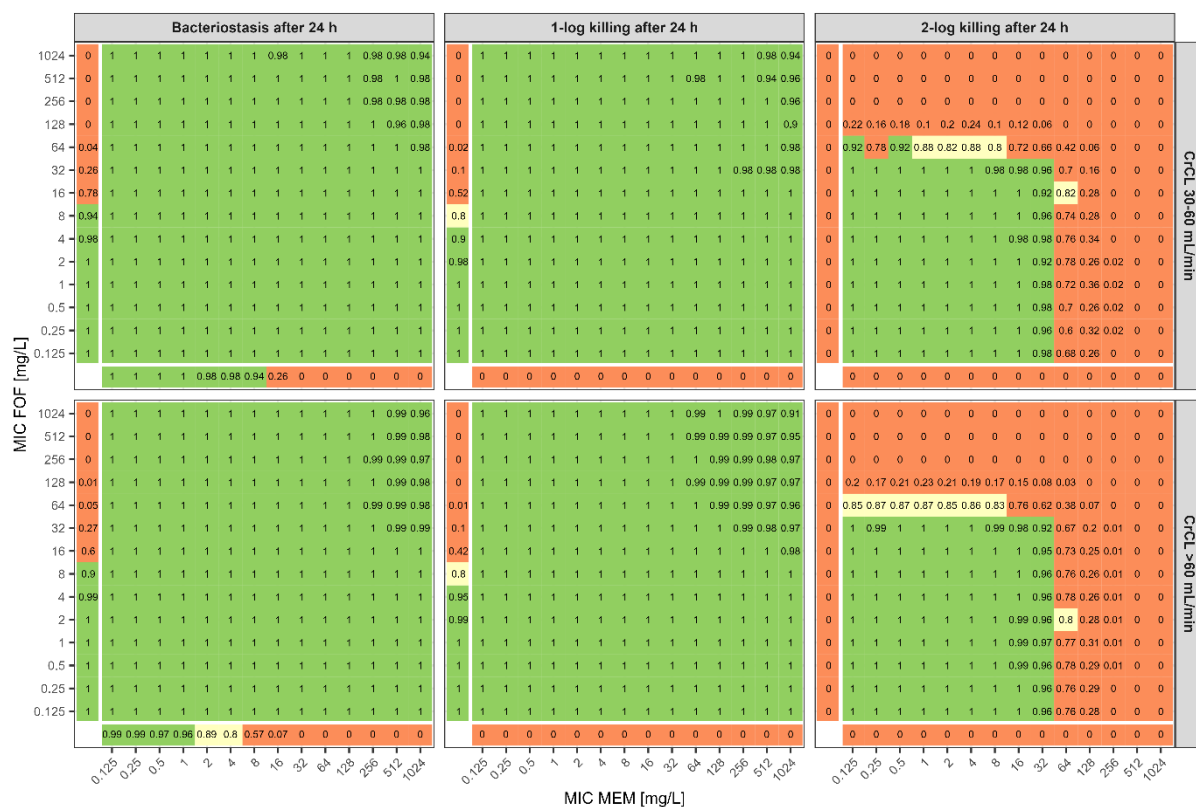


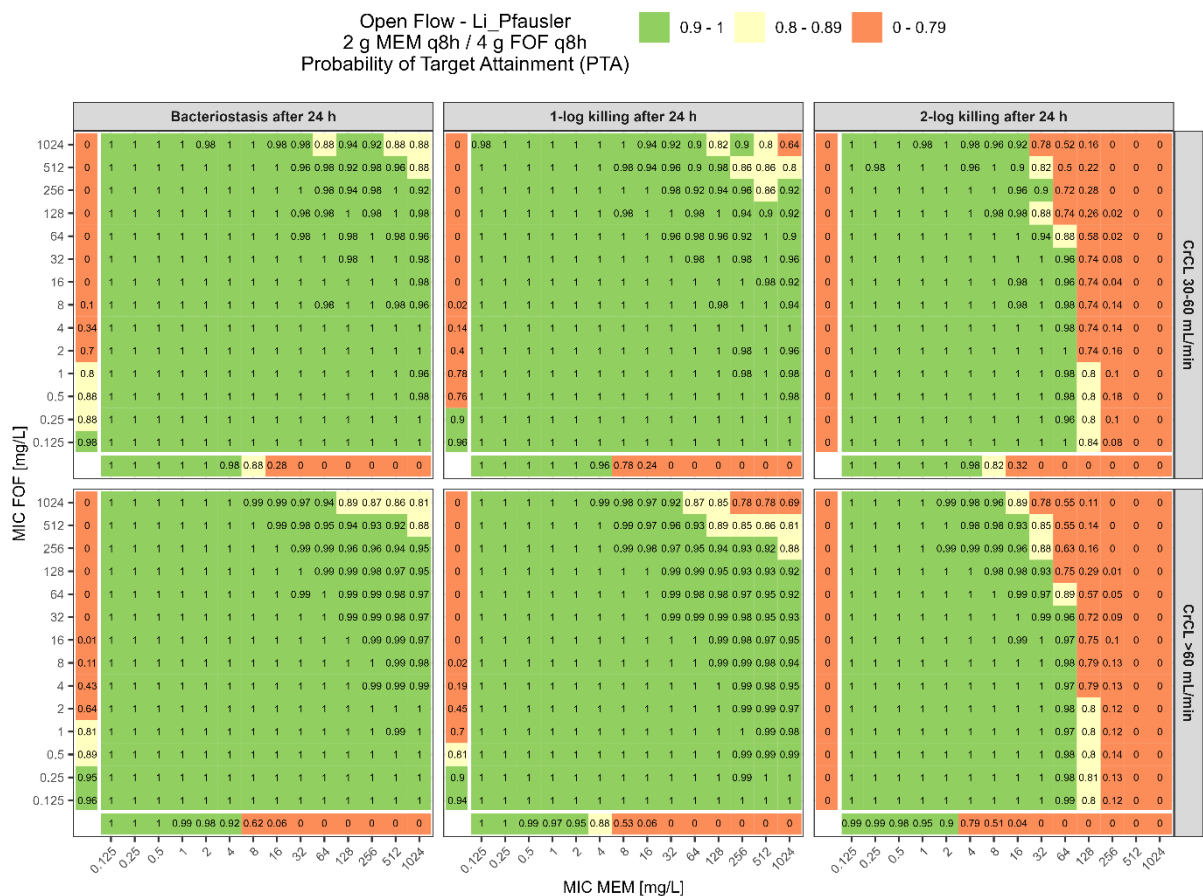
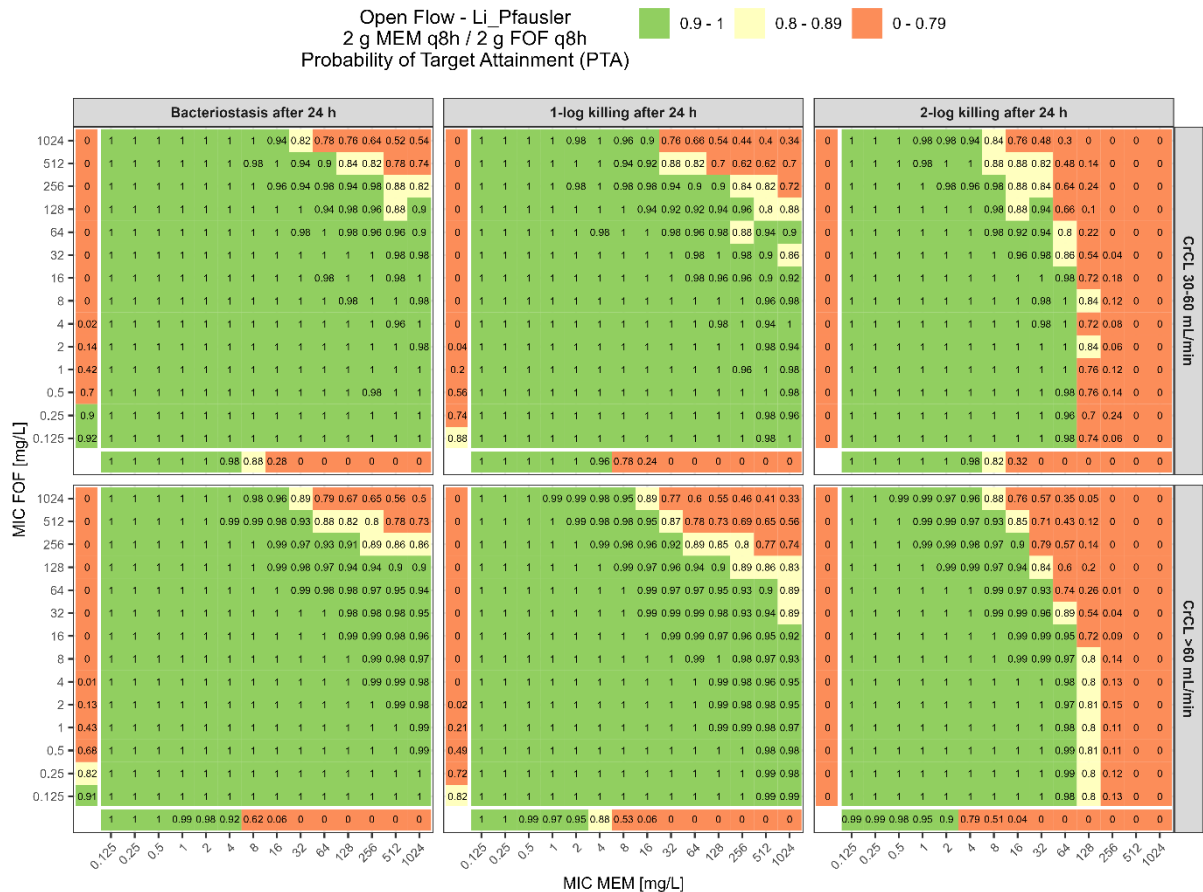


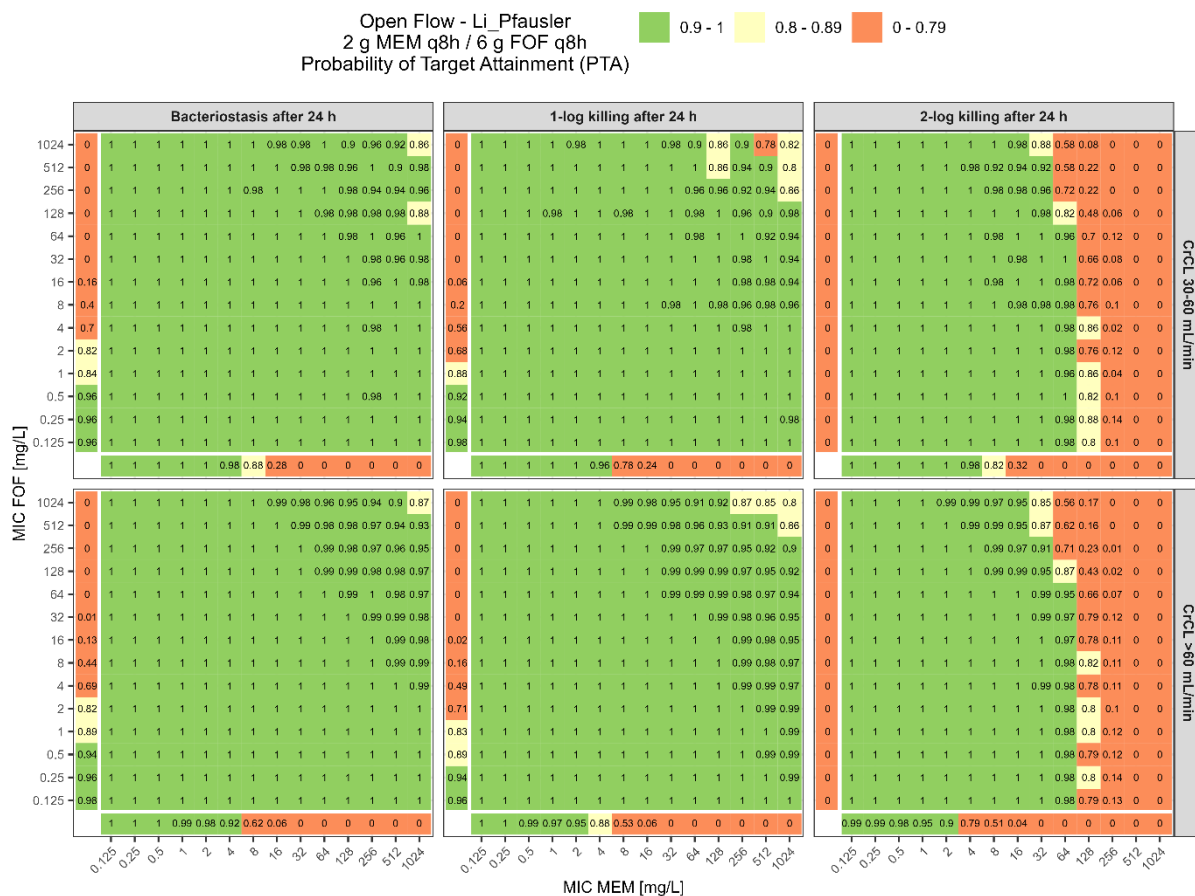
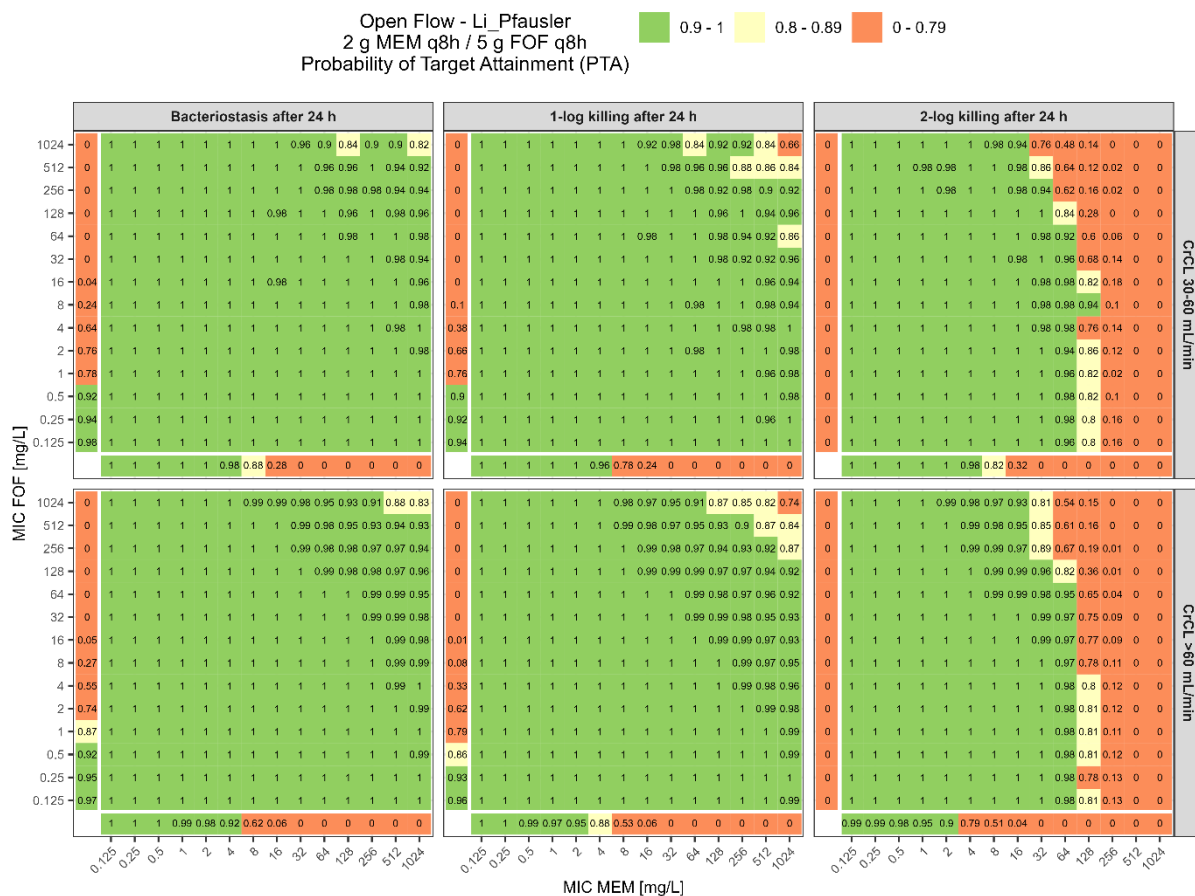


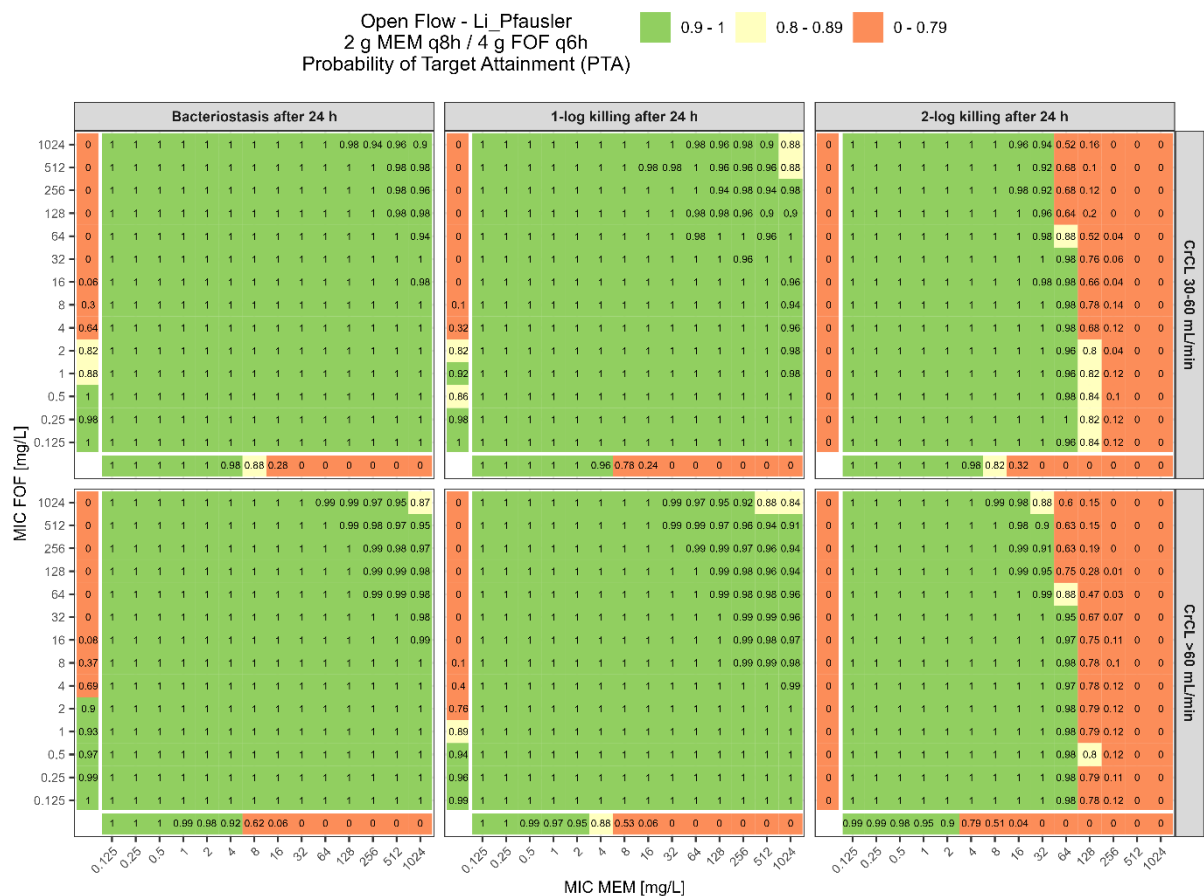
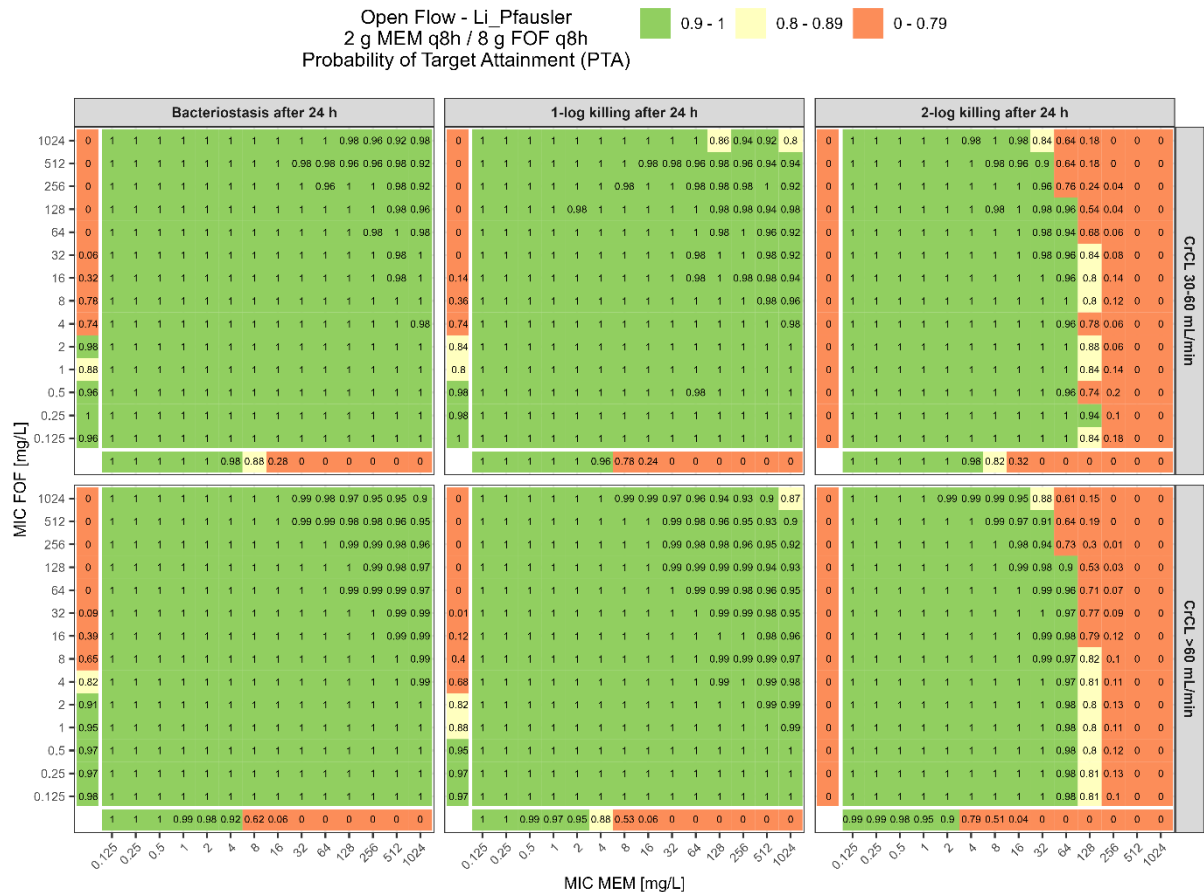
Hollow Fibre - Li_Pfausler
2 g MEM q8h / 8 g FOF bolus + 16 g cont.
Probability of Target Attainment (PTA)

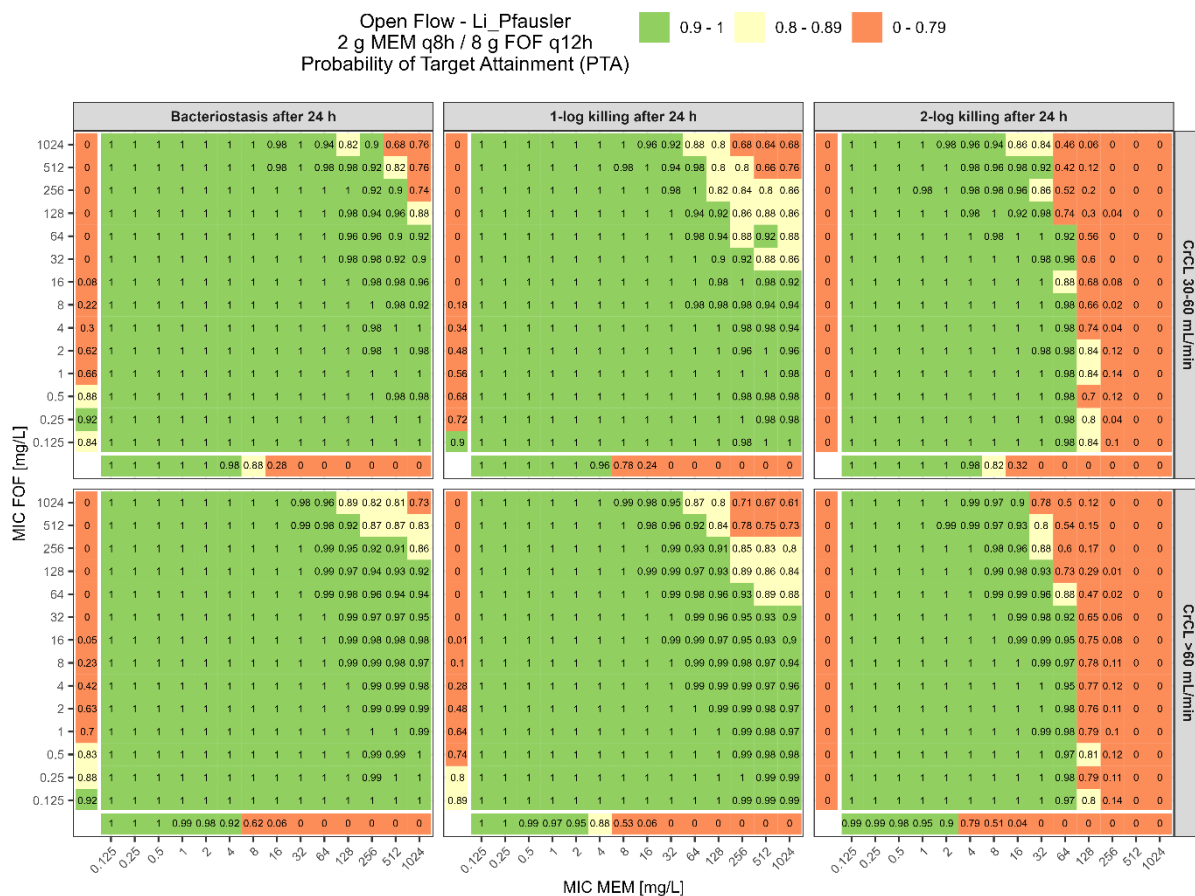
0.9 - 1 0.8 - 0.89 0 - 0.79









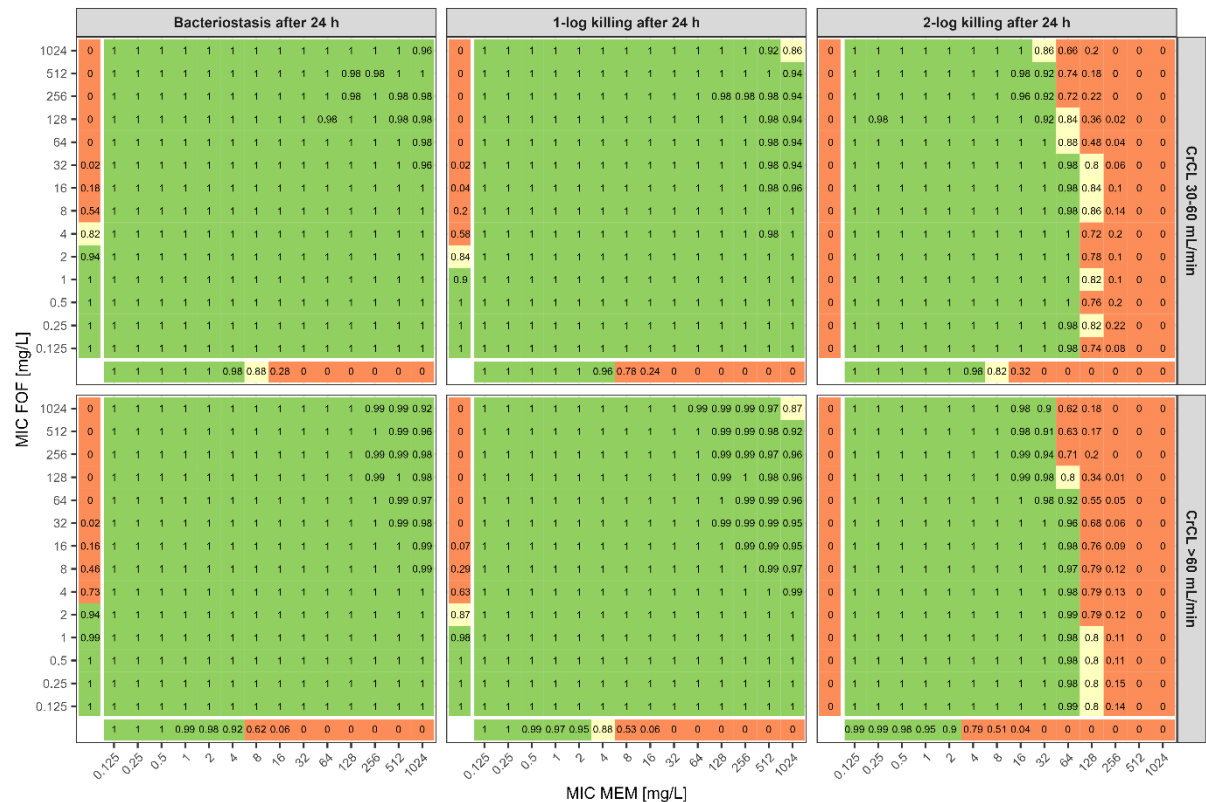


Open Flow - Li Pfausler
2 g MEM q8h / 8 g FOF bolus + 8 g cont.
Probability of Target Attainment (PTA)

0.9 - 1

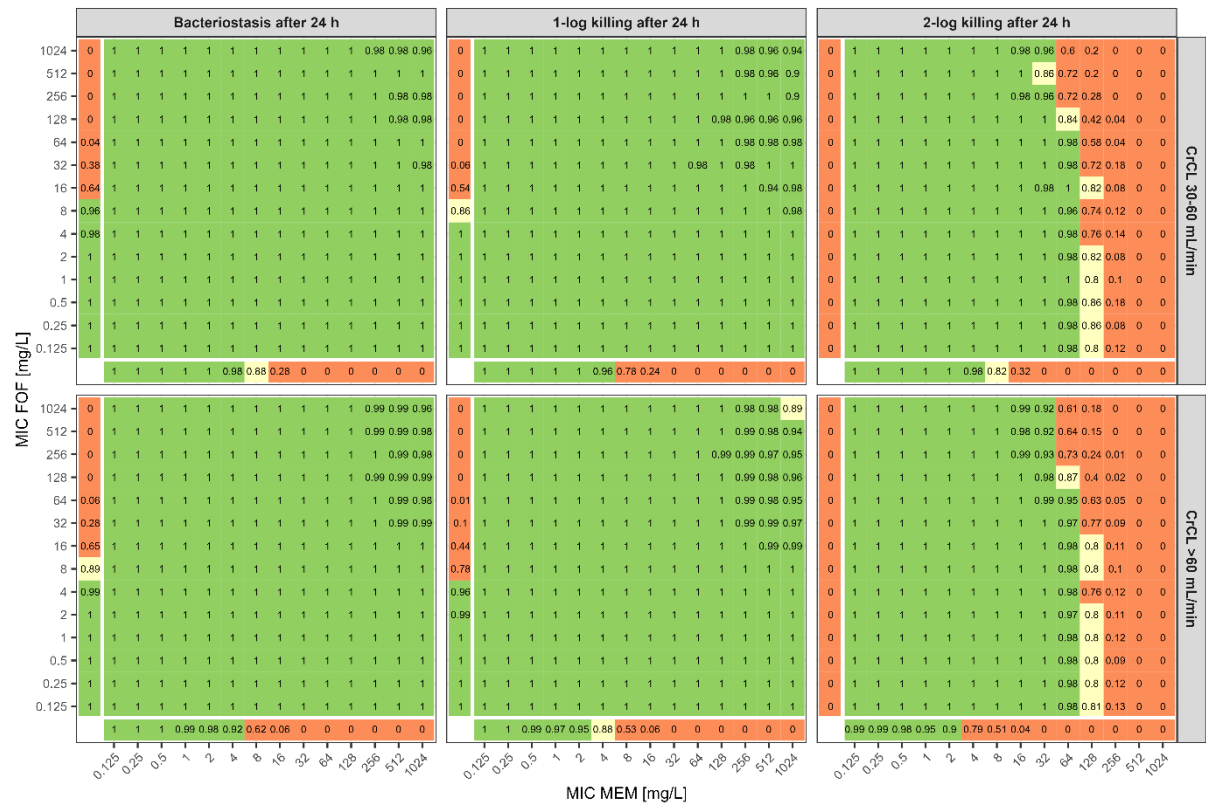
0.8 - 0.89

0 - 0.79



Open Flow - Li_Pfausler
2 g MEM q8h / 8 g FOF bolus + 16 g cont.
Probability of Target Attainment (PTA)

0.9 - 1 0.8 - 0.89 0 - 0.79



10 Hazardous materials

Table 1 List of the used hazardous substances in accordance with GHS including the GHS pictograms and hazard and precautionary statements

Substance	GHS pictogram	Signal word	Hazard statements	Precautionary statements
Acetonitrile		Danger	225, 302+312+332, 319	210, 280, 301+312, 303+361+353, 304+340+312, 305+351+338
Ascorbic acid	The substance is not classified by GHS according to regulation (EC) No 1272/2008.			
Avibactam		Warning	315, 319, 335	261, 264, 271, 280, 302+352, 304+340, 305+351+338, 312, 321, 362+364, 332+313, 337+313, 403+233, 405, 501
Ceftazidime		Danger	317, 334	261, 272, 280, 284, 302+352, 333+313
Ceftolozane		Danger	317, 334	261, 272, 280, 302+352, 333+313, 321, 362+364, 501, 284, 304+340, 342+311
Chloramphenicol		Warning	318, 351, 361fd	202, 280, 305+351+338, 308+313, 405, 501
Ethanol (absolute)		Danger	225, 319	210, 243, 280, 305+351+338, 403+235
Formic acid (98%)		Danger	226, 302, 314, 331	210, 280, 301+312, 303+361+353, 304+340+310, 305+351+338
Fosfomycin	The substance is not classified by GHS according to regulation (EC) No 1272/2008.			

Substance	GHS pictogram	Signal word	Hazard statements	Precautionary statements
Glucose-6-phosphate	The substance is not classified by GHS according to regulation (EC) No 1272/2008.			
Meropenem		Danger	317, 334	261, 272, 280, 284, 302+352, 333+313
Methanol		Danger	225, 301+311+331	210, 233, 280, 284, 302+352, 333+313
Pyruvate		Warning	317, 219	280, 302+352, 205+351+338, 337+313
Tazobactam		Danger	317, 334	261, 272, 280, 285, 302+353, 304+340, 333+313, 342+311, 362+364
Tigecycline		Warning	315, 317, 318, 360, 410	201, 202, 261, 264, 272, 273, 280, 302+352, 305+351+338, 308+313, 310, 321, 333+313, 362+364, 391, 405, 501
Tetracycline		Warning	361d, 411	201, 273, 308+313
Vaborbactam		Warning	302	264, 270, 301+312, 330, 501

11 Acknowledgements

In times of international conflicts and wars happening today in 2025, with ethnic groups trying to cleanse other ethnic groups, the little girl losing her father, the mother losing her little boy, the displaced trying to find safe shelter, the starving trying to find clean water or food, and at the same time most of the people in power closing their eyes over it...

I would like to thank Allah, the Owner of Majesty and Honour, that He has granted me a safe place to live, my family near to me, enough to eat and drink, and finally the privileged position of being able to educate myself and write this thesis. I hope I made enough efforts in using these privileges, and at the same time hope this work improves the situations of patients, and not only the situation of my own person and career.

I would like to thank Sebastian Wicha, an amazing supervisor and mentor, for teaching me a lot of things during this journey, especially how to conduct science the correct way. I will always proudly mention him as my first mentor in scientific practice!

I would like to thank my parents, Ammi and Papa, who tried to offer me and my siblings the world, only so that we can enjoy as much freedom as we want, in the best and most moral way possible.

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12 Eidesstattliche Versicherung

Hiermit versichere ich an Eides statt, die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt zu haben. Sofern im Zuge der Erstellung der vorliegenden Dissertationsschrift generative Künstliche Intelligenz (gKI) basierte elektronische Hilfsmittel verwendet wurden, versichere ich, dass meine eigene Leistung im Vordergrund stand und dass eine vollständige Dokumentation aller verwendeten Hilfsmittel gemäß der Guten wissenschaftlichen Praxis vorliegt. Ich trage die Verantwortung für eventuell durch die gKI generierte fehlerhafte oder verzerrte Inhalte, fehlerhafte Referenzen, Verstöße gegen das Datenschutz- und Urheberrecht oder Plagiate.

12. Mai 2026

Hamburg, den _____



Aneeq Farooq