2019

CARBAPENEM-RESISTANT *ENTEROBACTERIACEAE*: EPIDEMIOLOGY, GENETICS, *IN VITRO* ACTIVITY, AND PHARMACODYNAMIC MODELING

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Digital Object Identifier: https://doi.org/10.13023/etd.2019.009

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CARBAPENEM-RESISTANT *ENTEROBACTERIACEAE*: EPIDEMIOLOGY, GENETICS, *IN VITRO* ACTIVITY, AND PHARMACODYNAMIC MODELING

DISSERTATION

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Pharmacy at the University of Kentucky

By

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Lexington, Kentucky

Director: Dr. David Burgess, Professor of Pharmacy Practice and Science

Lexington, Kentucky

2019

ABSTRACT OF DISSERTATION

CARBAPENEM-RESISTANT *ENTEROBACTERIACEAE*: EPIDEMIOLOGY, GENETICS, *IN VITRO* ACTIVITY, AND PHARMACODYNAMIC MODELING

Background: Infections caused by carbapenem-resistant *Enterobacteriaceae* (CRE) such as *Escherichia coli* and *Klebsiella pneumoniae* are among the most urgent threats of the infectious disease realm. The incidence of these infections has been increasing over the years and due to very limited treatment options, mortality is estimated at about 50%. By 2050, mortality from antimicrobial resistant infections is expected to surpass cancer at 10 million deaths annually.

Methods: We evaluated 18 contemporary antimicrobials against 122 carbapenem-resistant *Enterobacteriaceae* using a variety of antimicrobial susceptibility testing methods according to Clinical Laboratory Standards Institute guidelines. Time-kill studies were performed on clinical isolates with variable resistance to meropenem, amikacin, and polymyxin B. Phenotypic expression assays were performed on all isolates and whole genome sequencing was performed on 8 isolates to characterize molecular resistance mechanisms. Pharmacodynamic modeling of meropenem and polymyxin B was also conducted.

Results: CRE were primarily *K. pneumoniae*, and *Enterobacter* spp. 60% expressed *Klebsiella pneumoniae carbapenemase* (KPC) only, 16% expressed Verona Integron-encoded Metallo-beta-lactamase (VIM) only, 5% expressed KPC and VIM, and 20% expressed other mechanisms of resistance. Antimicrobial susceptibility testing indicated the most active antimicrobials against CRE were ceftazidime/avibactam, imipenem/relebactam, amikacin, tigecycline, and the polymyxins. Etest® strips did not reliably measure polymyxin B resistance. The automated testing system, BD Phoenix™, consistently reported lower MICs than the gold standard broth microdilution. Time-kill studies showed regrowth at clinically achievable concentrations of meropenem alone (4, 16, and 64 mg/L), polymyxin B alone (0.25 and 1 mg/L), or amikacin alone (8 and 16 mg/L), but combinations of meropenem with either polymyxin B or amikacin were bactericidal and synergistic. Meropenem administered simultaneously or prior to polymyxin B exhibited superior activity to polymyxin B administered first.

Conclusions: Novel carbapenemase-inhibitor combinations (ceftazidime/avibactam and imipenem/relebactam) exhibit the best activity against KPC-producing CRE. The polymyxins, amikacin, and tigecycline exhibit the best activity against VIM-producing CRE. Meropenem in combination with polymyxin B is bactericidal and synergistic when the meropenem MIC is ≤32 mg/L, and meropenem should never be administered after polymyxin B. Meropenem and amikacin is bactericidal and synergistic when the amikacin MIC is ≤16 mg/L. Etest® strips should not be used for characterizing polymyxin B or colistin activity. Clinicians should be aware that automated testing systems may produce biased susceptibility results relative to the gold standard method, broth microdilution, which may influence interpretation of *in vitro* results.

KEYWORDS: Carbapenem-resistant *Enterobacteriaceae*, antimicrobial susceptibility testing, time-kill, whole-genome sequencing, pharmacodynamic modeling

Brandon Kulengowski

1/24/2019

CARBAPENEM-RESISTANT *ENTEROBACTERIACEAE*: EPIDEMIOLOGY, GENETICS, *IN VITRO* ACTIVITY, AND PHARMACODYNAMIC MODELING

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This dissertation is dedicated to Dr. David Burgess whose passion for research and leadership is only surpassed by his love for teaching and mentorship of students. I would not be where I am today without his vision, his patience, and his support of all that I have done. For this, I am most thankful and hope I have made him proud.

ACKNOWLEDGEMENTS

I am very grateful to the many contributions and insights provided by the faculty of the University of Kentucky College of Pharmacy. Specifically, I want to thank my dissertation director, Dr. David Burgess, for always knowing how to motivate and inspire me throughout both the research and the writing process. He always made himself available for discussion and has greatly facilitated my journey by assisting in design, implementation, presentation, and publication of experiments. I also thank the director of graduate studies and member of my dissertation committee, Dr. Dave Feola, for contributing to experimental design, intellectual discussion of projects, and publications. I thank Dr. Julie Ribes for collaboration leading to publication and for guidance on my dissertation project. Dr. Chee Ng also deserves my gratitude for his invaluable insights regarding mathematical modeling and for guiding the final stages of the pharmacodynamic mathematical model. I further thank Dr. Jeff Campion for providing not only valuable experimental design and interpretation guidance, but also personally assisting in some experiments and analyses. Furthermore, he contributed tremendously to the mathematical model and my understanding of the Win Nonlin modeling language as well as contributed to publications. Finally, I thank Dr. Grace Lee at the University of Texas at Austin for her guidance on whole genome sequencing, interpretation of genetic data, and for contributions leading to publication.

I am also thankful for those not directly involved in this project. First, Dr. Diane Snow, director of honors and undergraduate research, has been critical to my journey. She mentored me throughout my undergraduate career and introduced me to research. Without her time and early investment, I would not be where I am today. My family and friends also deserve acknowledgement for the love and support they provided throughout my student career. First, my wife Heather was always by my side to assist where needed– emotionally, and even technically in experiments. My mother and father contributed emotionally and financially which allowed me to dedicate the time needed to complete this project. Finally, my friends and colleagues are too numerous to name individually, but each one has contributed to some degree to the completion of this project whether by respite, intellectual discussion, technical assistance, and even formatting of the dissertation.

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Chapter One:

Antimicrobial Resistance

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Infectious disease treatment and management in patients is difficult when resistance to contemporary antimicrobial agents is involved. In fact, expression of resistance renders antimicrobial agents less effective and is associated with poor clinical outcomes, including increased mortality.[1-10] Unfortunately, resistance usually follows the development of any novel antimicrobial, given enough time (Figure 1.1).[11] Commonly known pathogens such as *Escherichia coli* and *Klebsiella pneumoniae* are among the most notorious for expression of drug resistance because they exist as part of the normal flora in the gastrointestinal tract of humans. Frequent antimicrobial consumption fosters development of drug resistance among these enteric bacteria for which novel antimicrobials are dwindling and currently available antimicrobials are few.[12] With this in mind, efforts such as the present work contribute information to questions such as: what antimicrobials alone or in combination provide patients with the greatest chance of survival when confronted by these highly resistant pathogens? Do particular agents work better together than others? How many antimicrobial agents are sufficient to ensure a high probability of recovery?

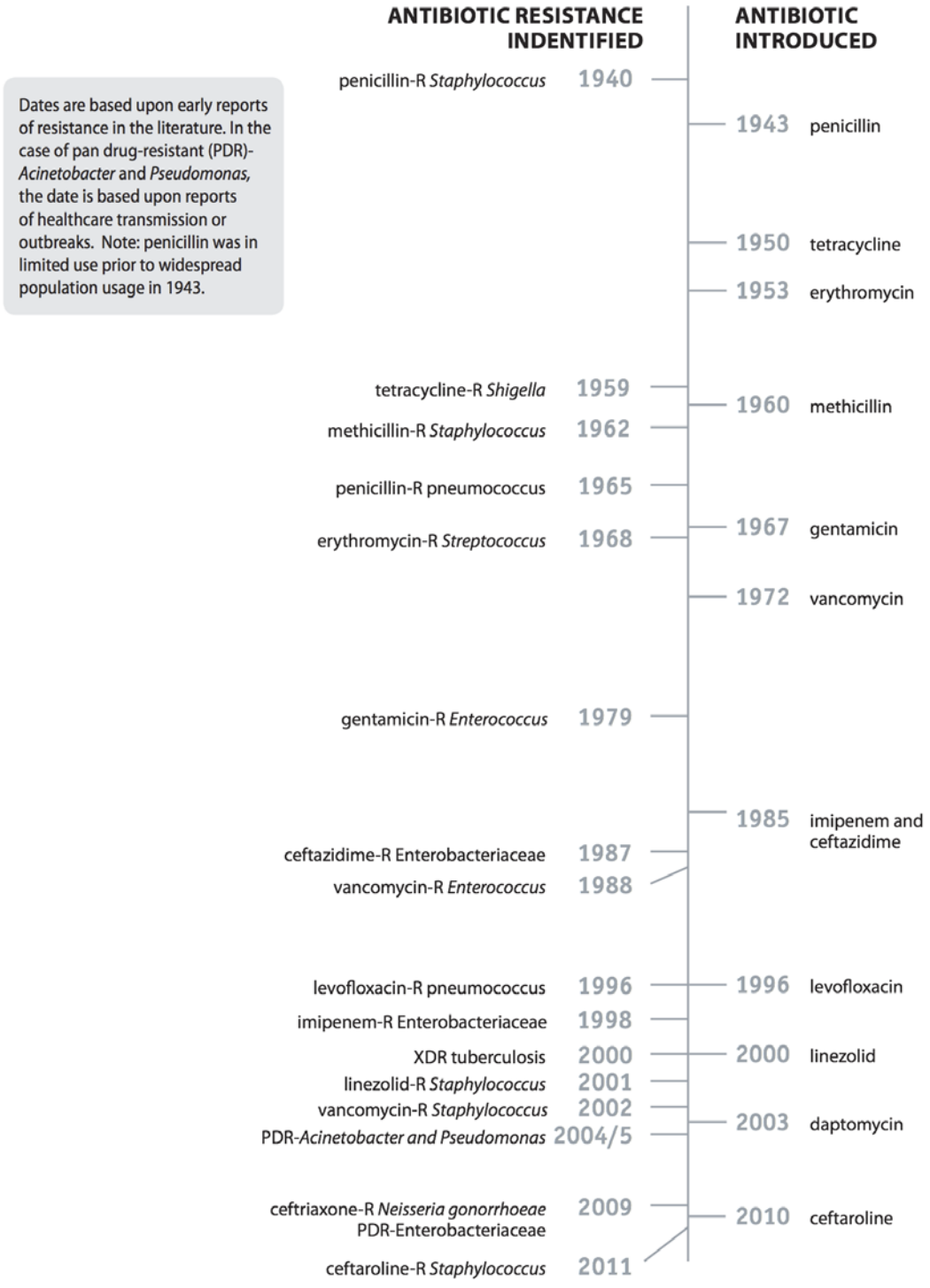


Figure 1.1: Antimicrobial Resistance Timeline. Reprinted[11]

In March 2015, the U.S. Department of Health and Human Services (HHS) established the President's Advisory Council on Combating Antibiotic-Resistant Bacteria (PACCARB), which is responsible for providing advice, information, and recommendations to the Secretary of Health and Human Services regarding programs and policies from the National Action Plan. Within this plan is the Centers for Disease Control and Prevention (CDC) 2013 report which categorized a variety of antimicrobial resistance problems based on seven factors associated with resistant infections—clinical impact, economic impact, incidence, 10-year projection of incidence, transmissibility, availability of effective antibiotics, and barriers to prevention.

Three threat levels were identified using these seven factors (Figure 1.2). Serious threats include organisms such as extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*, vancomycin-resistant *Enterococcus* (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), multidrug resistant *Pseudomonas aeruginosa*, and drug-resistant *Tuberculosis*, among others. Antimicrobial resistance in gram-negative organisms, specifically carbapenem-resistant *Enterobacteriaceae* (CRE), were among the highest of threat levels, designated as urgent (Figure 1.2).[11] The present work focuses on this group of urgent threat level pathogens.

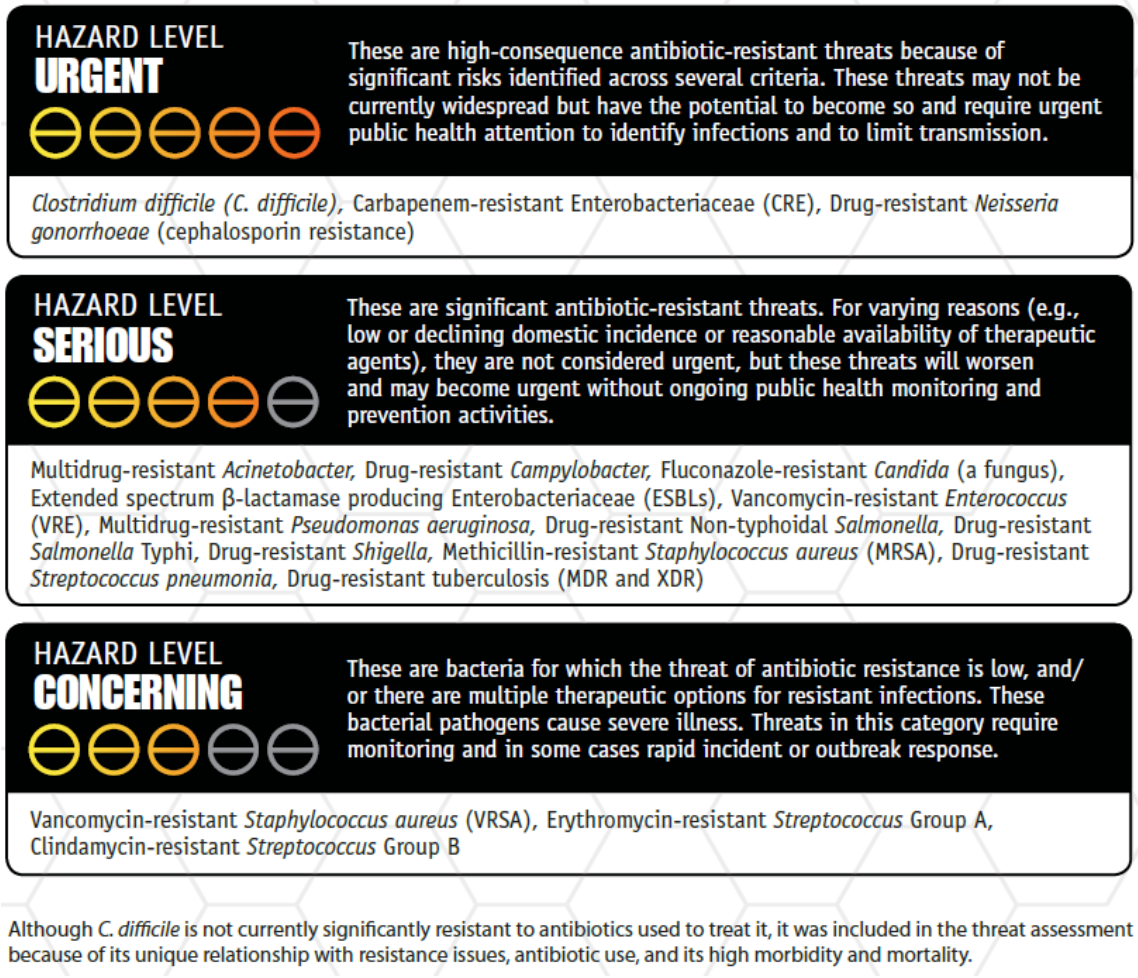


Figure 1.2: Antimicrobial Resistance Threat Levels. Reprinted[11]

Mechanisms of Resistance

Understanding mechanisms of resistance is important because these mechanisms shape the direction of research and the choice of therapy. For example, in the interest of obtaining clinically relevant information, it may be more advantageous to focus on characterizing the more common mechanisms of resistance rather than the least common where knowledge about a certain mechanism of resistance may afford therapeutic advantages (e.g. the sustained activity of aztreonam against exclusively metallo-β-lactamase producing organisms). For this reason, a general discussion of mechanisms of resistance is presented here, and more detail can be found in Chapter 2: Carbapenem-resistant *Enterobacteriaceae* (CRE).

Resistance mechanisms fall generally into four categories. 1) efflux pumps actively removing antimicrobials from the target site of action; 2) enzymatic degradation of the antimicrobial agent (e.g. β-lactamases);[13] 3) changes in cell wall permeability which may slow or prevent the antimicrobial from reaching the target site (e.g. mutations in porin channels); and 4) target site alterations that prevent the antimicrobial from binding (Figure 1.3). However, bacteria are not limited to only one mechanism. In fact, they often exhibit multiple mechanisms, which may confer resistance to multiple classes of antimicrobials. Concerning the focus of this dissertation, carbapenem-resistant *Enterobacteriaceae*, enzymatic degradation is the most common mechanism driving this phenotype.[14]

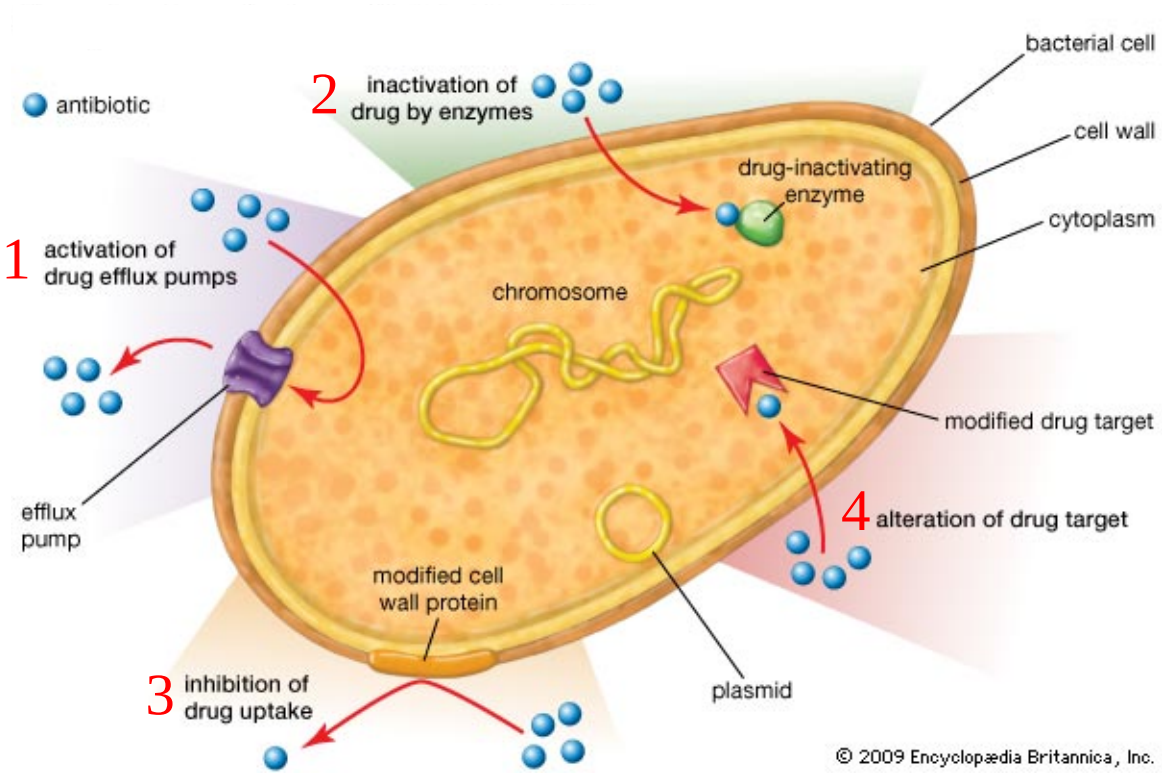


Figure 1.3: Mechanisms of Antibiotic Resistance**.** Reprinted[15]

History of β-lactam Resistance Development

Penicillin, the original β-lactam, was first administered to Anne Miller in 1942 as treatment for a streptococcal bloodstream infection. However, bacteria have been evolving to survive long before the introduction of antibiotics to humans. In fact, Edward Abraham and Ernst Chain identified a mechanism of penicillin resistance in 1940, two years before penicillin was administered to Anne Miller.[16] They discovered that a particular strain of *Escherichia coli* produced AmpC, an enzyme capable of inactivating penicillin (see Classification of β-lactamases). It was subsequently found that previously penicillin-sensitive *Staphylococcus aureus* could be made penicillin resistant after continuous subculture in the presence of penicillin *in vitro.*[17] By 1943, one year following human introduction to penicillin, four penicillin-resistant staphylococci strains were isolated from patients during the course of treatment.[18] The predominant mechanism of resistance among *Staphylococcus aureus* at this time was discovered to be production of β-lactamases, referred to now as "penicillinases."[19,20]

In response, the dose of penicillin was increased to compensate for reduced susceptibility;[21] but by 1947, a majority of hospital *Staphylococcus aureus* isolates were entirely resistant to penicillin.[22] To counter the growing resistance rates, chemists developed anti-staphylococcal penicillins (e.g. methicillin) for gram-positive organisms such as *Staphylococcus aureus* and *Staphylococcus epidermidis* and aminopenicillins (e.g. ampicillin) for gram-negative organisms such as *Escherichia coli, Klebsiella pneumoniae*, and *Serratia marcescens*.[23] These novel antimicrobials were not hydrolyzed by early penicillinase-producing organisms.

Eventually, β-lactamases developed in gram-negative organisms that conferred resistance to aminopenicillins (classified as TEM-1 and TEM-2 in organisms like *E. coli* and SHV-1 in *K. pneumoniae*; Figure 1.4). These enzymes were countered by the development of β-lactam/β-lactamase inhibitor combinations (e.g. ampicillin/sulbactam) and cephalosporins. These new compounds functioned in the presence of early β-lactamases such as TEM-1, TEM-2, and SHV-1, but once again, resistance developed with AmpC and ESBL-production. At first, ESBLs in the U.S. were point mutations of the TEM and SHV families, of which there are now hundreds of different subtypes. However, other ESBL families also developed and spread from other parts of the world, such as the current second-largest group—CTX-M—originally from the chromosome of *Kluyvera spp*.[24] OXA-type β-lactamases are another example of an ESBL, originally discovered in *Pseudomonas aeruginosa* isolated in Turkey.[25] There are myriad other β-lactamase families (PER,[26,27] VEB,[28] GES,[29,30] BES,[31] TLA,[32] SFO,[33] and IBC[34,35]), discovered from diverse geographic locations, and even some chromosomally located ESBLs.[36]

According to the latest report by the CDC, it is estimated that 19% of healthcare-associated *Enterobacteriaceae* infections in the U.S. are now caused by ESBL-producing organisms. Of the most common species of *Enterobacteriaceae*, 23% of *Klebsiella pneumoniae* and 14% of *Escherichia coli* infections now produce ESBL.[11] For reference, ten years prior, the National Healthcare Safety Network (NHSN) estimated 1% of *Klebsiella pneumoniae* infections and 0.5% of *E. coli* infections produced ESBLs. In other parts of the world, ESBL-producing bacteria are as high as 52% in Thailand,[37] and 70% in Egypt.[38] Eastern Europe has also reported rates as high as 25-50%.[39] Generally speaking, the rate of ESBLs in Europe is higher than that of the U.S., but lower than Latin American

or Asia.[40] The antimicrobial agents of choice to use against ESBL-producing organisms are the carbapenems, of which there are four – ertapenem, imipenem, meropenem, and doripenem. However, we have reached the latest era of antimicrobial resistance—carbapenemases. These will be discussed in more detail in Chapter 2: Carbapenem-resistant *Enterobacteriaceae* (CRE), but briefly, the two most common carbapenemases are *Klebsiella pneumoniae* carbapenemases (KPCs; more common in the U.S.) and metallo-β-lactamases (MBLs; more common in Europe and Southeast Asia).[13]

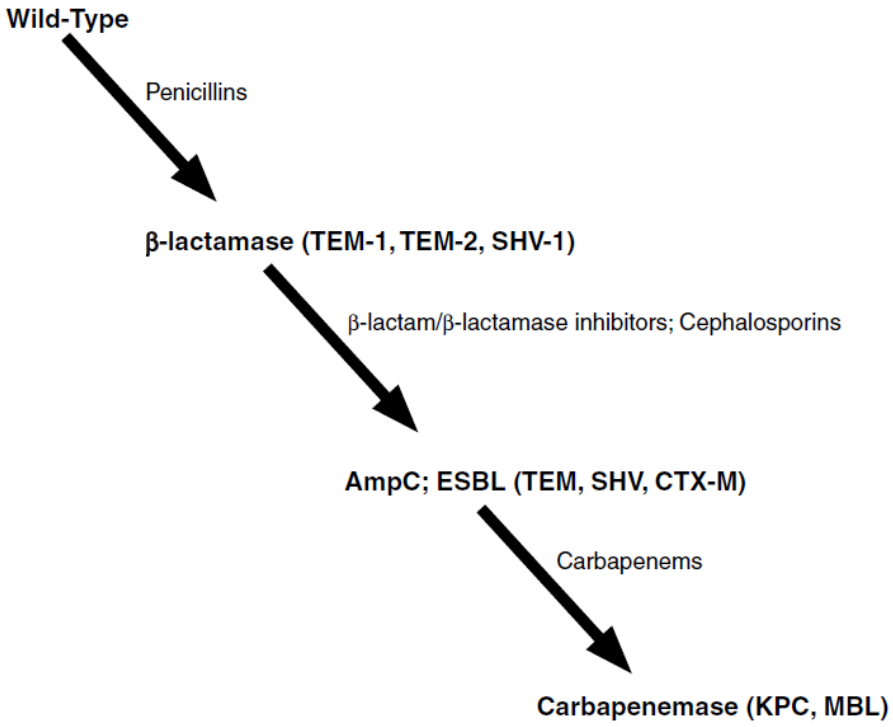


Figure 1.4: The Evolution of β-lactamases. Reprinted[13]
ESBL = extended-spectrum β-lactamase
KPC = *Klebsiella pneumoniae* carbapenemase
MBL = metallo-β-lactamase
TEM-1, TEM-2, SHV-1, TEM, SHV, CTX-M = types of β-lactamases

Classification of β-lactamases

To date, many attempts have been made to categorize β-lactamase enzymes, but these classification schemes can be summarized in two major approaches – classification based on biochemical and functional characteristics or classification based on molecular structure of the enzymes.[41] For the former, criteria such as the spectrum of antimicrobial substrates, hydrolysis rate (V_{max}), binding affinity (K_m), isoelectric focusing (pI), molecular weight, and amino acid composition have been used to develop classes/subclasses but will not be discussed here.[42,43] The simpler, molecular classification scheme uses four classes (Ambler classes A-D) which are described below.

Ambler class A is the broadest class and can most simply be thought of as a catch-all class to enzymes not fitting one of the other classes, consisting of β-lactamase enzymes that are located on plasmids, transposons, or chromosomes (e.g. TEM, SHV, PER, PSE, hundreds of others).[41,44,45] Class A enzymes range from hydrolyzing a narrow spectrum of β-lactams (e.g. penicillinases) to a broad spectrum (e.g. ESBL or carbapenemases). A very high degree of sequence variability and kinetic properties exist for this class.

Ambler class B enzymes, also called metallo-β-lactamases (MBLs), require zinc to carry out their function. Common MBLs in CRE organisms are imipenem-type carbapenemases (IMP), Verona integron-encoded metallo-β-lactamases (VIM), and New Delhi metallo-β-lactamase (NDM). Class B enzymes hydrolyze all β-lactam antimicrobials except monobactams (e.g. aztreonam) and are not inhibited by any current β-lactamase inhibitors such as clavulanic acid, sulbactam, tazobactam, avibactam, vaborbactam, and relebactam.

Ambler class C enzymes, commonly known as the cephalosporinases or AmpC enzymes, are typically chromosomally encoded with highly conserved sequences.[46-48] AmpC enzymes hydrolyze most extended spectrum β-lactams and β-lactam/β-lactamase inhibitor combinations such as ceftriaxone or piperacillin/tazobactam, but usually not carbapenems. However, CMY-10 was among the first AmpC enzymes that hydrolyze carbapenems.[49] Additionally, non-carbapenem hydrolyzing AmpC production in combination with porin channel mutations or efflux pumps can also confer resistance to carbapenems.[41]

Ambler class D enzymes are also known as oxacillinases (OXA) due to their ability to hydrolyze isoxazolyl β-lactamases such as oxacillin and methicillin.[50] There is a lot of structural similarity between class D and class A enzymes which can make differentiation, or even detection, difficult.[41] Class D enzymes are usually not inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam, but they are inhibited *in vitro* by sodium chloride concentrations of 100mM.[51-55] Additionally, these enzymes are relatively inactive against cephalosporins.[14] Although the OXA enzyme family consists of more than a hundred unique subtypes, 9 are considered ESBL and 37 are considered to be carbapenemases.[56]

Development of Resistance

Resistance to antimicrobials can be inherent. For example, this "natural" resistance can include inadequate uptake of an antimicrobial due to lack of transporters (e.g. *Pseudomonas aeruginosa* and tetracycline antimicrobials), lack of drug-activating mechanisms (e.g. metronidazole and aerobic organisms), or lack of target sites (e.g. penicillin binding proteins of enterococci and all cephalosporin class antimicrobials). Alternatively, resistance to antimicrobials can be acquired through normal mutation, vertically through reproduction, or horizontally through transformation, transduction, and conjugation (Figure 1.5). Sometimes, observable resistance requires a combination of these acquired resistance mechanisms.

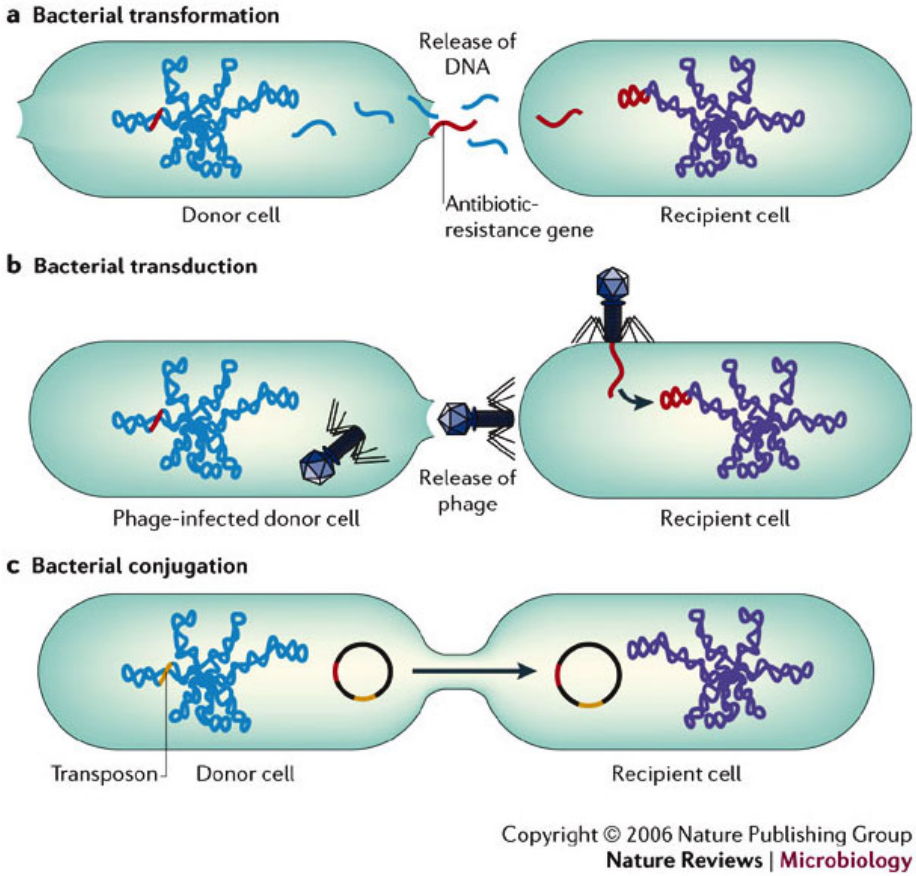


Figure 1.5: Horizontal Acquisition of Resistance. Reprinted[57]

Acquired resistance is an area of great concern because, unlike intrinsic resistance, these resistance profiles can be dynamic and unique for each species of bacteria and can change even during the course of therapy, making clinical decisions difficult and outcomes worse. Spontaneous mutation frequency that confers antimicrobial resistance is approximately on the order of 10^{-8} to 10^{-9} which means that one in every hundred billion to one trillion bacteria in an infection will develop resistance to an antimicrobial through random mutation.[58] Once exposed to an environment containing an antimicrobial, resistant organisms are preferentially selected for survival and this resistance can then be passed along through reproduction or through horizontal gene transfer.

In the setting of horizontal gene transfer, genetic material can be exchanged between individual bacteria of the same or different species.[58] One of the most common methods is conjugation where bacteria come into direct cell-to-cell contact and exchange small pieces of DNA called plasmids which may contribute to the explanation of why Ambler class A β-lactamases are so diverse in protein structure whereas class C β-lactamases (i.e. AmpC β-lactamases, which are typically located chromosomally) retain such highly conserved protein sequences. Another method of gene transfer is transformation where parts of DNA are taken up by bacteria from the environment which usually originated from the death or lysis of another bacterium.[58] The final method is transduction where bacteria-specific viruses called bacteriophages inject DNA into the bacteria cell (Figure 1.5).

Chapter Two:

Carbapenem-resistant *Enterobacteriaceae* (CRE)

Part of the research contained within this chapter has been published as Kulengowski, B. *In vitro* activity of polymyxin B and meropenem alone and in combination against carbapenem-resistant *Enterobacteriaceae*. 2016. *Theses and Dissertations—Pharmacy*. 57.

This chapter will discuss epidemiology, therapeutic agents, and current literature on carbapenem-resistant *Enterobacteriaceae* – the group of drug resistant pathogens labeled an urgent threat by the CDC.[11] *Enterobacteriaceae* include organisms such as *Escherichia coli, Klebsiella spp., Enterobacter spp., Salmonella spp., Shigella spp., Proteus spp., Serratia spp., Citrobacter spp.,* and *Yersinia pestis.* CRE-specific resistance mechanisms are often more complex, not always entirely understood, and can be multifaceted. One commonality between CRE and other gram-negative organisms is that enzyme production (e.g. β-lactamase) is still the most common resistance mechanism, but can also be present in combination with changes in cell wall permeability (e.g. porin channel mutations), upregulation of efflux pumps, or target site alterations which can further contribute to carbapenem resistance.[14] Additionally, β-lactamases without intrinsic carbapenemase activity but with cephalosporinase activity (e.g. DHA, ACT, CMY, SHV, CTX-M, etc.) can contribute to carbapenemase resistance when combined with other non-enzyme mediated mechanisms of resistance.[59-62]

Epidemiology of Carbapenem Resistance

Understanding the present epidemiology of CRE will attest to the relevance of the present work and help predict the ultimate direction of future studies. Interestingly, substantial geographic diversity exists for CRE. For example, in the United States, *Klebsiella pneumoniae* with KPC-2 or KPC-3 (two Ambler class A serine-based enzymes) compose about 80% of CRE cases.[14,63-65] In contrast, MBL (Ambler class B) or OXA-like (Ambler class D*)* enzymes are more clinically significant world-wide such as in India and Pakistan (NDM),[66] Greece and Italy (VIM),[67] or Turkey, Spain and North Africa (OXA-48).[14,68-72] Some countries (e.g. China) have low CRE prevalence, but significant diversity of carbapenemase enzymes.[73,74] Coexistence of various sequence types of MBLs and KPCs within the same *Klebsiella pneumoniae* strains has also been observed.[67,75-77] Ultimately, no two countries are the same when it comes to CRE characteristics and the prevalence can be drastically different, even between acute-care centers within the same country.[14]

Regarding the U.S. specifically, there are no national requirements to report CRE, and requirements for reporting CRE vary by state.[78] To facilitate data collection nationally, the CDC maintains two voluntary surveillance systems for CRE monitoring – Healthcare Associated Infections Community Interface (HAIC) which uses 10 sites across the nation for determining communities having or at risk for having CRE infections, and the National Healthcare Safety Network (NHSN) which tracks 17,000 facilities nationwide for healthcare-associated infections.[79] Figures 2.1-2.5 show the data reported to the CDC, separated by β-lactamase type (KPC, NDM, VIM, OXA-48) and by state.

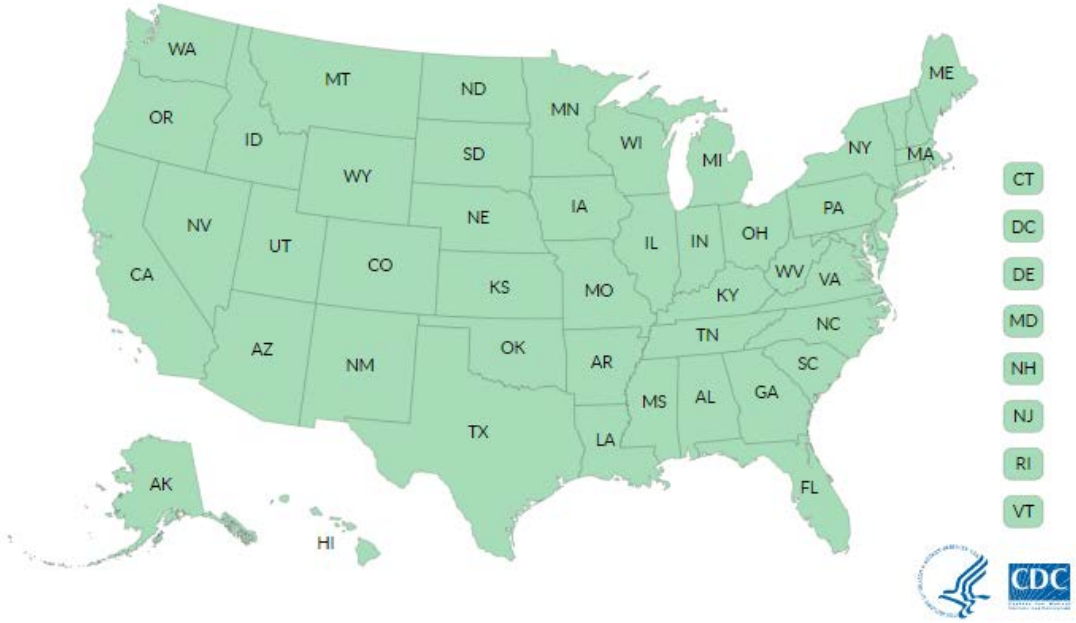


Figure 2.1: States with KPC-producing CRE Reported to the CDC. Reprinted[79]

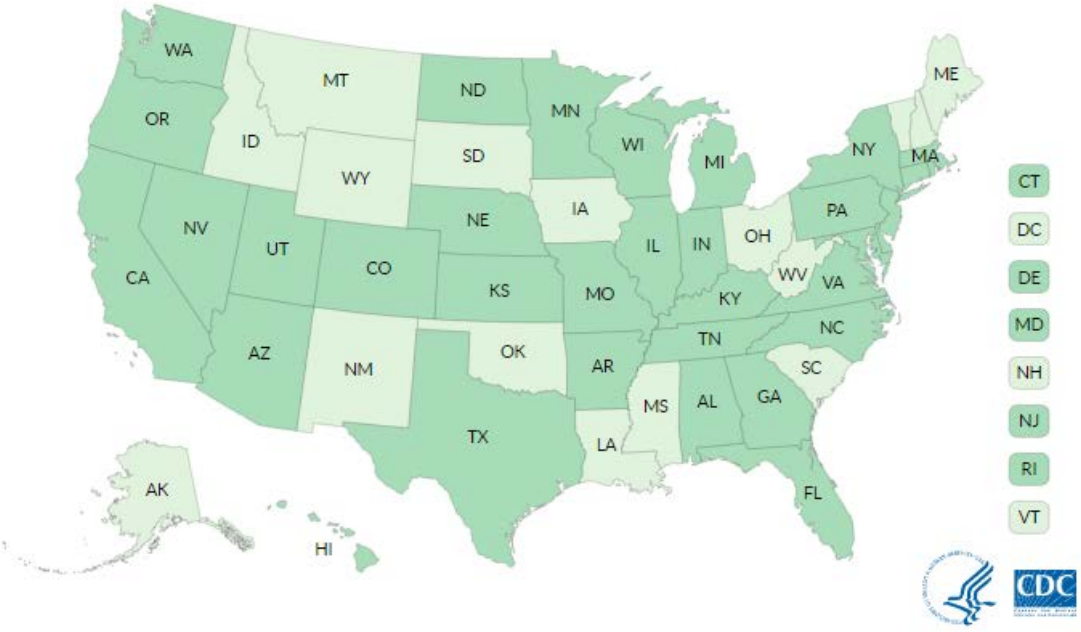


Figure 2.2: States with NDM-producing CRE (n=379) Reported to the CDC. Reprinted[79]

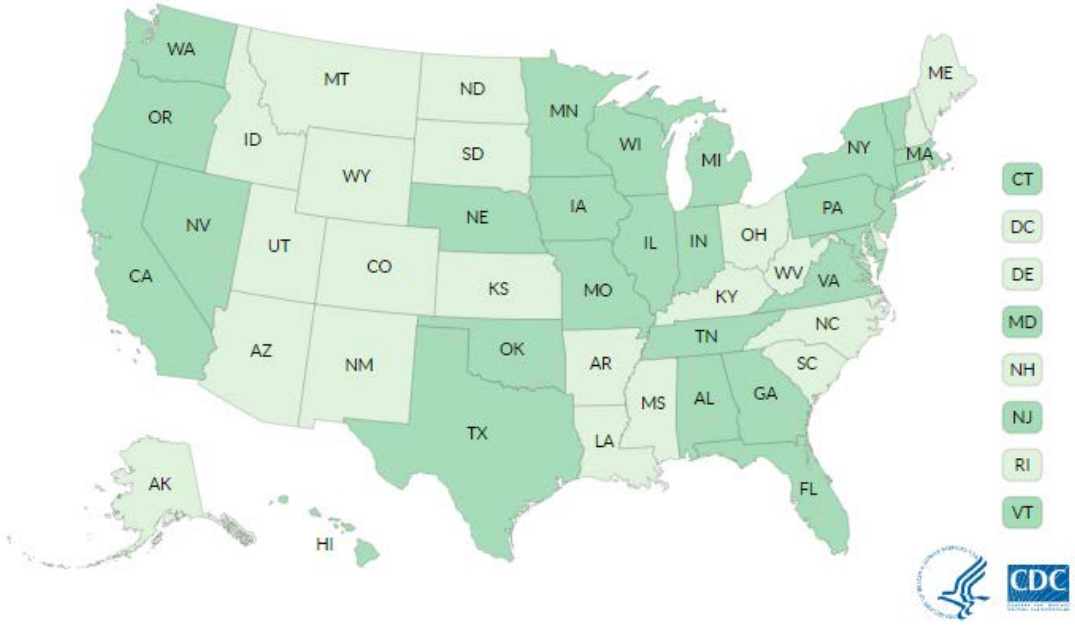


Figure 2.3: States with OXA-48-producing CRE (n=146) Reported to the CDC. Reprinted[79]

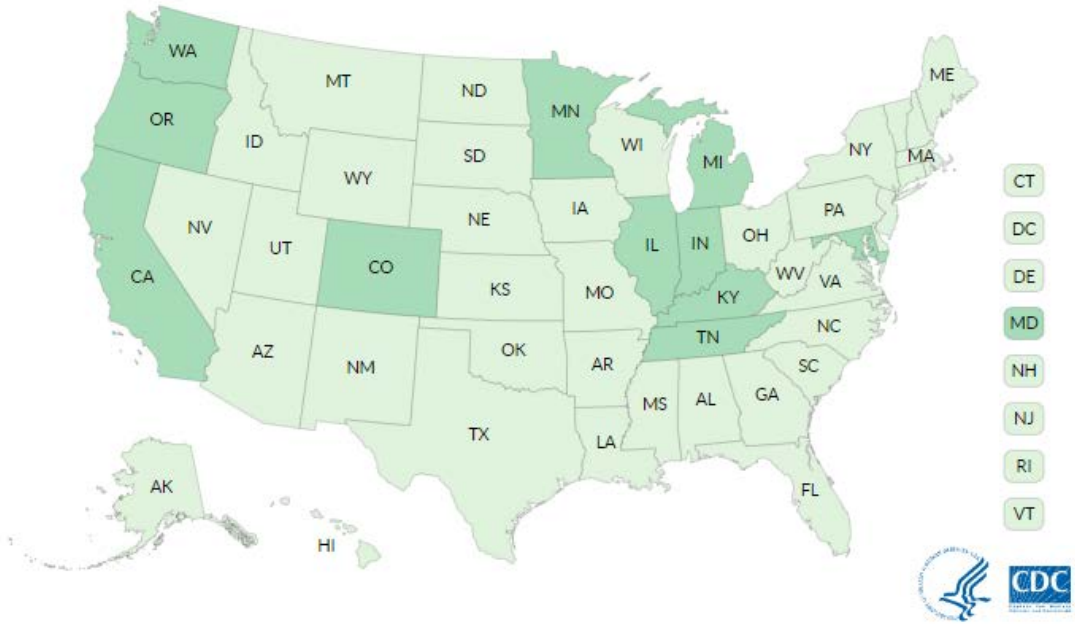


Figure 2.4: States with VIM-producing CRE (n=57) Reported to the CDC. Reprinted[79]

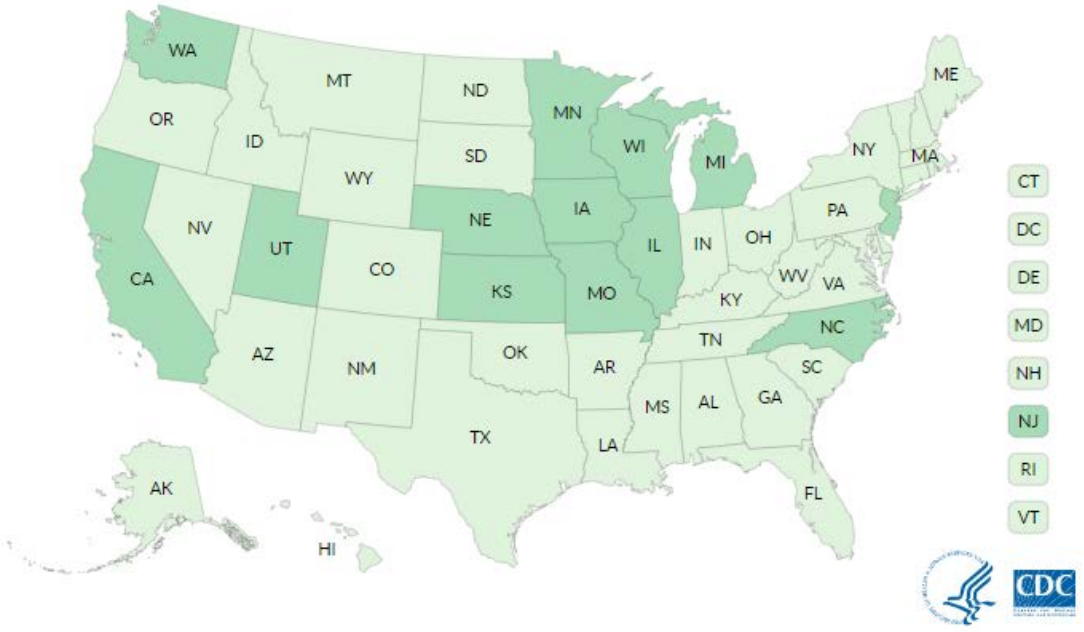


Figure 2.5: States with IMP-producing CRE (n=36) Reported to the CDC. Reprinted[79]

Klebsiella pneumoniae Carbapenemase (KPC; Class A)

A KPC-producing CRE was first identified in the United States from *Klebsiella pneumoniae* cultured from a patient in North Carolina in 1996, but reported in 2001.[80] Thereafter named KPC-1, it was not the first carbapenemase to be reported in *Enterobacteriaceae* because MBLs had been identified in *Enterobacteriaceae* in Japan as early as 1991.[81-83] It was, however, the first Ambler class A, serine-based carbapenemase to be found in *Enterobacteriaceae*. Subtype variants KPC-2 (later identified as identical to KPC-1) and KPC-3, initially concentrated in eastern states such as New York and New Jersey, but have since spread to all states (Figure 2.1).[3,80,84-90] Among CRE sent to the CDC, KPC has primarily been identified in *Klebsiella pneumoniae, Escherichia coli,* and

Enterobacter spp., and the KPC-2 and KPC-3 subtype variants are the most common in the United States.[91] As of October, 2018, Beta-Lactamase DataBase (BLDB) reports 36 KPC subtypes.[92]

Outside the U.S., the first KPC-producing *Enterobacteriaceae* was identified in 2005 from a hospital in Paris, France where a patient had been recently hospitalized in New York.[93] The legend for European carbapenemase enzyme type can be found in Figure 2.6. Currently, 34 of 38 surveyed European countries have reported KPC-producing CRE (Figure 2.7),[94,95] as well as Israel[96] and China.[97]

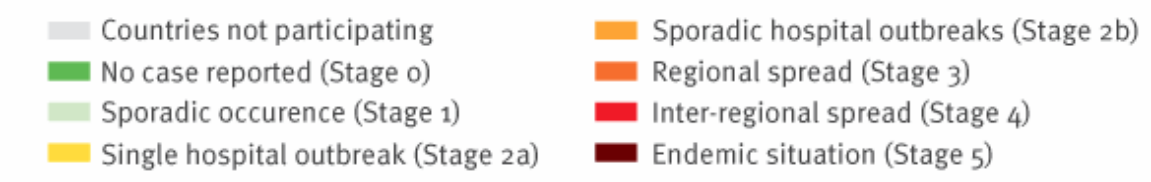


Figure 2.6: Epidemiological Stages by Color for European Carbapenem-resistant *Enterobacteriaceae*.

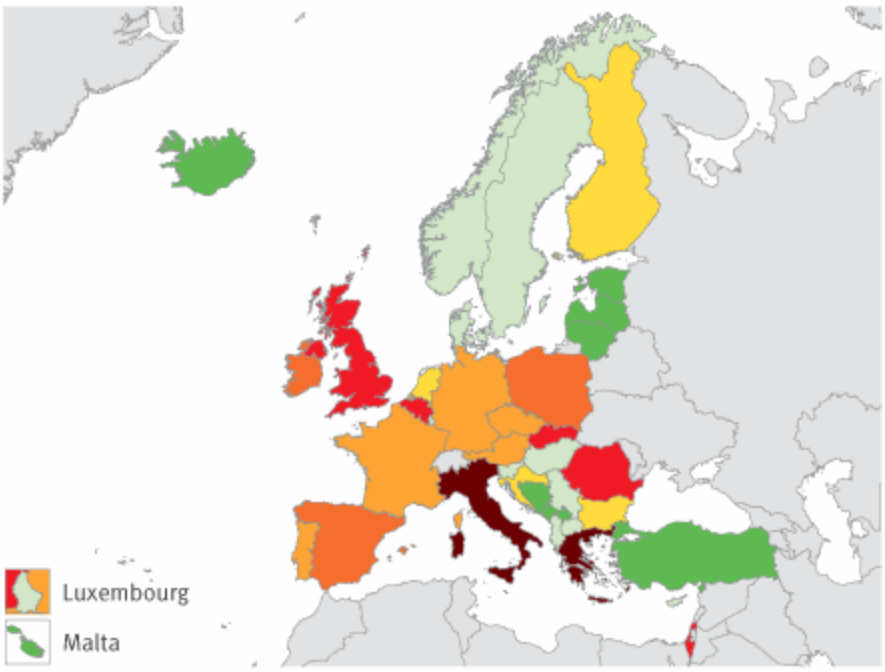


Figure 2.7: KPC-producing *Enterobacteriaceae* in 38 European Countries Based on Self-Assessment by National Experts. Reprinted[94]

Imipenem-like Carbapenemase (IMP; Class B)

In 1991, the very first MBL identified in *Enterobacteriaceae* was IMP-1, found in a *Serratia marcescens* clinical isolate in Japan.[81,82] IMP also emerged in Italy and Portugal in 1997 and 1998, respectively.[98] The differences in European IMP subtypes and Japanese IMP subtypes have led to the belief that European IMP-production emerged locally rather than global dissemination from Japan.[98] IMP has also been identified in Canada, China, Korea, Singapore, Taiwan and Australia to name a few more regions outside the U.S. IMP-producing CRE in Europe is depicted in Figure 2.8.[98]

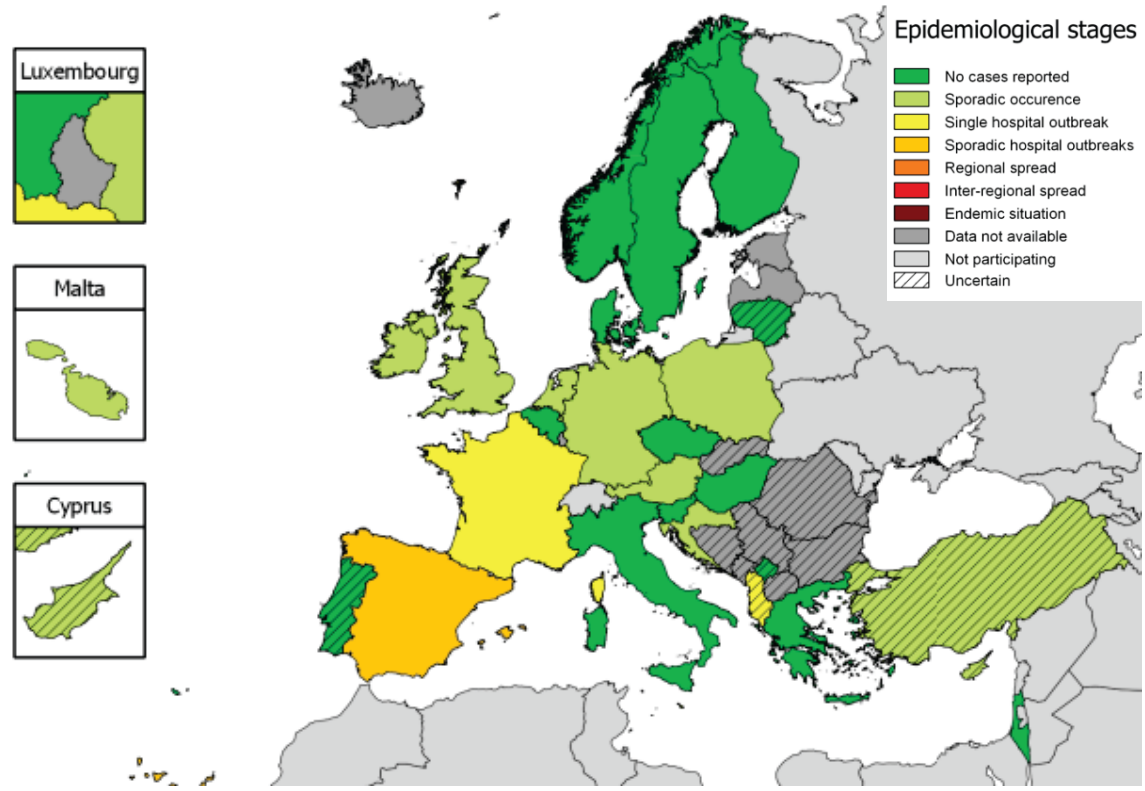


Figure 2.8: IMP-producing *Enterobacteriaceae* in 38 European Countries Based on Self-Assessment by National Experts. Reprinted[95]

From 2009 - 2010, the first IMP-producing CREs were isolated in the U.S. from three pediatric patients with no history of travel or receipt of medical care outside the United States.[99] Before this, the first IMP-producing isolate was *Pseudomonas aeruginosa*, reported in 2006.[100] As of October 2018, BLDB reports 78 subtypes of IMP-type β-lactamases.[92]

Verona Integron-encoded Metallo-β-lactamase (VIM; Class B)

A VIM-producing CRE was first identified in Greece from *Escherichia coli* in 2001,[101,102] and then later from other *E. coli* and *K. pneumoniae* isolates.[98,103] VIM-production has also been reported in Japan, South Korea, Portugal, Spain, Poland, Croatia, Chile, Venezuela, Argentina, Belgium and most recently in the United States.[104-110] Figure 2.9 depicts VIM-production in Europe whereas Figure 2.4 describes VIM-production in the United States, where Kentucky has reported more than all other states combined.[79]

The first VIM-producing CRE identified in the U.S. was in an adult patient with *Klebsiella pneumoniae* in 2006.[111] Additionally, a recent publication describes the first and only cluster of VIM-producing CRE in the U.S. Perirectal cultures of eight isolates (4 *E. cloacae*, 1 *Raoultella sp.*, 1, *E. coli*, 2 *Klebsiella pneumoniae*) from six patients were obtained – six from a neonatal intensive care unit, and two from an adult trauma and surgical intensive care unit.[112] To date, this is the only VIM-producing CRE colonization reported to include a neonatal population. Previous VIM-producing CRE have only involved a single species, and only one VIM-producing CRE-colonized patient had been reported (2013) in the same hospital. As of October 2018, BLDB reports 60 subtypes of VIM-type β-lactamases.[113]

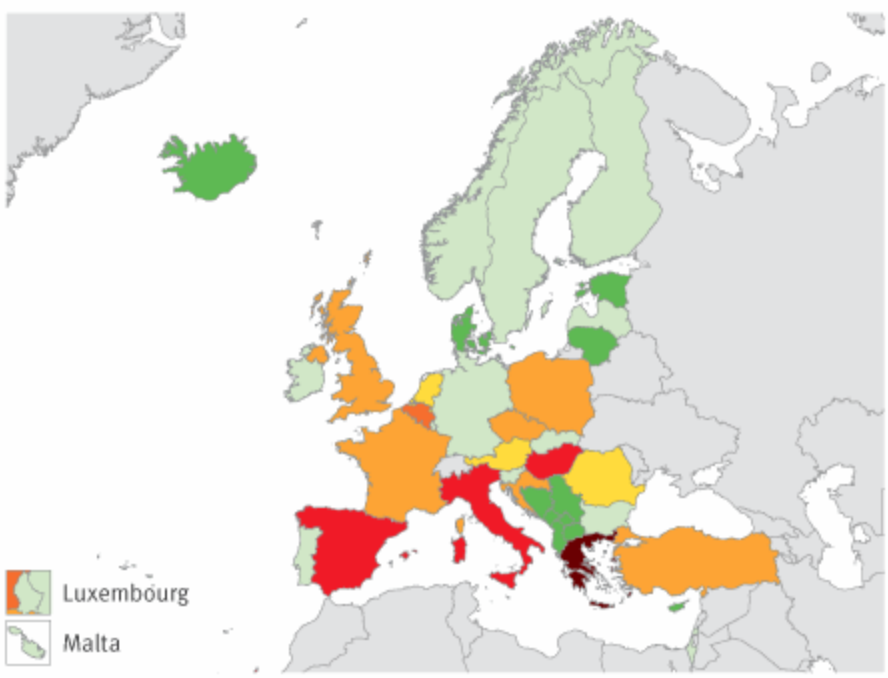


Figure 2.9: VIM-producing *Enterobacteriaceae* in 38 European Countries Based on Self-Assessment by National Experts. Reprinted[95]

Oxacillinase Group β-lactamase (OXA; Class D)

In 2001, OXA-48 was the first Ambler class D carbapenemase isolated in *Enterobacteriaceae*. It was first found in a *Klebsiella pneumoniae* isolate from Turkey.[54] Interestingly, this particular OXA enzyme has the highest hydrolysis rate of imipenem compared to all other published OXA enzymes.[56] OXA-48 has also been identified in Russia,[114] South Korea,[115] Argentina, India,[116] Taiwan,[117] North Africa,[65] and the U.S.[79] (Figure 2.3). Figure 2.10 depicts OXA-48 dissemination in Europe. As of October 2018, BLDB reports 856 subtypes of OXA-type β-lactamases.[113]

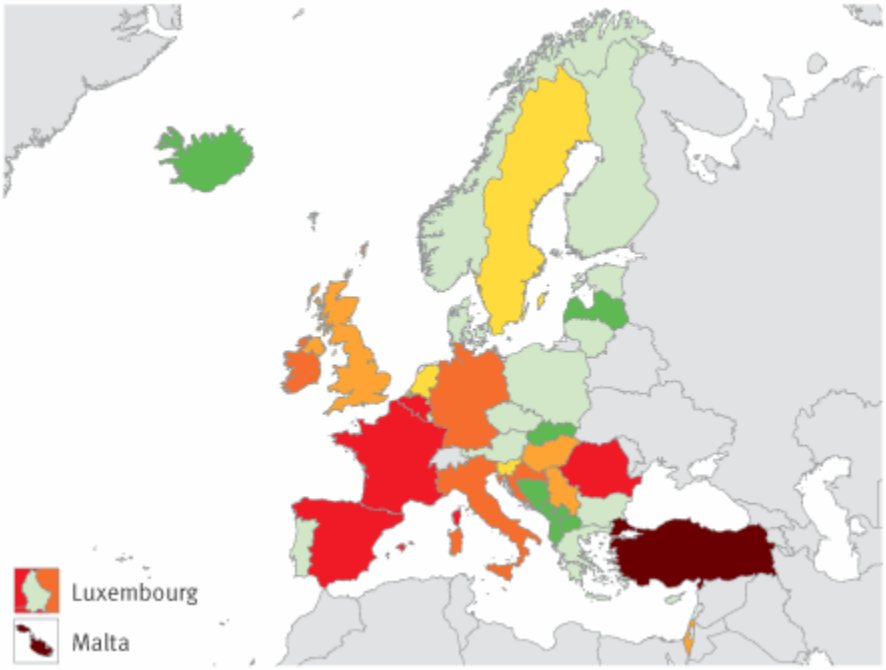


Figure 2.10: OXA-48-producing *Enterobacteriaceae* in 38 European Countries Based on Self-Assessment by National Experts. Reprinted[95]

New Delhi Metallo-β-lactamase (NDM; Class B)

In 2009, an NDM-producing CRE was first identified in Sweden from *Klebsiella pneumoniae*, cultured from a patient of Indian descent who had recently traveled to New Delhi, India and acquired a urinary tract infection (UTI).[118] This novel MBL was designated NDM-1. Currently, at least sporadic NDM-producing CRE has been reported in most European countries (Figure 2.11), but a more thorough description of NDM spread across Europe is described by Cantón *et al.*[119] As of October 2018, BLDB reports 24 NDM-type β-lactamases.[92]

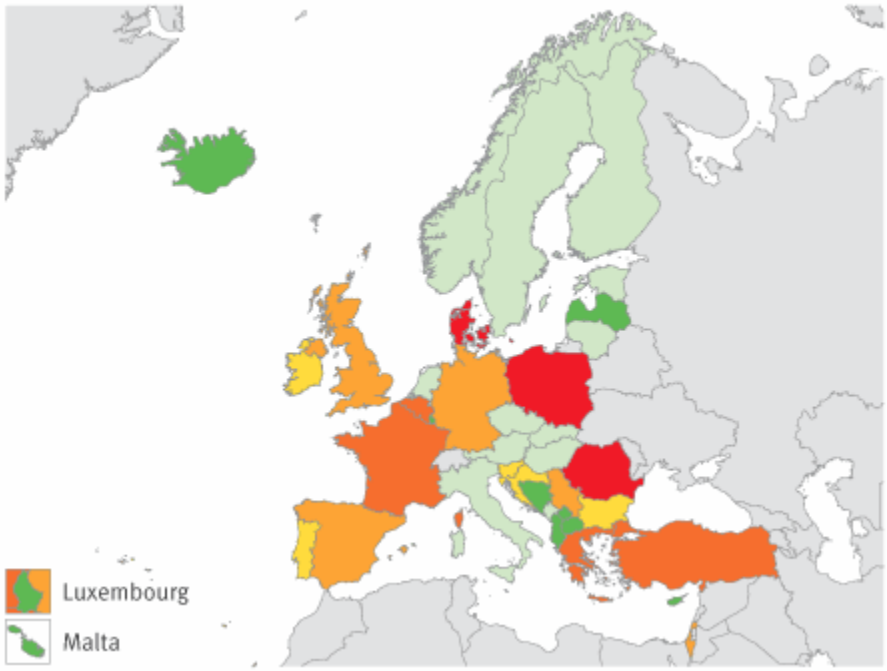


Figure 2.11: NDM-producing *Enterobacteriaceae* in 38 European Countries Based on Self-Assessment by National Experts. Reprinted[95]

In the U.S., the first NDM-producing *Enterobacteriaceae* was among nine isolated from 2009 - 2011 (5 *K. pneumoniae,* 2 *E. coli,* 1 *E. cloacae,* and 1 *Salmonella enterica*) from eight patients across five states (5 California, 1 Illinois, 1 Maryland, 1 Massachusetts, and 1 Virginia). All patients had recently been to India or Pakistan. Eight of these isolates were confirmed by the CDC to encode NDM-1, but the ninth isolate (*E. coli*) coded for what is now called NDM-6.[66] NDM-producing isolates were also being described in other parts of the world by this time, consistently in patients with recent travel to India or Pakistan.[91] Since 2012, the epidemiology in the U.S. appears to be changing as more NDM-producing CRE are being isolated from patients without recent travel outside country, suggesting local acquisition.[91]

CRE Incidence and Prevalence

Regarding the top 3 CRE reported in the U.S., 69% were *Klebsiella pneumoniae/oxytoca,* 18% were *E. coli,* and 13% were *Enterobacter* spp.[120] Nationwide, carbapenem resistance among *Klebsiella pneumoniae/oxytoca* was <1% in 2000,[121] but by 2010, the CDC reported carbapenem resistance up to 12.8% and 12.5% for central-line associated bloodstream infections (CLABSIs) and catheter-associated urinary tract infections (CAUTIs),[120] respectively. An academic medical center in New York reported carbapenem-resistant *Klebsiella pneumoniae* rates of 38% in 2008.[122] A collective report of 14 hospitals in New York also noted overall 38% *Klebsiella pneumoniae* carbapenem resistance in 2006, but has recently reported a decrease to 29% in 2009.[123]

Conventional Antimicrobial Agents

CRE are complex and diverse – what may work for some organisms may not be universally applicable to others. In general, CRE are resistant to all β-lactams and β-lactam/β-lactamase inhibitor combinations with the exception of ceftazidime/avibactam and meropenem/vaborbactam, newly approved β-lactam/β-lactamase-inhibitor combinations for KPC-producing gram-negative bacteria.[124] Additionally, exclusively MBL-producing CRE may be susceptible to aztreonam. However, due to the complexity and commonly multi-factored resistance that accompanies most CRE, this is seldomly applicable.[125]

Regarding other classes of antimicrobials, CRE are typically only susceptible (>85%) to colistin, polymyxin B, tigecycline, fosfomycin, and variably susceptible (35-75%) to aminoglycosides. There are limitations with each of the antimicrobials for which CRE are typically susceptible, including spectrum, pharmacologic characteristics, rapid resistance development, and toxicity/adverse events.[14] Newly approved antimicrobials like the novel beta-lactam/beta-lactamase inhibitor combinations will be discussed briefly in Conventional Antimicrobial Agents against Carbapenem-resistant *Enterobacteriaceae*. The most recently approved aminoglycoside (plazomicin; July 2018) and tetracycline (eravacycline; August 2018) will not be discussed beyond mention in this dissertation.

Polymyxins

The polymyxin class of antibiotics was introduced in the mid-1950s, consisting now of two agents – polymyxin B and polymyxin E (colistin). Both agents are cationic polypeptides that share a ring of amino acids and a fatty acid tail (Figure 2.12). The structural difference of colistin involves a substitution of the phenylalanine in polymyxin B with D-leucine. Additionally, both polymyxins have two major components based on the fatty acid chain length – polymyxin B1 and B2 and colistin A and B.[14]

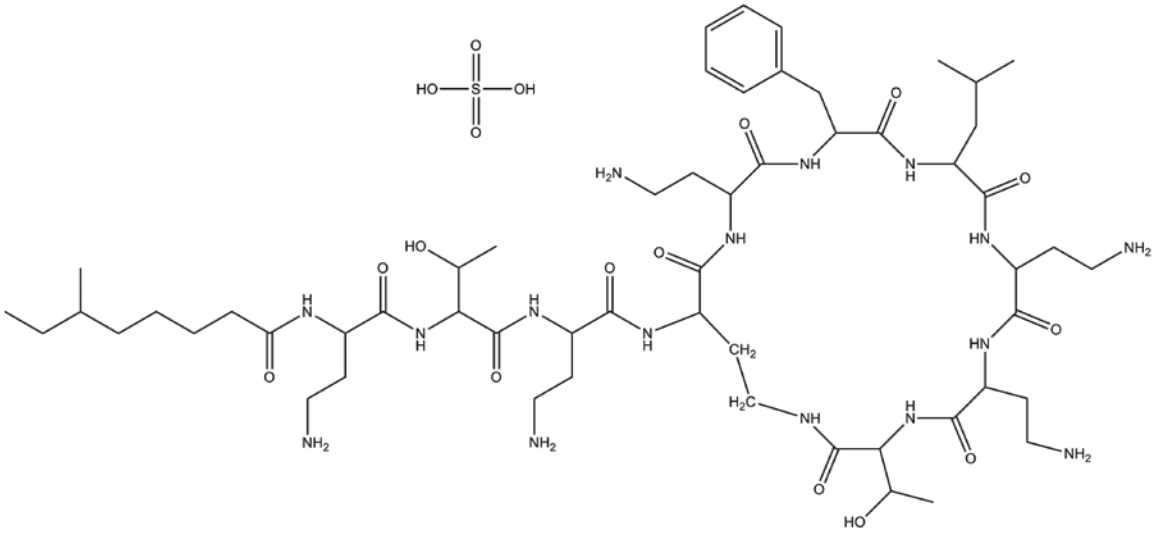


Figure 2.12: Structure of Polymyxin B. Reprinted[126]

The mechanism of action of the polymyxin class involves binding to negatively-charged moieties in the lipopolysaccharide (LPS) present in the outermost membrane of gram-negative bacteria (Figure 2.13). This interaction results in the loss of intracellular products, killing the bacteria.

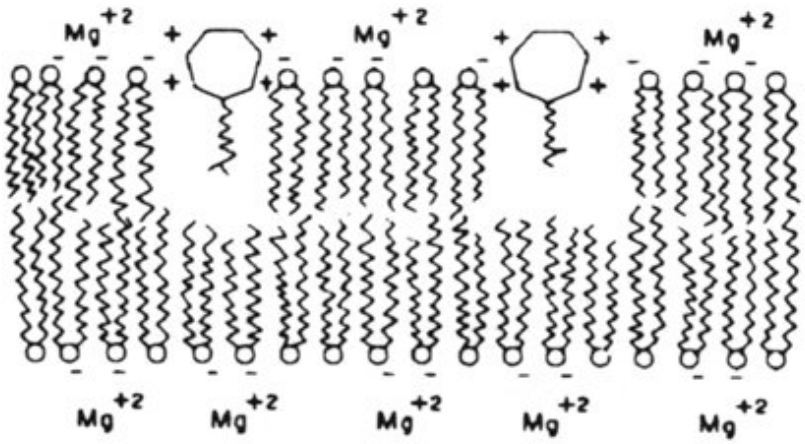


Figure 2.13: Polymyxin B/Colistin Mechanism of Action. Reprinted[127]

Polymyxins have a broad gram-negative spectrum of activity – including *Enterobacteriaceae* (except *Proteus spp.* and *Serratia spp.*), *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. However, their utility had been limited by the development of safer antimicrobials like the aminoglycosides and cephalosporins. In fact, this antimicrobial class was primarily reserved for cystic fibrosis patients, gastrointestinal (GI) tract decontamination, and topical antimicrobial therapy.[14] In the '90s, this class was "reintroduced" to address problems with carbapenem-resistant organisms. One of the first published successes of polymyxin treatment for CRE involved a critically ill patient with a carbapenem-resistant *Klebsiella pneumoniae* bloodstream infection.[128]

Polymyxin B and colistin differ significantly in their pharmacokinetics. However, routes of administration are similar between the polymyxins with the exception that polymyxin B cannot be administered orally. Otherwise, both polymyxins can be administered intravenously, intramuscularly, intrathecally, topically, or by inhalation. Clinically, colistin is administered as a prodrug (colistimethate sodium; CMS) which must

be first hydrolyzed into various derivatives (e.g. colistin) before having any antimicrobial effect, whereas polymyxin B is administered as an active agent. The rates of hydrolysis are variable according to the physical environment within the patient (e.g. pH and temperature). Differences in rates and extents of hydrolysis have been observed brand-to-brand or even batch-to-batch.[129] Since there is no appreciable antimicrobial activity from the parent compound, rational dosing of CMS is very challenging.[130,131]

Additional challenges in CMS dosing exist in its elimination, which is primarily renal, and its conversion to colistin, which is non-renal. In fact, patients with normal renal function are often so efficient at eliminating CMS that a dose 4 to 5 times that which is needed to attain required plasma concentrations of colistin must be administered.[131] Colistin can also be found concentrated in the urine, but this is primarily due to post-renal conversion of the parent compound because colistin is primarily eliminated through a non-renal mechanism. As a result, large interpatient and intrapatient variability exists for CMS dosing.[132] In stark contrast, polymyxin B, which is administered as an active agent, is eliminated mainly by non-renal mechanisms and very little polymyxin B can be found in the urine.[133,134] Figure 2.14 summarizes the different elimination pathways for colistin vs. polymyxin B.[131]

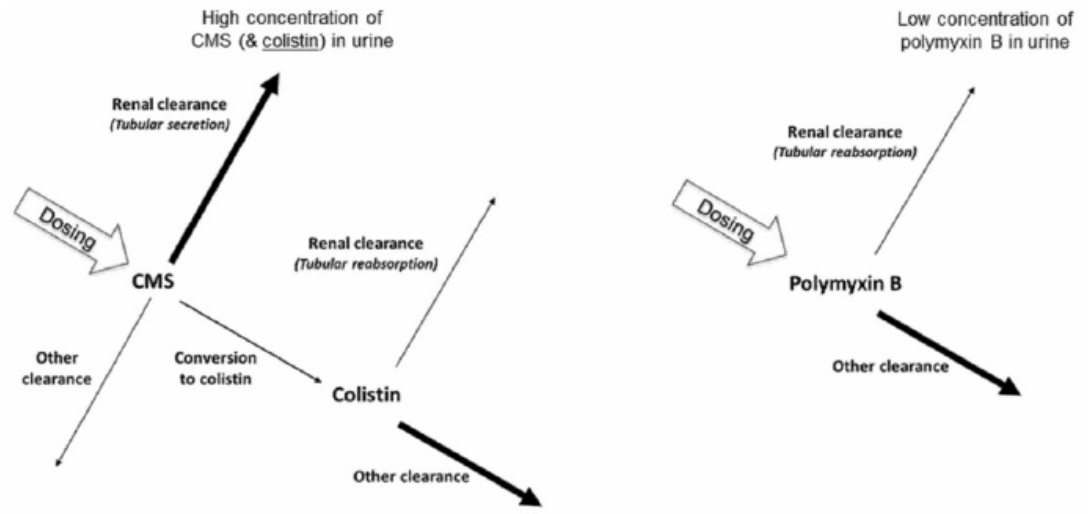


Figure 2.14: Elimination Pathways for Colistin and Polymyxin B. Reprinted[131]
Thickness of the arrows indicates relative magnitude of clearance mechanism

The clinical implication of these pharmacokinetic differences are that, even with a loading dose of CMS, it takes several hours to achieve effective plasma levels of colistin and delay in appropriate antimicrobial therapy has been associated with increased mortality in critically ill patients.[135,136] Furthermore, low colistin plasma levels have been associated with growth of colistin-resistant subpopulations.[137-140] As renal function improves (or declines), the rate and extent of conversion of parent drug to colistin changes, and dosing strategies accounting for this change have not yet been perfected, rending it impossible to reliably achieve effective steady-state plasma concentrations in patients with creatinine clearance above 80 mL/min.[132,141] For example, at the maximal approved dose of CMS (300 mg colistin base activity / day), patients with creatinine clearance > 80 mL/min achieved plasma concentrations < 2 mg/L.[132] As a result, more recent studies have used larger doses of colistin at approximately 5 mg/kg loading dose and 2.5 mg/kg every 12 hours.[124] Nephrotoxicity, another independent predictor of mortality, is the dose limiting

adverse effect of colistin.[142] Polymyxin B, however, can rapidly achieve desired plasma levels that can be effectively maintained[134] and nephrotoxicity with polymyxin B has been reported to be lower than with CMS,[143,144] being closer to about 14%,[145] compared to colistin which has been reported closer to 45% using RIFLE criteria.[142] However, at our institution, nephrotoxicity of polymyxin B and colistin have ranged 40-50%.[146]

Polymyxin-resistant CRE infections are another concern altogether. Rapid resistance development has been observed when colistin or polymyxin B are used alone.[137-139] Colistin-resistant (MIC >2 μg/mL) CRE have occurred in various parts of the globe including Italy,[147] Greece,[148] Spain,[72] and the United States.[149,150] In fact, a tertiary center in Spain reports an increase from 13.5% to 31.7% in colistin resistance among *Klebsiella pneumoniae* isolates.[72] A retrospective multi-center observational study in Italy showed a threefold increase in colistin-resistant KPC-producing *Klebsiella pneumoniae* from 2010 to 2013 (20% resistance overall), and colistin-resistance was determined to be an independent risk factor for 14-day mortality (47% vs. 31%; P = 0.001).[151]

Mechanisms that lead to resistance to polymyxins in *Enterobacteriaceae* are not fully understood. One proposal is that modifications in components that make up the LPS layer of gram-negative organisms, like lipid A, may play a role in polymyxin-resistance. Specifically, *phoP/phoQ* and *pmrA/pmrB* can be activated by environmental stimuli (e.g. low magnesium concentrations or polymyxin exposure) or can harbor mutations which typically lead to constitutive expression. One result is that phosphate head-groups in lipid A are substituted with 4-amino-4-deoxy-L-arabinose (L-Ara4N) which inhibits polymyxin binding. Figure 2.15 shows possible mutations (red star symbols) and how the *phoP/phoQ*

and *pmrA/pmrB* system modifies the LPS layer, which ultimately leads to lower binding affinity (resistance) to polymyxin class antibiotics.

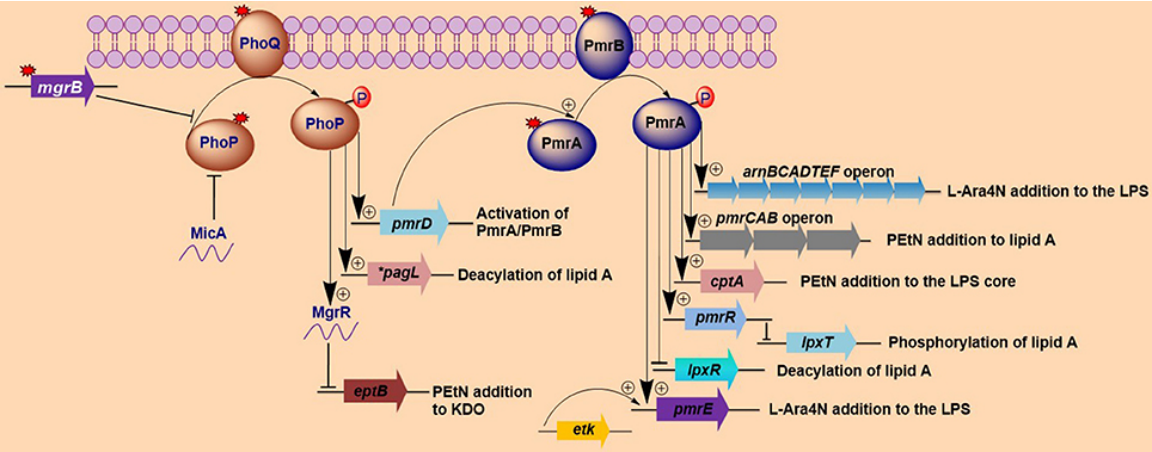


Figure 2.15: Proposed Polymyxin Resistance Pathway in Gram-negative Organisms. Reprinted[152]

The mobilized colistin resistance (mcr-1) gene was first identified in *E. coli* in Chinese pigs.[153] This was the first polymyxin resistance gene identified which was capable of horizontal gene transfer. Additional mechanisms of resistance include modification of outer membrane proteins (e.g. OprH) which can block polymyxin merger with the cell membrane as well as efflux pumps.[152] Regarding *K. pneumoniae* specifically, alterations in the mgrB gene (removal a negative feedback loop on the *phoP/phoQ* system) has been associated with an epidemic dissemination of colistin-resistant CRE in Italian hospitals[154,155] but has also been identified in other parts of Europe, Asia, Africa and the United States.[154] As a final addition, lipid A modifications in *K. pneumoniae* have been associated with cross-resistance to host defense systems as well,[156] which may contribute to the observed increase in mortality associated with colistin-resistant CRE.

Tigecycline

Tigecycline is a glycylcycline (Figure 2.16), a class related to the tetracyclines, that also inhibits protein synthesis by binding to the 30S ribosomal subunit (Figure 2.17).[157-159] The charged aminoacyl-tRNA can no longer bind to the ribosome in the presence of tigecycline due to the distorted ribosomal acceptor site, which halts the reproduction process of the bacteria. As a result, tigecycline is bacteriostatic and has a broad spectrum of activity including both gram-positive organisms and gram-negative organisms, even anaerobic and atypical organisms, but it does not clinically impact *Pseudomonas spp.* or *Proteus spp*.[160,161]

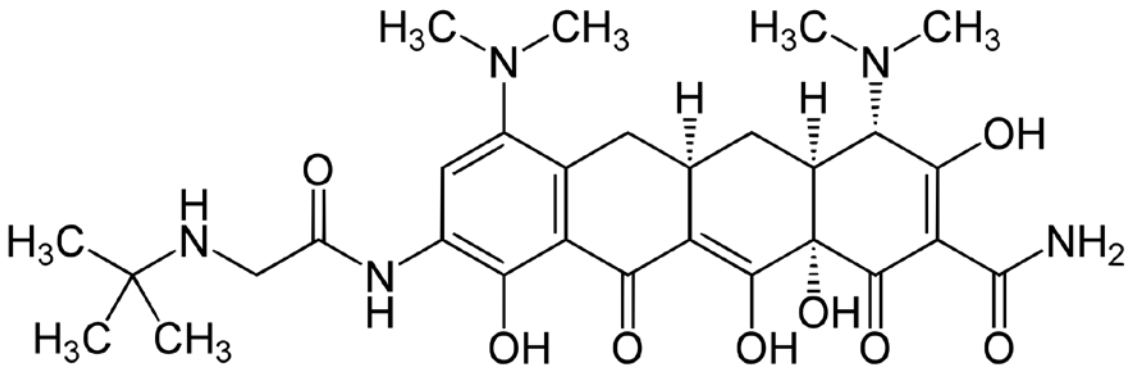


Figure 2.16: Structure of Tigecycline. Reprinted[162]

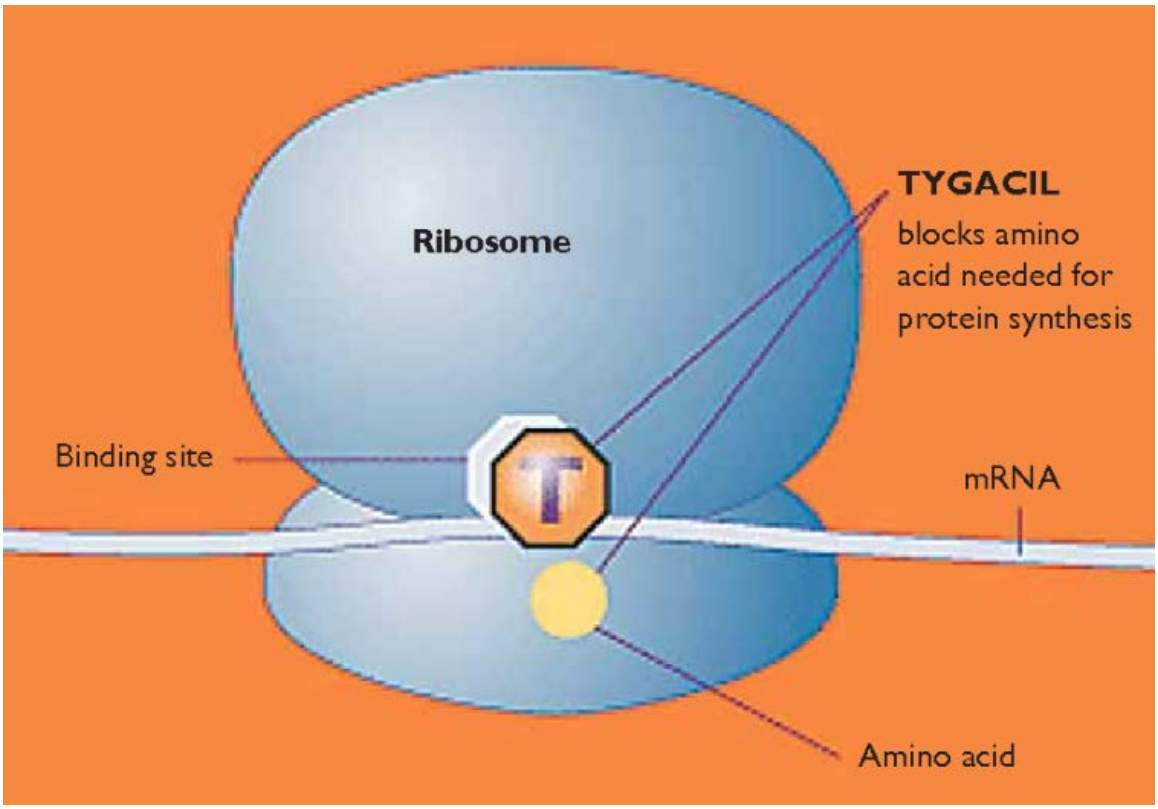


Figure 2.17: Tigecycline Mechanism of Action. Reprinted[163]

Clinical experience with tigecycline has set its role in complicated skin and skin structure infections (cSSSIs) and complicated intra-abdominal infections (cIAIs).[164] Tigecycline has been associated with increased mortality when compared to other antimicrobial agents and so is typically reserved after failure of other antimicrobials or in situations where antimicrobial choices are limited such as in CRE or other multidrug resistant (MDR) organisms.[165,166] An AUC/MIC ratio > 12.5 correlates with clinical outcome in cSSSI, which suggests clinical breakpoints of 0.25-0.5, but CLSI currently has not established a breakpoint for *Enterobacteriaceae*.[167] The susceptibility breakpoint established by the FDA is ≤2 µg/mL.[168] Of note, peak serum concentrations of 0.60 µg/mL

after a 100 mg infusion render tigecycline unable to effectively treat bloodstream infections.[14]

Resistance to tigecycline (MIC >2 µg/mL) is not common, but when it has been characterized, it is usually associated with mutations in the *ramA* gene which leads to upregulation of AcrAB-TolC, a multidrug efflux pump in *Enterobacteriaceae*.[169] Additionally, overproduction of *marA*, *rarA*, *acrAB,* and *oqxAB* genes can lead to tigecycline resistant phenotypes.[170] Tigecycline resistance has been recently reported in China,[171] Europe,[172-174] and the United States.[173,175]

Fosfomycin

Fosfomycin is bacterial cell-wall inhibitor, discovered in Spain in 1969, and it is relatively unique in structure by containing an epoxide (Figure 2.18).[176,177] Peptidoglycan synthesis is inhibited by fosfomycin which blocks the formation of N-acetylmuramic acid by competitively inhibiting phosphoenol pyruvate synthetase (Figure 2.19).[14]

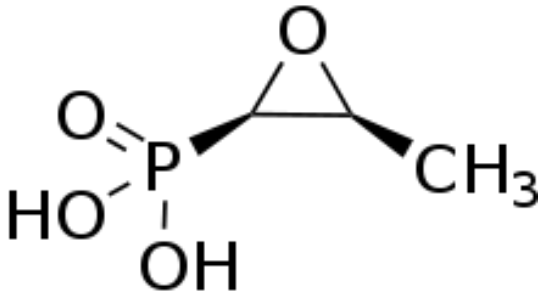


Figure 2.18: Structure of Fosfomycin. Reprinted[178]

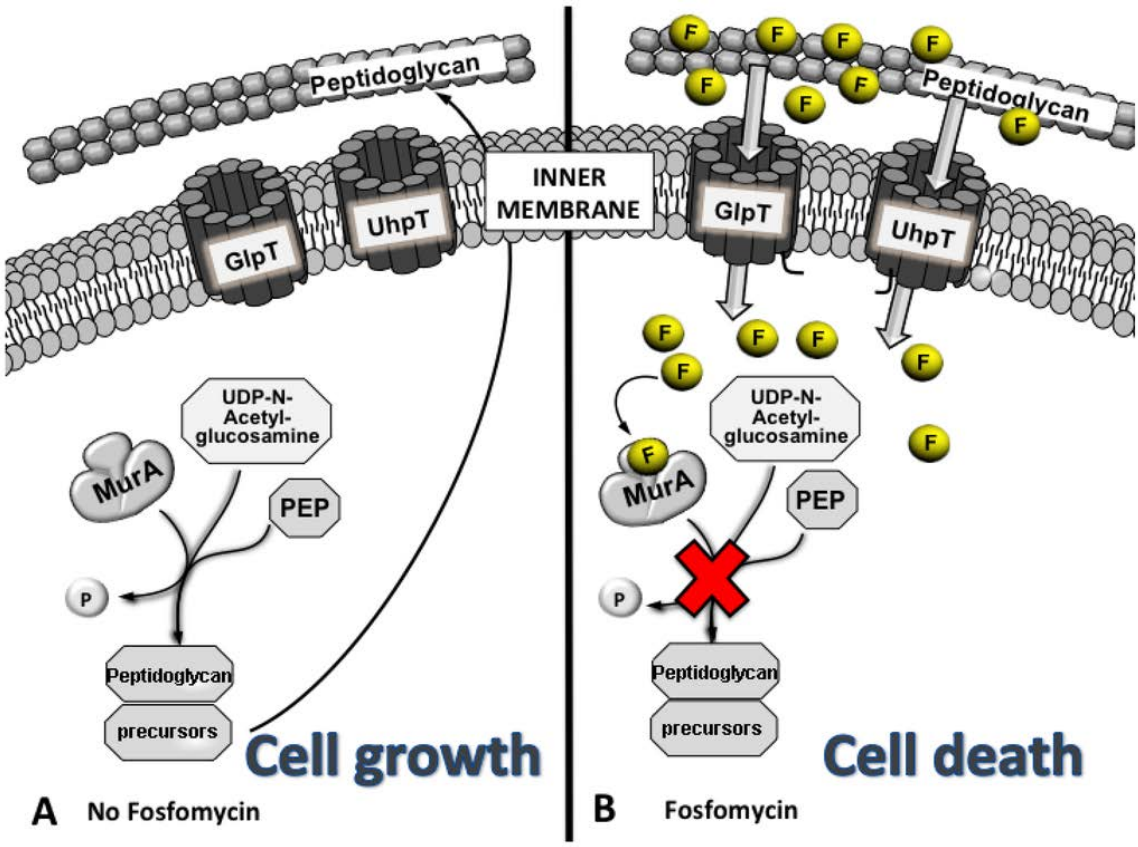


Figure 2.19: Fosfomycin Mechanism of Action. Reprinted[179]

Like other cell-wall inhibitors (e.g. β-lactam antibiotics), fosfomycin is bactericidal and has a broad spectrum of activity that includes both gram-positive and gram-negative bacteria. In Europe, fosfomycin is primarily used in combination with other antimicrobials for CRE treatment, particularly strains with reduced susceptibility to colistin and tigecycline.[180,181] In the U.S., fosfomycin is only approved orally for uncomplicated urinary tract infections due to its excellent genitourinary penetration. The optimal dosing strategy for treatment is still unclear. The FDA label indicates a single 3 gm daily dose whereas other clinical trials have evaluated 3 gm every 2 or 3 days for urinary tract infections.[182] For non-urinary infections, doses of 6-8g over 2-4h every 8h have been used in Europe.[124]

In CRE infections, a multicenter (11 ICUs), prospective case-series study from Greece showed favorable outcomes when fosfomycin was used in combination with another antimicrobial (usually colistin or tigecycline) in a majority of patients with fosfomycin-susceptible carbapenem-resistant infections, a majority of which (41 out of 68) were *Klebsiella pneumoniae*. Bacterial eradication was observed in 56.3% of cases overall and in 60% of cases caused by colistin-resistant CRE. Fosfomycin resistance developed during the course of treatment in three cases.[183]

Fosfomycin resistance has been characterized, and usually results from either mutations in the transport systems (GlpT and UhpT) that are located on the chromosome of bacteria or through inactivating enzymes (*fos*A family) located on bacterial plasmids. The chromosomal mutations prevent the uptake of fosfomycin into the cell and, although these mutations are relatively quickly acquired, a high fitness cost is observed in *E. coli* which limits fosfomycin resistance when not under direct antimicrobial pressure.[184] The same fitness cost has not been observed in *Klebsiella spp.* and *Enterobacter spp.*, and therefore fosfomycin monotherapy may select for resistant isolates among these *Enterobacteriaceae*.[185] Regarding plasmid-mediated resistance, a plasmid carrying both $bla_{\text{KPC-2}}$ and *fos*A3 is circulating among carbapenem-resistant *Klebsiella pneumoniae* in China and accounts for 60% of the observed fosfomycin resistance in that country.[186]

Aminoglycosides

The aminoglycoside class was introduced in the 1940s with streptomycin.[14] Today, three aminoglycosides are primarily used (four in Europe)–tobramycin, gentamicin, amikacin and kanamycin (in Europe). However, plazomicin was recently approved and exhibits improved activity against CRE relative to the other aminoglycosides. Although aminoglycosides are generally similar in structure (Figure 2.20) and function, those structural differences that do exist among this class often confer differences in stability against a variety of aminoglycoside modifying enzymes that would inactive these antimicrobials.

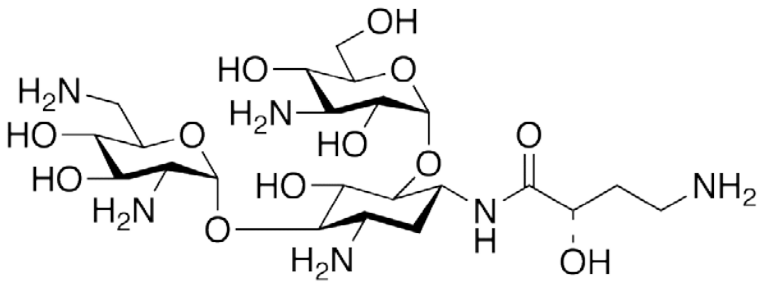


Figure 2.20: Structure of Amikacin. Reprinted[187]

Like tigecycline, aminoglycosides function by binding to the 30S ribosomal subunit, but these antimicrobials can additionally facilitate the insertion of incorrect amino acid sequences into proteins rather than only preventing their translation (Figure 2.21). As a result, aminoglycosides exhibit bactericidal activity against gram-positive and gram-negative organisms, including *Pseudomonas spp.*, but have little affect against anaerobes.

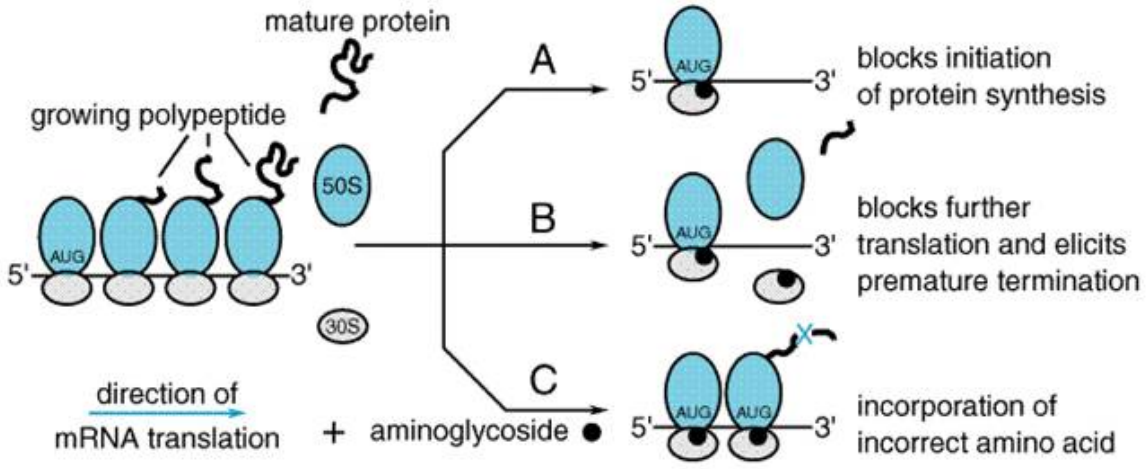


Figure 2.21: Aminoglycoside Mechanism of Action. Reprinted[188]

Aminoglycosides are sometimes a viable option against aminoglycoside-susceptible CRE. In fact, a superior rate of microbiologic clearance was observed in a retrospective cohort study of CRE bacteriuria when an aminoglycoside was used (88%) compared to either polymyxin B (64%) or tigecycline (43%).[189] Additionally, aminoglycosides are associated with less nephrotoxicity (~10-20%)[190] than colistin (~45%),[142,145,146,191,192] while maintaining bactericidality and the ability to treat bloodstream infections over tigecycline.[14]

Resistance to aminoglycosides is primarily mediated by aminoglycoside-modifying enzymes (analogous to β-lactamase production) but is highly variable, showing regional dependence as well as differences among hospitals within the same geographic region. Resistance rates can even vary by strain, but in general, rates of non-susceptibility have been reported as ranging from 35% to 63% for gentamicin, 61% to 98% for tobramycin, and 16% to 82% for amikacin.[193-195] Other resistance mechanisms have been identified as

well, including modification of the ribosome target,[196] reduced permeability of the bacterial cell wall, and also efflux pumps.[197,198]

Conventional Antimicrobial Agents against Carbapenem-resistant *Enterobacteriaceae*

A majority of cases, cases series, and the published experience of medical centers indicate that combination therapy might provide a mortality benefit when compared to monotherapy.[10,68,124,185,199-203] However, the data utilized in these studies often predates the newest approved agents – ceftazidime/avibactam, meropenem/vaborbactam, plazomicin, and eravacycline. Additionally, polymyxin B, colistin, tigecycline, and fosfomycin have all demonstrated rapid selection for resistance when used as monotherapy against CRE.[185,204] There are very few randomized-controlled clinical trials comparing single agents or their combinations, so reliance on retrospective analyses is frequent which results in significant limitations. Some experts conclude that a systematic review and meta-analysis is not possible regarding CRE treatment due to the heterogeneity of available evidence.[205]

In Vitro Studies

Polymyxin Combinations

The interaction between antimicrobial agents has primarily been characterized by time-kill methodology, where polymyxins are most frequently investigated in combination with either a carbapenem, tigecycline, fosfomycin, rifampin, an aminoglycoside, or sometimes with three or four agents from multiple drug classes.[204] The goal of *in vitro* testing in this setting is to quickly evaluate combinations of antimicrobials that might show synergistic interactions when used to treat CRE. Most *in vitro* testing has been performed on KPC-producing CRE whereas MBL- and OXA-48- producing CRE have more limited data.[14]

In KPC-producing *Enterobacteriaceae*, time-kill studies have shown polymyxin B exhibiting synergistic activity ($\geq 10^2$ CFU/mL more killing than the more active agent alone at 24 hours) when in combination with rifampin and when in combination with imipenem.[206] Polymyxin B in combination with both doripenem and rifampin were determined to interact synergistically and exhibit bactericidal activity ($\geq 10^3$ CFU/mL killing at 24 hours).[207] Colistin and tigecycline have also been evaluated in combination together and determined to be synergistic.[208] In a broth microdilution checkerboard assay of 12 KPC-producing *Klebsiella pneumoniae* isolates, polymyxin B was synergistic in combination with either tigecycline, doxycycline or rifampin, but no synergy was detected for combinations with imipenem or gentamicin.[209]

In 42 VIM-producing *K. pneumoniae* isolates from Greece, colistin was found to be synergistic with imipenem in about 50% of colistin-susceptible isolates, regardless of

imipenem MIC, and indifferent (CFU/mL killing is the same as the more active agent alone at 24 hours) in the rest. For the colistin-non-susceptible isolates, the combination was antagonistic (CFU/mL killing was less than the more active agent alone at 24 hours) for 56% of the isolates and synergistic for only 11%.[210]

In nine colistin-resistant KPC-producing *Klebsiella pneumoniae* and three colistin-susceptible KPC-producing *Klebsiella pneumoniae*, colistin in combination with two carbapenems (doripenem and ertapenem) showed synergy in 8 of 12 isolates. Colistin in combination with one carbapenem (doripenem) showed synergy in 6 of 12 isolates and colistin in combination with the other carbapenem (ertapenem) showed synergy in 5 of 12 isolates. Interestingly, the authors noticed an association between synergy of the triple combination of colistin-doripenem-ertapenem and porin expression levels. Specifically, the eight isolates showing synergy had the highest porin expression, and receiver operator characteristic (ROC) analysis designated this group of eight as significantly different from the remaining four in terms of porin expression ($P = 0.002$). The authors speculated that permeability for both carbapenems was limited by porin channel expression and that higher expression provided easier access to the sites of action of both carbapenems.[211]

A systematic review and meta-analysis of *in vitro* interactions between polymyxins and any carbapenem against polymyxin-susceptible CRE found an overall synergy rate of 55%. This analysis also indicated that Etest® and checkerboard synergy testing typically reported lower than did time-kill methodology, and that the use of combination therapy led to less resistance development *in vitro* when post-exposure resistance testing was performed.[212]

Colistin (CST), meropenem (MEM), and tigecycline (TIG) interactions were evaluated using a 3-D checkerboard assay in 20 carbapenem-resistant *K. pneumoniae* clinical isolates. Among these, 13 were resistant to colistin and 6 were resistant to tigecycline. Synergy rates were 10% for MEM and TIG; 30% for CST and MEM; 30% for CST and TIG; and 30% CST, MEM, and TIG. It was noted by the authors that synergy was correlated with higher TIG MICs (>2 μg/mL) and higher CST MICs (>8 μg/mL), there was no antagonism, and addition of a third antimicrobial agent did not contribute to synergy.[213] CST, MEM, and TIG were also evaluated in a time-kill study of eight CRE clinical isolates (4 *K. pneumoniae*, 2 *E. coli*, 1 *E. cloacae*, 1 *S. marcescens*). MEM and TIG were not synergistic in any of the eight strains. TIG and CST showed synergy at concentrations above the MICs for most strains.[208]

Other Combinations

Time-kill assays involving double and triple antimicrobial combinations of aztreonam, ciprofloxacin, colistin, daptomycin, fosfomycin, meropenem, rifampin, telavancin, tigecycline, and vancomycin against MBL-producing (2 VIM and 2 NDM) polymyxin-susceptible *K. pneumoniae* isolates were used to evaluate potential combination therapy against MBL-producing CRE. Sample times were 0, 1, and 24 hours. Synergy was found in double combinations of colistin with either aztreonam, fosfomycin, meropenem, or rifampin and in triple combinations with colistin and meropenem with either aztreonam, fosfomycin, or rifampin. The most effective combination was meropenem, colistin, and rifampin demonstrating bactericidal and synergistic activity throughout 24 hours for all

four strains. Ciprofloxacin, tigecycline, daptomycin, telavancin, and vancomycin alone and in combination with colistin was without synergy or bactericidal activity at 24 hours.[214]

In KPC-producing *Klebsiella pneumoniae*, fosfomycin in combination with either meropenem or colistin was synergistic in 64.7% and 11.8% of isolates, respectively. Fosfomycin in combination with gentamicin was indifferent.[215] Synergy was evaluated in another study with fosfomycin in combination with imipenem (74%), meropenem (70%), doripenem (74%), colistin (36%), netilmicin (42%), and tigecycline (30%) for 50 KPC-producing *Klebsiella pneumoniae*.[216]

Amikacin (AMK) 16 µg/mL was evaluated alone and in combination with ertapenem (ERT) 2 µg/mL, imipenem (IPM) 4 µg/mL, and meropenem (MEM) 4 µg/mL against four *K. pneumoniae* clinical isolates resistant to all four antimicrobials (MICs >8 µg/mL for ERT, IPM, and MEM; MIC 32 µg/mL for AMK). Alone, none of the antimicrobials achieved bactericidal activity. Synergy was found in combinations of AMK with either MEM or IPM throughout 24 hours in all isolates. Bactericidal activity was found in 2 of 4 isolates for MEM and AMK and 1 of 4 isolates for IPM and AMK. ERT with AMK was not synergistic or bactericidal in any isolate.[217]

In Vitro Pharmacodynamic Models

Human pharmacokinetics of meropenem were simulated to optimize meropenem dosing against carbapenemase-producing *Klebsiella pneumoniae* using a one-compartment, chemostat model. An advantage to the 0.5 hour infusion of 1 gm every 8 hours was found in a high dose/prolonged infusion regimen (3 hour infusion of 2 gm every

8 hours). Using this regimen, bactericidal activity ($\geq 10^3$ CFU/mL killing) was obtained by 6 hours against all KPC-producing *Klebsiella pneumoniae* isolates. However, regrowth was observed for 9 of 11 isolates with meropenem MICs $\geq$ 8 µg/mL, but not for two isolates whose meropenem MICs were 2 and 8 µg/mL. Measured meropenem levels were lower than expected using the model, but this was attributed to the production of carbapenemase enzymes by the *K. pneumoniae* isolate.[218]

Human pharmacokinetics of tigecycline (as 50 mg every 12 hours) in combination with either meropenem (as 2 gm infused over 3 hours every 8 hours)[218] or rifampin (as 600 mg every 12 hours) in lung-epithelial fluid were modeled using a one-compartment, chemostat model. Tigecycline alone and in combination with rifampin against carbapenemase-producing *Klebsiella pneumoniae* isolates showed little activity when used against isolates with meropenem MICs $\leq$ 2 µg/mL. However, when tigecycline was used in combination with meropenem, a synergistic, bactericidal effect was observed for isolates with tigecycline MICs up to and including 2 µg/mL and meropenem MICs up to and including 16 µg/mL. However, none of the regimens maintained bactericidal activity for the full 48-hour study period.[219]

Using time-kill methodology and a 3-dimensional response model, six 2-agent combinations of amikacin (AMK), doripenem (DOR), levofloxacin (LEV) and rifampin (RIF) were evaluated against a KPC-2-producing *K. pneumoniae* (MICs AMK: 64 µg/mL, DOR: 16 µg/mL, RIF: >64 µg/mL, LEV: 128 µg/mL) and a KPC-3-producing *K. pneumoniae* (MICs AMK: 32 µg/mL, DOR: 32 µg/mL, RIF: >256 µg/mL, LEV: 8 µg/mL). Clinically obtainable concentrations (AMK: 4-80 µg/mL; DOR 4-32 µg/mL; LEV 0.5-10 µg/mL; RIF 0.25-6 µg/mL) were used in combinations to determine synergy based on the

3-D response model and 24-hour colony count. DOR and AMK was the only combination determined to be synergistic; DOR and RIF, DOR and LEV, and LEV and RIF were additive; AMK and RIF, and AMI and LEV were antagonistic. Murine pneumonia models were used to confirm results obtained through the time-kill experiments and the model for DOR and AMK, and AMK and LEV. As predicted by the model, DOR and AMK showed improved survival for both isolates whereas AMK and LEV displayed inferior survival rates. Although limited in design, this study is one of few models that analyzed polymyxin-sparing regimens.[220]

Dual-carbapenem Therapy

Doripenem and ertapenem alone and in combination were evaluated against a carbapenemase-producing *K. pneumoniae* clinical isolate (doripenem MIC 4 µg/mL; ertapenem MIC 64 µg/mL) using a one-compartment chemostat model. The free doripenem concentrations simulated a 3-hour infusion of 2 gm every 8 hours in humans, and the free ertapenem concentrations simulated a dose of 1 gm every 24 hours in humans. Adding doripenem to ertapenem extended the bactericidal activity from 6h with monotherapy of either agent to 16h in the combination. Doripenem levels were above the MIC of the organism for a majority of the dosing interval. The primary mechanism of interaction of these two carbapenems was that ertapenem acted as a suicide inhibitor of the carbapenemases, since it is most easily hydrolyzed, leaving doripenem to exact its bactericidal activity against CRE.[221]

Animal Studies

KPC-producing *Enterobacteriaceae*

In both an immunocompetent and a neutropenic murine thigh model, doripenem was administered to simulate human administration of a 4-hour infusion of 1 gm and 2 gm doripenem every 8 hours. These regimens were evaluated against KPC-producing *Klebsiella pneumoniae* with doripenem MICs ranging from 4 to 32 µg/mL. 1- and 2- gm doses of doripenem achieved bacteristasis in both models against *K. pneumoniae* isolates with doripenem MICs up to and including 8 and 16 µg/mL, respectively. Expectedly, there was significantly more killing (0.5-1 CFU/mL) in the immunocompetent murine model compared to the neutropenic murine model at 24 hours.[222]

Doripenem and ertapenem alone and in combination were evaluated against three KPC-producing *K. pneumoniae* clinical isolates (doripenem MICs 8, 16, and 32 µg/mL; ertapenem MICs >64 µg/mL) using a murine thigh model of both immunocompetent and neutropenic mice. The free doripenem concentrations simulated a 4-hour infusion of 2 gm every 8 hours in humans, and the free ertapenem concentrations simulated a dose of 1 gm every 24 hours in humans. Although a higher degree of bacterial killing was observed in the combination regimens when compared to monotherapy, only the combination against the lowest doripenem MIC isolate (MIC 8 µg/mL) in the immunocompetent mice was statistically significant, and only at 72 hours (not 24 or 48).[223]

Meropenem, tigecycline, and polymyxin B were evaluated in a rat model alone and in double and triple regimen combinations (n=10 for each regimen) against KPC-2-producing *Klebsiella pneumoniae*. Additionally, time-kill assays were performed on each

agent alone and in combination. No pharmacokinetic studies were performed to verify equivalent human dosing, but all combinations involving polymyxin B showed significantly superior results in terms of mortality (Figure 2.22) and culture clearance. Interestingly, meropenem and tigecycline combinations were antagonistic by time-kill analysis, but this interaction was seemingly overcome by the addition of polymyxin B. This was observed in the rat model as well, but there was not an observable advantage in triple-combination therapy compared to polymyxin B in combination with either meropenem or tigecycline.[224]

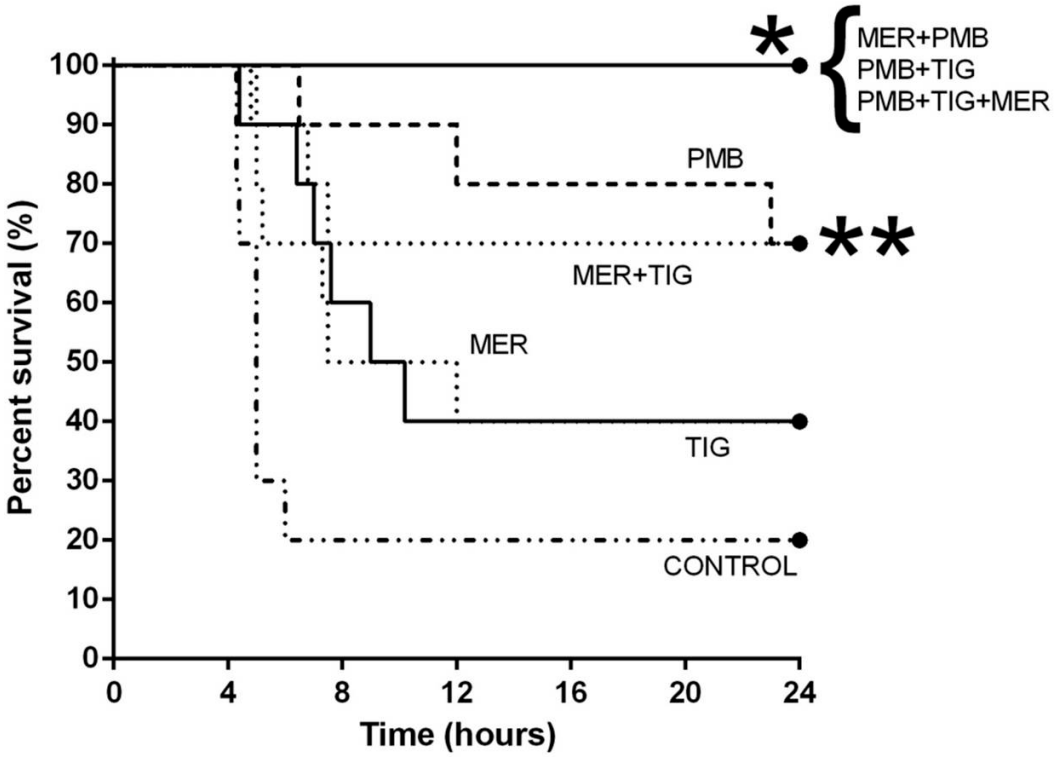


Figure 2.22: Survival Curves of Rats Infected with KPC-2-producing *Klebsiella pneumoniae*. Reprinted[224]
* P-value of <0.05 compared with other groups
** P-value of <0.05 compared with control

MBL-producing *Enterobacteriaceae*

Two animal models (one murine, one rabbit) have evaluated carbapenem monotherapy in VIM-1-producing *K. pneumoniae* or *E. coli*. In both studies, isolate MICs to carbapenems were relatively low (imipenem MICs ≤4 μg/mL for all but one isolate). Dosing regimens were selected to simulate dosing in humans and optimize T>MIC, the pharmacodynamic index correlating with clinical outcome.[225] In both studies, carbapenems were effective in significantly reduced colony counts (CFU/mL) compared to placebo, but were either not as effective as observed in the non-VIM producing isolate,[226] or were surpassed by aztreonam activity, which is relatively stable in the presence of MBL enzymes.[227] These data suggest that while carbapenems are still active as monotherapy against MBL-producing CRE, there may be other factors than time above the MIC that play a role in optimizing treatment.

Doripenem and ertapenem as monotherapy were evaluated in a murine thigh model against a wild-type *K. pneumoniae,* and an isogenically derived NDM-1- and a KPC-2-producing *K. pneumoniae*. Four clinical isolates of NDM-1-producing *K. pneumoniae* were also included for comparison. Dosing regimens of doripenem and ertapenem simulated a 4-hour infusion of 2 gm every 8 hours and 1 gm every 24 hours, respectively. Interestingly, at least 10^1 CFU/mL killing was observed at 24 hours for the wild-type *K. pneumoniae,* the isogenic NDM-1- and the NDM-1-producing clinical isolates with doripenem MICs ≤ 8 μg/mL. However, the isogenic KPC-producing *K. pneumoniae* showed growth (Figure 2.23), despite a 4-fold lower MIC to ertapenem and doripenem.[228] The results of the KPC-producing isolate were consistent however with previous work performed in this lab.[223]

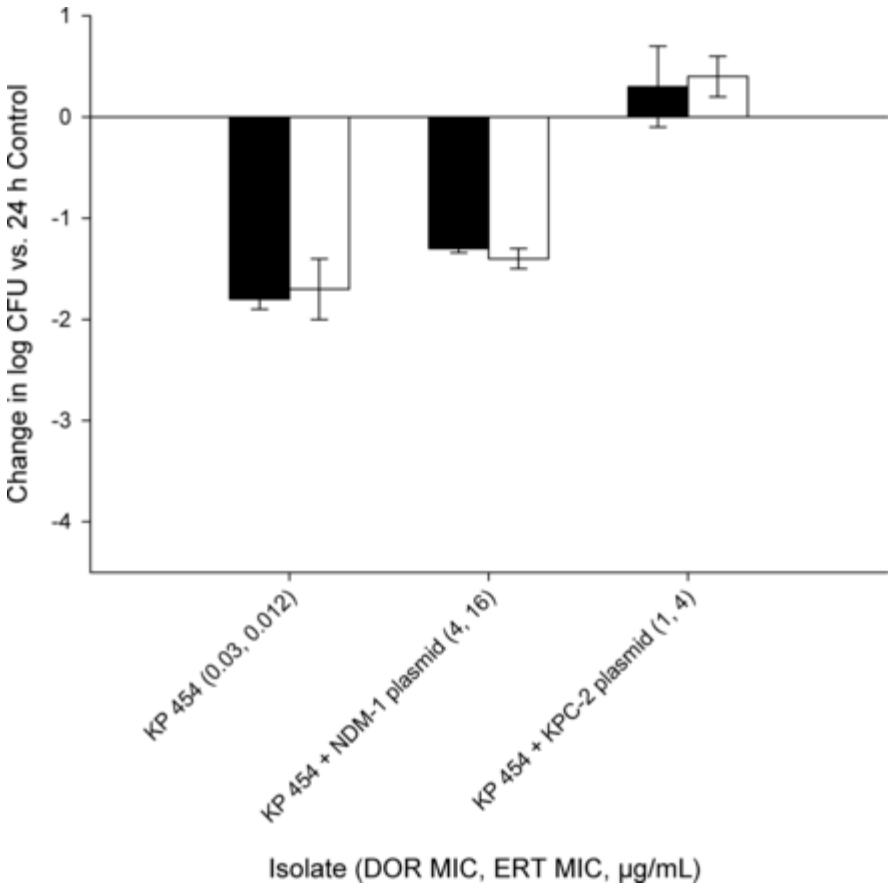


Figure 2.23: Change in $\log_{10}$ CFU/mL after 24 Hours. Reprinted[228]
Wild-type *K. pneumoniae* strain and its derived isogenic strains harboring either an NDM-1 or a KPC-2 plasmid after treatment with either doripenem at 2 gm every 8 hours (black) or ertapenem at 1 gm every 24 hours (white) in an immunocompetent mouse thigh infection model. Each value is the mean ± standard deviation for infected thighs for each isolate.

OXA-48-producing *Enterobacteriaceae*

Doripenem, ertapenem, ceftazidime, and levofloxacin were evaluated in a murine thigh model against an isogenic pair of wild-type *K. pneumoniae* and OXA-48-producing *K. pneumoniae* as well as six OXA-48-producing *Enterobacteriaceae* clinical isolates, with and without other ESBLs (doripenem MICs 0.38 - 8 µg/mL). Levofloxacin, ertapenem and

ceftazidime exhibited efficacy correlating with pharmacodynamic targets and *in vitro* MIC. However, similar to experiments involving isolates producing NDM-1,[228] the observed efficacy of doripenem treatment was surprising. However, whereas doripenem seemed efficacious against low-MIC NDM-1-producing isolates,[228] there was variable efficacy observed by doripenem across all OXA-48-producing isolates, despite achieving the pharmacodynamic target of at least 40% T>MIC.[225] It was concluded by the authors that genotypic expression may be more important than phenotypic MIC and pharmacodynamic targets in selecting appropriate therapy.[229]

Other Studies

In a rather unique study, gene transcription levels of carbapenemase enzymes were analyzed in clinical isolates harboring either CTX-M-15 (ESBL; 1 *K. pneumoniae* and *1 E. coli*), OXA-48 (*E. coli)*, NDM-1 (*K. pneumoniae*) or KPC-2 (*Salmonella spp.*) after infecting mice or inoculating test tubes. The aim of the study was to determine carbapenemase enzyme induction, inhibition, or lack of effect by single antimicrobials or combinations. For the mice studies, rifampin alone, colistin alone and colistin in combination with ertapenem, meropenem, imipenem, fosfomycin, kanamycin, tigecycline, ceftazidime, or rifampin were evaluated. For *in vitro* studies, colistin, meropenem, rifampin and tigecycline alone were evaluated as well as colistin in combination with meropenem, fosfomycin, rifampin, or tigecycline.[230]

The authors listed likely beneficial combinations based on carbapenemase transcription levels observed *in vitro* and *in vivo* and mortality observed *in vivo* according

to enzyme type. For OXA-48, colistin in combination with a carbapenem, rifampin, fosfomycin, or tigecycline seemed most beneficial, but monotherapy with any agent was not recommended. For NDM-1, colistin in combination with rifampin, fosfomycin, or tigecycline were most effective, but again, monotherapy was not recommended with any agent. Finally, for KPC, colistin in combination with a carbapenem, fosfomycin or kanamycin were most beneficial.[230]

Human Studies

Reiterating, comparisons between monotherapy and combination therapy for CRE treatment in humans is limited by frequent retrospective design; small sample sizes; varying geographical locations; lack of appropriate control for confounding variables; and heterogeneous definitions of primary endpoints, sources of infection, infecting genus/species, and patient demographics. However, most recent studies (2016-present) conclude that combination therapy is preferred (Table 2.1). Perez *et al.* selectively compared retrospective reports of CRE bloodstream infections in hundreds of patients receiving either combination or monotherapy. Their analysis concluded a mortality risk reduction of approximately 50% when combination antimicrobials were used compared to monotherapy. These studies were primarily in KPC-producing *K. pneumoniae* and combinations were usually carbapenem-containing in addition to a polymyxin or tigecycline.[14]

Table 2.1: Summary of Monotherapy vs. Combination Therapy in Patients against CRE

Reference	Study Design	Mechanisms of Resistance	Definition and Rate of Mortality	Significantly Associated with Survival
Zarkotou, 2011[10]	Retrospective	KPC-2	**Infection-related, in-hospital; M: 7/15 C: 0/20**	C
Qureshi, 2012[199]	Retrospective	KPC-2; KPC-3	**28-day M: 11/19 C: 2/15**	C
Tumbarello, 2012[200]	Retrospective	KPC-2; KPC-3	**30-day M: 25/46 C: 27/71**	C
Capone, 2013[231]	Prospective	KPC-3; VIM-1; CTX-M-15 w/ porin	**In-hospital M: 8/37 C: 17/54**	Neither
Navarro-San Francisco, 2013[232]	Retrospective	OXA-48	**30-day M: 2/7 C: 13/27**	M*
Balkan, 2014[68]	Retrospective	OXA-48	**28-day M: 2/5 C: 16/31**	Neither
Daikos, 2014[203]	Retrospective	KPC-2; VIM-1; KPC-2 and VIM-1	**28-day M: 32/73 C: 28/103**	C
de Oliveira, 2014[233]	Retrospective	KPC	**30-day M: 21/57 C: 32/61**	Neither
Kontopidou, 2014[234]	Retrospective	KPC; VIM; KPC and VIM	**14-day M: 16/64 C: 8/43**	Neither
Chang, 2015[235]	Retrospective	AmpC or ESBL w/ porin; KPC-2; IMP-8; NDM-1; VIM-1	**30-day M: 7/23 C: 5/10**	Neither
Freire, 2015[236]	Retrospective	KPC-2	**30-day M: 8/22 C: 21/38**	Neither
Katsiari, 2015[237]	Prospective	KPC-2; VIM	**Infection-related, in-hospital M: 2/7 C: 12/25**	Neither

Table 2.1 (continued): Summary of Monotherapy vs. Combination Therapy in Patients against CRE

Reference	Study Design	Mechanisms of Resistance	Definition and Rate of Mortality	Significantly Associated with Survival
Lowman, 2015[238]	Retrospective	OXA-48	**In-hospital M: 2/6 C: 5/13**	Neither
Tumbarello, 2015[151]	Retrospective	KPC-2; KPC-3	**14-day M: 118/307 C: 107/354**	C
de Maio Carrilho, 2016[239]	Prospective	KPC; few other	**Infection-related, in-hospital M: 6/29 C: 38/98**	Neither
Gomez Simmonds, 2016[240]	Retrospective	KPC; few other	**30-day M: 34/44 C: 28/73**	Neither
Falcone, 2016[241]	Retrospective	KPC	**30-day M: 34/44 C: 7/64**	C
Tofas, 2016[242]	Retrospective	KPC; n=2 VIM	**14-day M: 5/10 C: 11/30**	C
Trecarichi, 2016[243]	Prospective	Unspecified	**21-day M: 69/77 C: 40/72**	C
Villegas, 2016[244]	Retrospective	KPC; VIM; NDM	**Infection-related, in-hospital M: 5/8 C: 17/29**	Neither
Gutiérrez-Gutiérrez, 2017[245]	Retrospective	KPC; OXA-48; VIM; few other	**30-day M: 85/208 C: 47/135**	C
Machuca, 2017[246]	Prospective	KPC	**30-day M: 14/32 C: 18/72**	C
Papadimitriou-Olivgeris, 2017[247]	Retrospective	KPC; n=3 VIM; n=1 NDM; KPC and VIM	**30-day M: 18/57 C: 7/38**	C

Table 2.1 (continued): Summary of Monotherapy vs. Combination Therapy in Patients against CRE

Reference	Study Design	Mechanisms of Resistance	Definition and Rate of Mortality	Significantly Associated with Survival
Satlin, 2017[248]	Retrospective	KPC-2; KPC-3; n=2 NDM-1; n=2 OXA-48	**30-day M: 21/55 C: 22/43**	Neither
Tuon, 2017[249]	Retrospective	Unspecified (likely KPC)	**30-day M: 40/66 C: 6/17**	C

Adapted from Tumbarello et al.[124]
*High prevalence of patients presenting with less severe infections in monotherapy arm
M-Monotherapy
C-Combination therapy

Despite known resistance to carbapenems, when CRE are treated with combinations containing a carbapenem, there appears to be added benefit on top of the benefit for combination therapy, particularly in strains with lower carbapenem MIC's (MIC ≤ 8 µg/mL). Zouvelekis *et al.* systematically evaluated studies using monotherapy with carbapenems (meropenem or imipenem) and determined that the failure rate of 50 CRE patients across 15 studies was found to be proportional to the MIC for the respective carbapenem used. Note: clinical failure definitions varied from physician to physician and study to study. Some definitions were patient death, superinfection or reinfection with same organism, prolonged hospital stay, and resistance development while on antimicrobial therapy, but overall clinical failure was estimated to be 75% for CRE infections with carbapenem MICs above 8 µg/mL. This failure rate decreased to 33.3%, 28.6%, and 25% when carbapenem MICs were 8, 4, and 2 µg/mL or less, respectively.[204]

This observation fits in the context of the PK/PD studies in humans and the pharmacodynamic index for carbapenems – 40% to 50% time above the MIC (T>MIC) in

that higher carbapenem MICs render target attainment of 50% T>MIC more difficult. It is estimated that for a meropenem MIC of 4 µg/mL, the probability of attaining 50% T>MIC is 69% for a dosing regimen of a 30 minute infusion of 1 gm every 8 hours. When a high dose/prolonged infusion is used (e.g. 3-hour infusion of 2 gm every 8 hours), the probability of target attainment increases to 100%. When the MIC is 8, the probability of attaining 50% T>MIC of a high dose/prolonged infusion of meropenem is 85%.[250]

Adding to the evidence of carbapenem-based combination regimens are two articles evaluating CRE treatment in Greece. For the first study, 103 *K. pneumoniae* isolates producing either VIM or KPC were treated with combination therapy (30% carbapenem-based) and 72 isolates were treated with monotherapy and mortality was significantly lower in the combination therapy group (27.2% vs. 44.4%, p=0.018). Lower mortality was observed for carbapenem-containing regimens when compared to regimens without carbapenems (19.3% vs. 30.6%).[203] For the second study, 132 VIM-producing *K. pneumoniae* and 102 KPC-producing *K. pneumoniae* isolates were included across nine studies where it was determined combination therapy was superior to monotherapy (p = 0.01; odds ratio 2.41; 95% confidence interval 1.2-4.7) and those regimens that included carbapenems were associated with a 6.7% failure rate compared to a 26.9% failure rate of those regimens without a carbapenem (P-value 0.04).[185]

In Italy, 14 day mortality was assessed in 661 patients with KPC-producing *K. pneumoniae*. Independent predictors of 14 day mortality were determined to be bloodstream infection, presentation with septic shock, inadequate empirical antimicrobial therapy, chronic renal failure, high APACHE III score, and colistin resistance. Combination therapy with at least two drugs showing *in vitro* activity against the isolate

was associated with lower mortality (odds ratio 0.52; (95% confidence interval 0.35-0.77). Combinations that included meropenem were associated with significantly higher survival rates when the meropenem MIC was ≤ 8 μg/mL.[151]

A review article of published case reports and case series from 2001-2011 included 105 total cases of KPC-producing infections (101 of which were *Enterobacteriaceae*). Cases receiving monotherapy were 49 (47%) whereas cases receiving combination therapy were 56 (53%), 19 (34%) of which included a carbapenem. Treatment failure was associated more with monotherapy than combination therapy (49% vs. 25%; p = 0.01). Other significant differences were between monotherapy vs. combination therapy involving pulmonary infections, polymyxins, or carbapenems, (Table 2.2).[201]

Table 2.2 Treatment Failure: Monotherapy vs. Combination Therapy.

	Monotherapy (%)	**Combination (%)**	**P**
Overall Treatment Failure	24/49 (49)	14/56 (25)	**0.01**
Source:			
Blood	12/24 (50)	9/32 (28)	0.09
Pulmonary	10/15 (67)	5/17 (29)	**0.03**
Urine	1/8 (13)	0/3 (0)	0.4
Polymyxin Treatment Failure	8/11 (73)	10/34 (29)	**0.02**
Carbapenem Treatment Failure	12/20 (60)	5/19 (26)	**0.03**
Tigecycline Treatment Failure	2/7 (29)	7/19 (37)	0.4
Aminoglycoside Treatment Failure	0/6 (0)	4/24 (17)	0.6

Reprinted[201]

Another review article systematically obtained CRE case reports, case series, and observational studies from the across the globe (e.g. U.S., Spain, Ireland, Columbia, China, Israel, Brazil, Taiwan, Switzerland, and Greece). A total of 301 patients infected with *Klebsiella pneumoniae* were identified, about half KPC-producing and half MBL-producing. Patients were stratified into seven groups based on treatment regimens (Figure 2.21). Once more, combination therapy with a carbapenem was significantly superior to alternative combinations analyzed.[204]

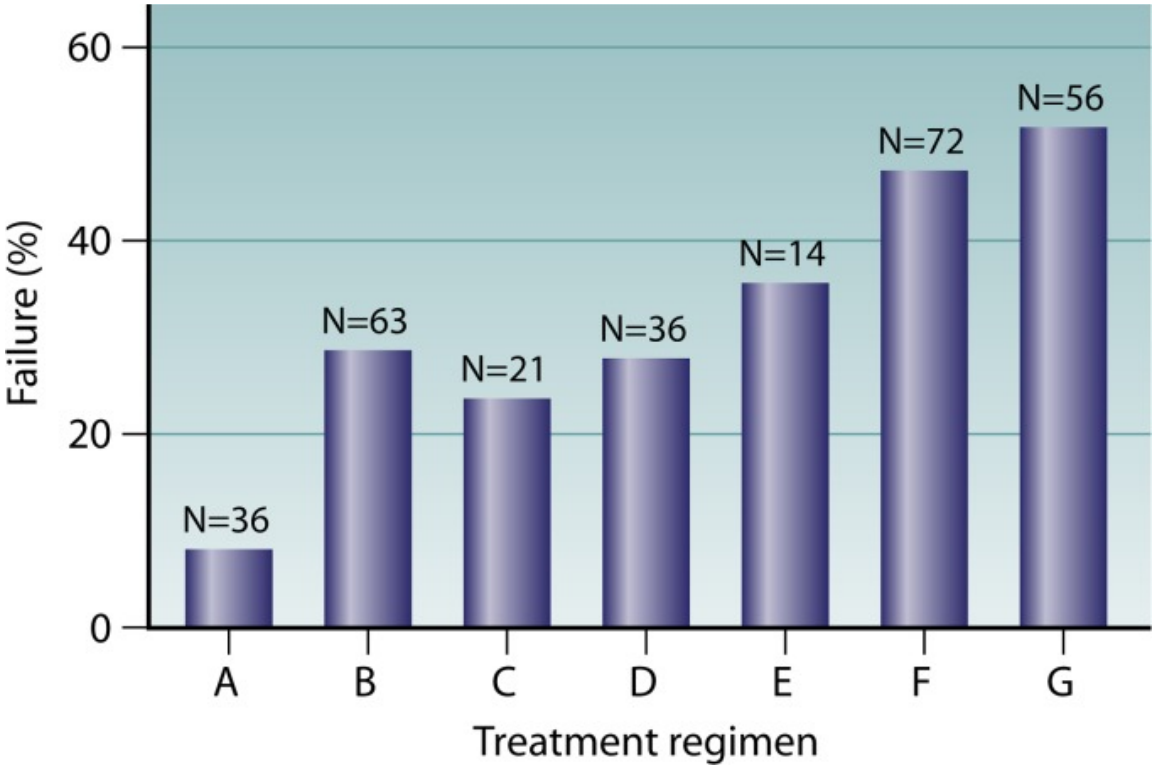


Figure 2.24: Outcomes of Infections Caused by Carbapenemase-producing *Klebsiella pneumoniae*, According to Treatment Regimen. Reprinted[204]
Regimen A, combination therapy with ≥2 active drugs, one of which was a carbapenem; regimen B, combination therapy with ≥2 active drugs, not including a carbapenem; regimen C, monotherapy with an aminoglycoside; regimen D, monotherapy with a carbapenem; regimen E, monotherapy with tigecycline; regimen F, monotherapy with colistin; regimen G, inappropriate therapy. Regimen A was superior to regimens B, E, F, and G (for A versus B, E, F, and G, the *P* value was 0.02, 0.03, <0.0001, and <0.0001, respectively). Regimens B, C, and D were superior to regimen G (for B versus G, *P* = 0.014; for C versus G, *P* = 0.04; and for D versus G, *P* = 0.03).

Finally, another recent meta-analysis by Zusman et al. indicated that antimicrobial combinations containing a polymyxin antimicrobial are associated with lower mortality than polymyxin monotherapy for carbapenem-resistant infections.[251] This study combined the results of 22 other studies, including 3 randomized controlled clinical trials, without heterogeneity, for a total of 537 patients. Some limitations do exist regarding the applicability of these data to CRE, however. Specifically, all three randomized controlled trials included in the meta analysis were primarily treating *Acinetobacter spp.* Next, very low quality (but certainly not low quantity) evidence support polymyxin combination therapy over polymyxin monotherapy against carbapenem-resistant *Enterobacteriaceae*.

Few human studies have evaluated fosfomycin against CRE. One is a multicenter case-series of 41 carbapenemase-producing *K. pneumoniae* and 17 carbapenemase-producing *P. aeruginosa*. Fosfomycin (median dose 24g/day) was usually combined with either colistin or tigecycline with a clinical success rate at day 14 of 54%. 28 day mortality was 37.5%. Interestingly, resistance to fosfomycin developed in only three cases.[183] The second study followed 11 ICU patients infected with fosfomycin susceptible, carbapenem-resistant *K. pneumoniae* where fosfomycin was administered in combination with colistin (6 patients), gentamicin (3 patients), or piperacillin/tazobactam (1 patient). The combination used for the 11th patient was not mentioned by the authors. All-cause in-hospital mortality was 18.2% (2/11 ICU patients).[252]

Recently Approved Antimicrobials against Carbapenem-resistant *Enterobacteriaceae*

Ceftazidime/avibactam

Ceftazidime/avibactam was the first beta-lactam/beta-lactamase inhibitor combination with activity against CRE, and the FDA approved its use in February 2015. Its spectrum of activity is similar to ceftazidime (i.e. wild-type *Enterobacteriaceae* and *Pseudomonas spp.*) but avibactam (a diazabicyclooctanase)[253] adds Ambler class A[254] and D[255] carbapenemase-producing *Enterobacteriaceae* in addition to ESBL-producing *Enterobacteriaceae* with porin channel mutations to the spectrum.[254] However, limited activity against MBLs has been observed. Ceftazidime/avibactam has also been tested *in vitro* against KPC-producing isolates with OmpK36 porin channel mutations. All 72 KPC-producing isolates studied were resistant to ceftazidime (MICs >64 µg/mL) but tested susceptible (ceftazidime MICs <4 µg/mL) with the addition of avibactam.[256]

Shields et al. published a landmark retrospective study comparing for the first time ceftazidime/avibactam with (n=5) or without (n=8) gentamicin to conventional treatment against CRE.[257] Most isolates (106/109) harbored KPC-2 or KPC-3 enzymes, and conventional treatment was primarily a carbapenem with an aminoglycoside (CB +AG; n=25), a carbapenem with colistin (CB+COL; n=30), or other therapies including some monotherapy. Clinical success was defined as 30-day survival, resolution of signs and symptoms of infection, sterilization of blood cultures within 7 days of treatment initiation, and absence of recurrent infections (Figure 2.25).

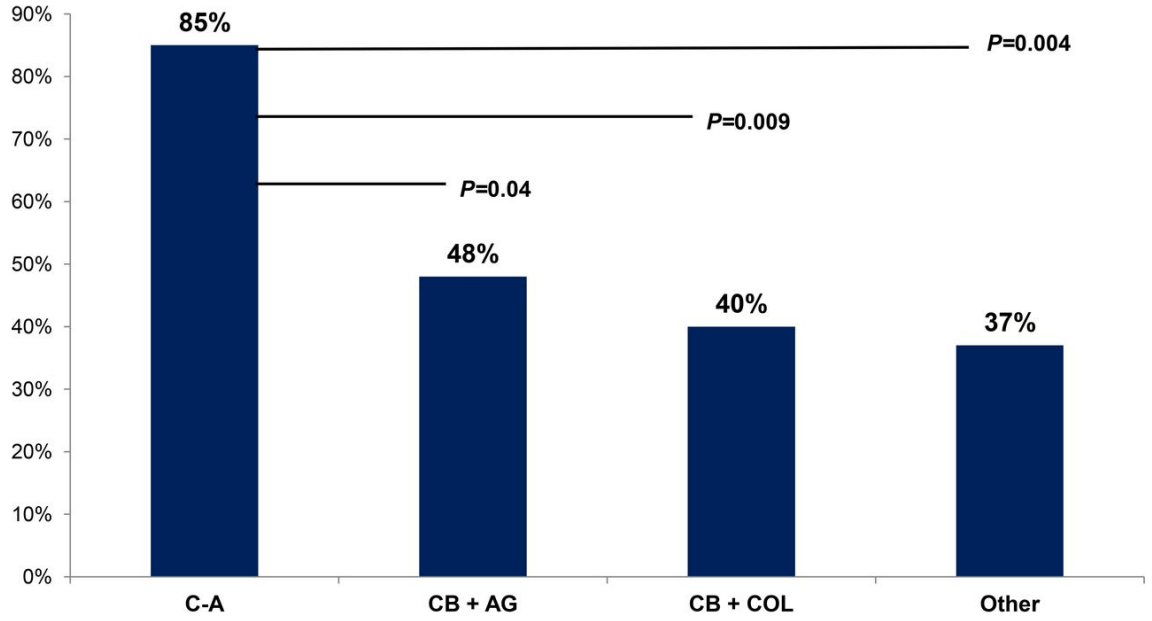


Figure 2.25: 30-day Clinical Success Across Treatment Regimens. Reprinted.[257] C-A – ceftazidime/avibactam; CB + AG – carbapenem + aminoglycosides; CB + COL – carbapenem + colistin; other – aminoglycoside, carbapenem, colistin, tigecycline, or ciprofloxacin monotherapy or colistin/tigecycline combination therapy

However, a follow-up study has reported ceftazidime/avibactam resistance in 3/10 recurrent infections in patients previously treated with ceftazidime/avibactam[258] due to mutations in the bla_{KPC-3} gene.[259] Additional data are needed to determine how rapid ceftazidime/avibactam resistance develops, the occurrence frequency of ceftazidime/avibactam resistance, and whether this phenomenon is associated with ceftazidime/avibactam monotherapy, inappropriate dosing, or high-inoculum infections. A more complete overview of clinical data with ceftazidime/avibactam is presented by Rodriguez-Bano et al.[260]

Meropenem/vaborbactam

Meropenem/vaborbactam was the next antimicrobial agent approved by the FDA (August 2017) to combat infections caused by CRE and was the first carbapenem/beta-lactamase inhibitor combination. In fact, the randomized, controlled clinical trial (TANGO 2) was terminated early after an interim analysis showed superiority of meropenem/vaborbactam compared to best available therapy. Its spectrum of activity includes all organisms for which meropenem alone exhibits activity, and vaborbactam adds activity against organisms producing Ambler class A carbapenemases (i.e. KPC). Unlike ceftazidime/avibactam, very limited activity is seen against organisms producing class D enzymes. Vaborbactam also does not restore activity to meropenem in isolates expressing MBL enzymes, so alternative approaches are warranted in this case. TANGO 2 is continuing to recruit patients in the meropenem/vaborbactam arm to gather additional data, and observational studies are lending further support to its superiority over best available therapy (e.g. combinations of conventional antimicrobial agents). However, data is still very much limited at this time. Additional information on this novel antimicrobial is discussed by Cho et al.[261]

Summary

1. CRE are among the top threats in infectious disease according to the CDC and the President's Advisory Council on Combating Antibiotic-Resistant Bacteria.[11]

2. CRE are present throughout the world, but the characteristics of carbapenem resistance can vary widely depending on the country, state, or even acute care center. Within the U.S., KPC-producing *Enterobacteriaceae* compose 80% of CRE cases.[14]

3. Significant mortality is associated with CRE infection, ranging from 24-70%.[1-10]

4. CRE are challenging to treat, often only being susceptible to polymyxins, fosfomycin, tigecycline, and sometimes aminoglycosides for which there are limited data from randomized controlled trials directing antimicrobial therapy.

5. A review of the literature favors combination therapy, usually with a carbapenem and/or a polymyxin, but type of carbapenemase and appropriate pharmacodynamic targets likely play a significant role on optimal therapy.

6. There are few antimicrobials in development against CRE, and those that have been approved have limited data guiding their appropriate use against CRE.

Hypotheses

1. Different antimicrobial susceptibility testing methods can be utilized to evaluate and characterize antimicrobial activity against carbapenem-resistant *Enterobacteriaceae*

2. Novel carbapenemase inhibitor combinations, tigecycline, and the polymyxins would exhibit high activity against carbapenem-resistant *Enterobacteriaceae*

3. Whole genome sequencing and phenotypic assays to identify resistance mechanisms can guide appropriate selection of antimicrobial therapy

4. Antimicrobial agents used alone against carbapenem-resistant *Enterobacteriaceae* would be insufficient to prevent bacterial growth and prevent the emergence of resistance

5. Meropenem in combination with polymyxin B or an aminoglycoside would exhibit synergistic, bactericidal activity against carbapenem-resistant *Enterobacteriaceae* having low and high levels of carbapenem resistance, described by the minimum inhibitory concentration of meropenem

6. Sequencing and timing of antimicrobials in combination and the degree of carbapenem resistance will impact the observed interaction

7. A pharmacodynamic mathematical model can describe observed behavior of carbapenem-resistant *Enterobacteriaceae* under variable antimicrobial conditions

Specific aims

1. To evaluate the activity of novel and conventional antimicrobial agents against carbapenem-resistant *Enterobacteriaceae* clinical isolates from the University of Kentucky Chandler Hospital using broth microdilution, Etest®, disk diffusion, and the BD Phoenix™ system

2. To assess genotypic and phenotypic expression of antimicrobial resistance mechanisms by whole genome sequencing and disk diffusion methodology and their association with corresponding antimicrobial activity

3. To describe the growth of different carbapenem-resistant *Enterobacteriaceae* when exposed to meropenem, amikacin, and polymyxin B alone and in combination at various concentrations using time-kill assays and microfiltration techniques

4. To mathematically model the pharmacodynamic relationship of meropenem and polymyxin B against *Enterobacteriaceae* that represent different degrees of carbapenem resistance, indicated by the meropenem minimum inhibitor concentration

Chapter Three:

Methods

Part of the research contained within this chapter has been published as Kulengowski, B. *In vitro* activity of polymyxin B and meropenem alone and in combination against carbapenem-resistant *Enterobacteriaceae*. 2016. *Theses and Dissertations—Pharmacy*. 57.

In Vitro Susceptibility Testing

Antimicrobial Agents

Antimicrobial powders were obtained from the manufacturers or supply companies listed in Table 3.1. After adjusting for potency, these powders were used in all studies.

Table 3.1: Sources of Antimicrobial Powders

Antimicrobial	Manufacturer/Supply Company	
Amikacin	Sigma-Aldrich®	St. Louis, MO
Aztreonam	Sigma-Aldrich®	St. Louis, MO
Cefepime	USP	Rockville, MD
Ceftazidime	Sigma-Aldrich®	St. Louis, MO
Colistin	Sigma-Aldrich®	St. Louis, MO
Ertapenem	Sigma-Aldrich®	St. Louis, MO
Gentamicin	Sigma-Aldrich®	St. Louis, MO

Table 3.1 (continued): Sources of Antimicrobial Powders

Antimicrobial	Manufacturer/Supply Company	
Imipenem	Sigma-Aldrich®	St. Louis, MO
Levofloxacin	Sigma-Aldrich®	St. Louis, MO
Meropenem	USP	Rockville, MD
Minocycline	Sigma-Aldrich®	St. Louis, MO
Nitrofurantoin	Sigma-Aldrich®	St. Louis, MO
Piperacillin	Sigma-Aldrich®	St. Louis, MO
Polymyxin B	Sigma-Aldrich®	St. Louis, MO
Relebactam	Merck & Co.	Kenilworth, NJ
Tazobactam	LKT Laboratories, Inc.	St. Paul, MN
Tigecycline	Sigma-Aldrich®	St. Louis, MO
Tobramycin	Sigma-Aldrich®	St. Louis, MO

Antimicrobial Solution Preparation

Stock solutions were prepared for each antimicrobial agent using CLSI recommended diluents (usually sterile water).[262] For water sterilization, filtered water (Q-POD® Millipore using a 0.22 µm Millipak® 40 filter; Merck KGaA, Darmstadt, Germany) was autoclaved at 121˚C for 30 minutes in 1 L batches. Primary stock solutions were prepared using the antimicrobial powders described previously (see Antimicrobial Agents). 10 mL of each antimicrobial agent was prepared using a volumetric flask (Kimax® 10 mL volumetric flask; Kimble; Cole-Parmer®, Vernon Hills, IL) and powder was weighed using an analytical balance (Mettler AE200, Figure 3.1; Marshall Scientific, Hampton, NH) according to purity (Table 3.2).

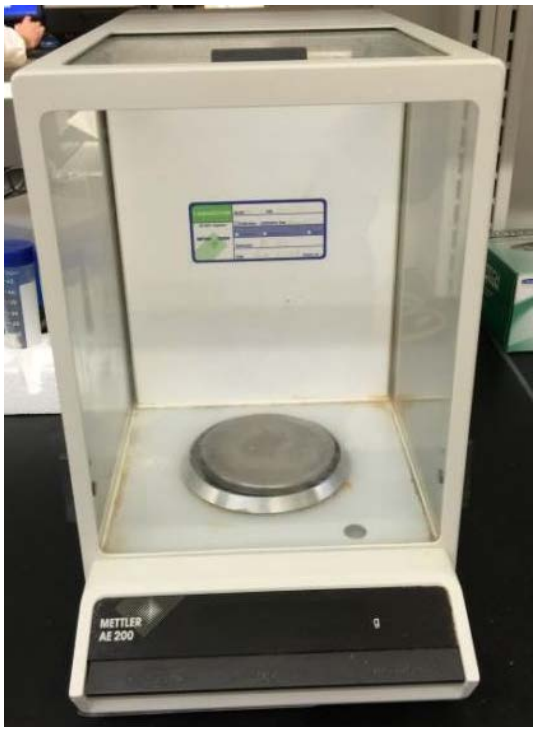


Figure 3.1: Analytical Balance; Marshall Scientific, Hampton, NH.

Table 3.2: Primary Antimicrobial Stock Solutions

Antimicrobial	Powder (mg)	Potency (mg/mg)	Concentration (mg/mL)
Amikacin	263.9	0.776	20.48
Aztreonam	212.7	0.963	20.48
Cefepime	124.0	0.826	10.24
Ceftazidime	232.2	0.882	20.48
Colistin	32.7	0.783	2.56
Ertapenem	26.6	0.962	2.56
Gentamicin	78.0	0.655	5.12
Imipenem	59.1	0.866	5.12
Levofloxacin	12.9	0.99	1.28
Meropenem	5.39	0.95	5.12
Minocycline	28.7	0.892	2.56
Nitrofurantoin	205	0.999	20.48
Piperacillin	216.5	0.946	20.48
Polymyxin B	34.4	0.745	2.56
Relebactam	16.0	1.0	1.6
Tazobactam	16.1	0.994	1.60
Tigecycline	12.9	0.994	1.28
Tobramycin	55.23	0.927	5.12

Exceptions to using sterile water as the only diluent include the following antimicrobials: aztreonam, cefepime, ceftazidime, ertapenem, imipenem, and nitrofurantoin. Aztreonam stock was made by dissolving the weighed powder into a

saturated sodium bicarbonate solution and then filling the rest of the volumetric flask with sterile water. Cefepime was dissolved in a 0.1 M phosphate buffer at pH 6. Ceftazidime was dissolved in a solution containing sodium carbonate in an amount equal to 10% of the ceftazidime powder after correcting for purity (Table 3.2). Ertapenem and imipenem were dissolved in a 0.01 M phosphate buffer at pH 7.2. Nitrofurantoin was dissolved in DMSO.

Phosphate buffer stock solutions were previously prepared from a 1 M dibasic potassium phosphate solution (Dry Powder; Sigma-Aldrich®, St. Louis, MO) and a 1M monobasic potassium phosphate solution (Dry Powder; Sigma-Aldrich®, St. Louis, MO) using proportions outlined in Table 3.3, and subsequently diluted in sterile water to 0.1 M or 0.01 M appropriately. Primary stock solutions were frozen at -20˚C until needed for an experiment, frozen and thawed no more than 5 times, and were not used beyond 1 month after making.[263]

Table 3.3: Preparation of 1 M Phosphate Buffer Solution

pH	Volume of 1 M K_2HPO_4 (mL)	Volume of 1 M K_1H2PO_4 (mL)
6.0	0.132	0.868
7.2	0.717	0.283
8.0	0.940	0.060

All prepared antimicrobial stock solutions were stored in plastic conical vials (15 mL polypropylene conical centrifuge tubes; USA Scientific, Ocala, FL). Secondary stock solutions were prepared using 9 parts broth to 1 part primary stock solution on the day of testing in similar plastic conical vials and used immediately following preparation. For antimicrobials that were to be on the same 96-well tray as antimicrobials requiring inhibitors (e.g. piperacillin or imipenem), 8.75 parts broth was used to 1.25 parts primary

stock to account for the extra 25 µL of volume at the end of the experiment (see Broth Microdilution Procedure). Inhibitors required being diluted 99 parts broth to 1 part primary stock solution due to the minimum weighable quantity of the analytical balance used and the low concentration (4 mg/L) needed in each well (see Broth Microdilution Procedure). Table 3.4 contains secondary stock concentrations assuming antimicrobials were diluted 1:10 using broth. See Broth Microdilution Procedure for addition of antibiotics to the microtiter trays (Costar® non-treated, sterile, polystyrene 96-well; Sigma-Aldrich®, St. Louis, MO).

Table 3.4: Antimicrobial Secondary Stock Concentrations and Testable MIC range

Antimicrobial	Concentration (mg/mL)	Concentration Range (mg/L)
Amikacin	2.048	0.25 – 512
Aztreonam	2.048	0.25 – 512
Cefepime	1.024	0.125 – 256
Ceftazidime	2.048	0.25 – 512
Colistin	0.256	0.03 - 64
Ertapenem	0.256	0.03 - 64
Gentamicin	0.512	0.06 – 128
Imipenem	0.512	0.06 – 128
Levofloxacin	0.128	0.015 - 32
Meropenem	0.512	0.06 – 128
Minocycline	0.256	0.03 - 64
Nitrofurantoin	2.048	0.25 – 512
Piperacillin	2.048	0.25 – 512

Table 3.4 (continued): Antimicrobial Secondary Stock Concentrations and Testable MIC range

Polymyxin B	**0.256**	**0.03 - 64**
Relebactam	0.016	4
Tazobactam	0.016	4
Tigecycline	0.128	0.015 - 32
Tobramycin	0.512	0.06 – 128

Bacterial Isolates

Clinical isolates of 612 non-duplicate, multidrug resistant (MDR), gram-negative organisms were collected between November 9, 2008 and October 1, 2018 from the Clinical Microbiology Laboratory at the University of Kentucky Chandler Medical Center in Lexington, Kentucky. All isolates were cultured and identified during routine testing in the clinical laboratory according to guidelines from the Clinical and Laboratory Standards Institute (CLSI).[264] All isolates were frozen at -80˚C in 10% glycerol in water solution until needed for study.[265] Multidrug resistance was defined as non-susceptibility to at least one agent in three or more antibiotic classes.[166] Isolates were designated carbapenem-resistant *Enterobacteriaceae* (CRE) if they were *Enterobacteriaceae* with documented carbapenemase production or non-susceptibility to any of the carbapenem antimicrobials (ertapenem, imipenem, meropenem, or doripenem; Table 3.10).[79]

Each isolate was subcultured twice in cation-adjusted Mueller-Hinton broth (CAMHB) prior to conducting experiments to ensure log phase growth. Inoculation of

conical tubes (15 mL polypropylene conical centrifuge tubes; USA Scientific, Ocala, FL) containing approximately 5 mL of CAMHB was accomplished using a sterile loop applicator (Fisherbrand™; ThermoFisher Scientific, Waltham, MA). These cultures were then incubated at 35˚C in a shake incubator (Figure 3.2; MaxQ 6000; ThermoFisher Scientific, Waltham, MA) at 220 oscillations per minute until turbid. All isolate manipulations were performed in the Lee T. Todd, Jr. Building (TODD), formerly Biological-Pharmaceutical Complex (BPC), room 374B. ATCC® quality control (QC) organisms were used: *E. coli* ATCC® 25922, *P. aeruginosa* ATCC® 27853, *K. pneumoniae* ATCC® 700603, *K. pneumoniae* ATCC® BAA-1705, *K. pneumoniae* ATCC® BAA-1706, *K. pneumoniae* ATCC® BAA-2146, and *E. coli* NCTC 13846. The QC *E. coli* and *P. aeruginosa* strains are recommended for antimicrobial susceptibility testing on *Enterobacteriaceae*. QC *K. pneumoniae* 700603 is a negative control for MBL testing by Etest® (Etest® Procedure) and MBL/KPC phenotypic testing (Modified Disk Diffusion Procedure for the Detection of MBL and KPC Enzymes).[262] QC *K. pneumoniae* 1705, 1706, and 2146 are additional control strains for MBL/KPC phenotypic testing (Modified Disk Diffusion Procedure for the Detection of MBL and KPC Enzymes).[266] Respectively, these are KPC positive, wild type, and NDM positive controls. There was no available ATCC control strain that produces both a KPC and MBL enzyme at the time of study. QC *E. coli* NCTC 13846 is an mcr-1 positive control strain for polymyxin B resistance.[267]

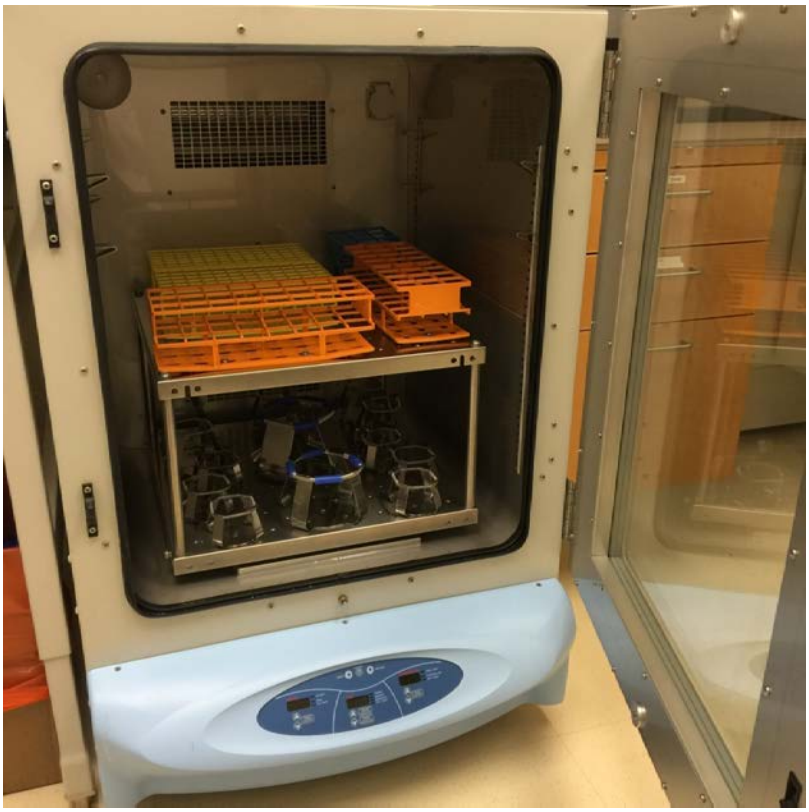


Figure 3.2: MaxQ 6000; ThermoFisher Scientific, Waltham, MA.

Broth Microdilution Susceptibility Testing

In vitro susceptibility testing was performed in duplicate on at least two separate occasions using broth microdilution according to CLSI guidelines (see Broth Microdilution Procedure and Minimum Inhibitory Concentration (MIC) Determination).[264] Broth microdilution susceptibility testing was performed on 122 CRE as part of an investigator proposed research study funded by Merck and Co. (MISP #56367). MICs were determined for amikacin, gentamicin, tobramycin, colistin, polymyxin B, minocycline, tigecycline, ertapenem, meropenem, imipenem, levofloxacin, nitrofurantoin, cefepime, ceftazidime, aztreonam, piperacillin/tazobactam, and a novel β-lactam/β-lactamase inhibitor combination – imipenem/relebactam (Figure 3.3). Quality control organisms used in each experiment were *E. coli* ATCC® 25922 or *P. aeruginosa* ATCC® 27853 according to CLSI guidelines.[268]

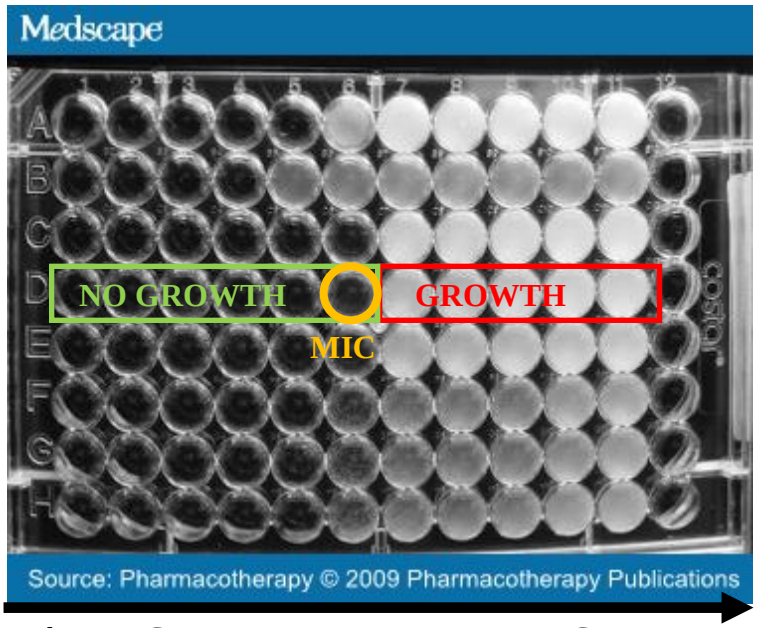


Figure 3.3: Broth Microdilution 96-well Microtiter Tray. The green box surrounds a wells without visible antimicrobial growth. The red box surrounds wells with visible antimicrobial growth. The yellow circle indicates the well containing the lowest tested concentration of antimicrobial that inhibited bacterial growth to the unaided eye.

Automated Susceptibility Testing

BD Phoenix™ (Becton, Dickinson and Company; Franklin Lakes, NJ) is an automated system designed to identify the organism and perform antimicrobial susceptibility testing (Figure 3.4). The University of Kentucky Clinical Microbiology Laboratory evaluated each isolate with BD Phoenix™ in order to obtain MICs for amikacin, ampicillin, ampicillin/sulbactam, aztreonam, cefazolin, cefepime, cefoxitin, ceftazidime, ceftriaxone, cefuroxime, ciprofloxacin, ertapenem, gentamicin, levofloxacin, meropenem, nitrofurantoin, piperacillin/tazobactam, tetracycline, tobramycin, and sulfamethoxazole/trimethoprim. This data was originally reported for clinical use in the management of patients infected with these isolates. This data is available in Appendix A.

Figure 3.4: BD Phoenix™; Bection, Dickinson and Company, Franklin Lakes, NJ. Reprinted[269]

Kirby-Bauer Disk Diffusion Susceptibility Testing

Kirby-Bauer disk diffusion was used to evaluate susceptibility of CRE isolates to ceftazidime/avibactam according to CLSI guidelines because avibactam powder was not commercially available for study.[270] This method does not measure MICs, but instead indicates whether isolates are susceptible, intermediate, or resistant to the antibiotic of choice based on the diameter of the zone of inhibition (see Kirby-Bauer Disk Diffusion Procedure and Figure 3.5). *P. aeruginosa* ATCC® 27853 was used as a quality control strain. A modified disk diffusion assay was also utilized to detect whether isolates produce KPC, MBL, both, or neither enzyme (see Modified Disk Diffusion Procedure for the Detection of MBL and KPC Enzymes).[266] *K. pneumoniae* ATCC® 700603, *K. pneumoniae* ATCC® BAA-1705, *K. pneumoniae* ATCC® BAA-1706, and *K. pneumoniae* ATCC® BAA-2146 were used as quality control strains.

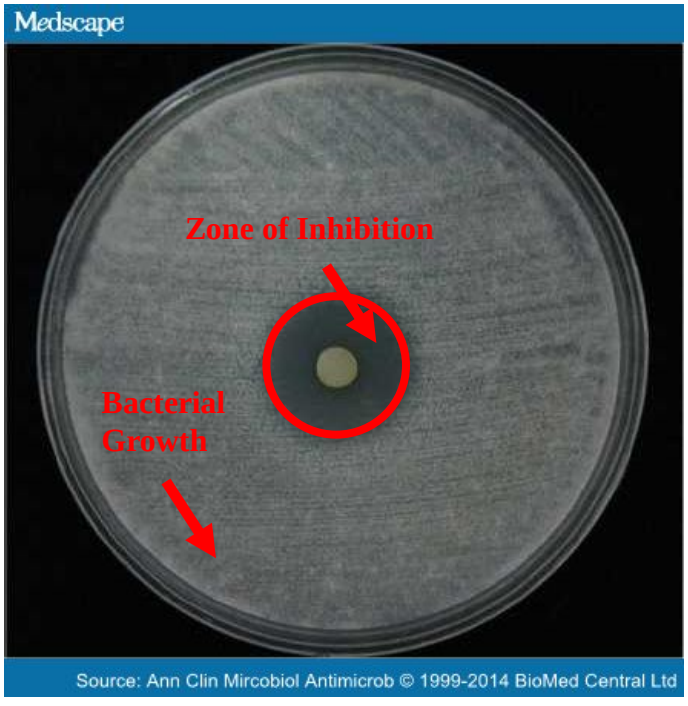
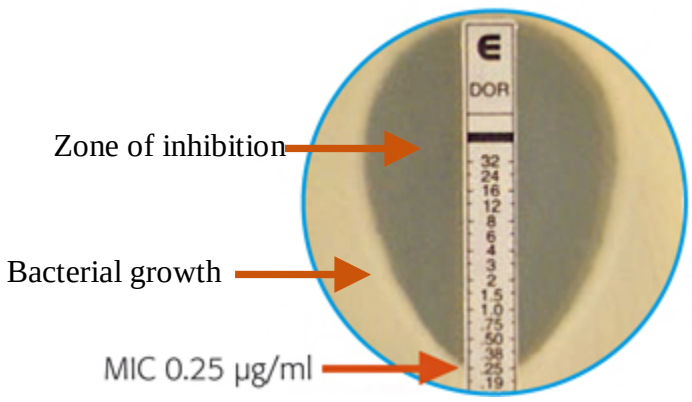


Figure 3.5: Kirby-Bauer Disk Diffusion.

Etest® Susceptibility Testing

Etest® strips (bioMérieux, Inc., Durham, NC) were utilized to detect metallo β-lactamase (MBL) production in CRE isolates non-susceptible to ceftazidime/avibactam from Kirby-Bauer testing (see Kirby-Bauer Disk Diffusion Susceptibility Testing). Etest® strips were also utilized to measure MICs (Figure 3.6) like broth microdilution and were used for comparing polymyxin B MICs measured by Etest® to polymyxin B MICs measured by broth microdilution (see Polymyxin B Etest® Compared to Gold-standard Broth Microdilution in Carbapenem-resistant *Enterobacteriaceae* Exhibiting a Wide Range of Polymyxin B MICs).[271] *Klebsiella pneumoniae* ATCC® 700603 was used as a quality negative control for MBL Etest® strips, and *P. aeruginosa* ATCC 27853 and *E. coli* NCTC 13846 were used as quality control organisms for polymyxin B MIC testing with Etest® strips.



Source: bioMérieux, Inc., Durham, NC

Figure 3.6: Doripenem Etest®.

Glassware, Plastic Tubing, and Pipette Tip Preparation

All glassware and autoclavable plastic were either provided pre-sterilized by the manufacturer or were sterilized by autoclave at 121°C for at least 20 minutes and verified by autoclave indicator tape (Fisherbrand™, ThermoFisher Scientific, Waltham, MA) and corresponding autoclave quality control reports prior to their utilization in experiments. Equipment that could not be autoclaved (e.g. automated plate pourer or the laser colony counter) was sterilized by 70% ethanol in a spray bottle. A dispenser (Oxford®; Cole-Parmer®, Vernon Hills, IL) was sterilized by two 70% ethanol washes followed by two 0.22 µm filtered (Millipak® 40 filter; Merck KGaA, Darmstadt, Germany), distilled water washes and verified by negative growth of water dispensed onto an agar plate. A pipette controller (Ovation® Ali-Q™; VistaLab, Brewster, NY) that utilized disposable serological pipettes eventually removed the necessity of the previously mentioned Oxford dispenser.

Media Preparation

Cation-adjusted Mueller-Hinton broth (Difco™; Becton Dickinson, Sparks, MD) was used for antimicrobial dilution and bacterial culture. Preparation involved dissolving 21 grams of broth powder in 1 liter of filtered, distilled water (Q-POD® Millipore using a 0.22 µm Millipak® 40 filter; Merck KGaA, Darmstadt, Germany). The solution was then autoclaved at 121˚C for 30 minutes. The manufacturer reports reconstituted Mueller-Hinton broth solutions are stable for up to one year after reconstitution (Becton, Dickinson and Company; personal communication, February 24, 2016). However, broth was typically utilized within one to two weeks of reconstitution.

A 10 mg/mL stock solution of calcium and a 10 mg/mL stock solution of magnesium were prepared by adding 3.68 g of $CaCl_2*2H_2O$ to 100 mL of filtered, distilled water and adding 8.36 g of $MgCl_2*6H_2O$ to 100 mL of filtered, distilled water. Each stock solution was filter sterilized again using a 0.22 micron filter (Corning® 150 mL Bottle Top Filter 0.22 µm; Corning Inc., Corning, NY). For every liter of Mueller-Hinton broth, 2.5 mL of calcium chloride stock solution and 1.25 mL of the magnesium sulfate stock solution were added for a final concentration of 25 mg/L calcium chloride and 12.5 mg/L magnesium sulfate.

Mueller-Hinton agar (Difco™; Becton Dickinson, Sparks, MD) was prepared by suspending 38 grams of agar powder in 1 L of filtered, distilled water. The suspension was then autoclaved at 121˚C for 30 minutes. Following sterilization, the suspension was poured by an automated machine (Figure 3.7; MP-1000 PourMatic 100mm; John Morris Scientific, Chatswood, Sydney, Australia) onto Petri dishes (Falcon® 100x15 mm sterile petri dishes; Corning Inc., Corning, NY) which were subsequently sealed in manufacturer supplied bags and stored in a walk-in refrigerator at 4°C until needed for use. The manufacturer reports Mueller-Hinton agar is stable for up to 3-5 months after reconstitution (Becton, Dickinson and Company; personal communication, February 24, 2016). Agar plates were typically utilized within one month of being reconstituted.

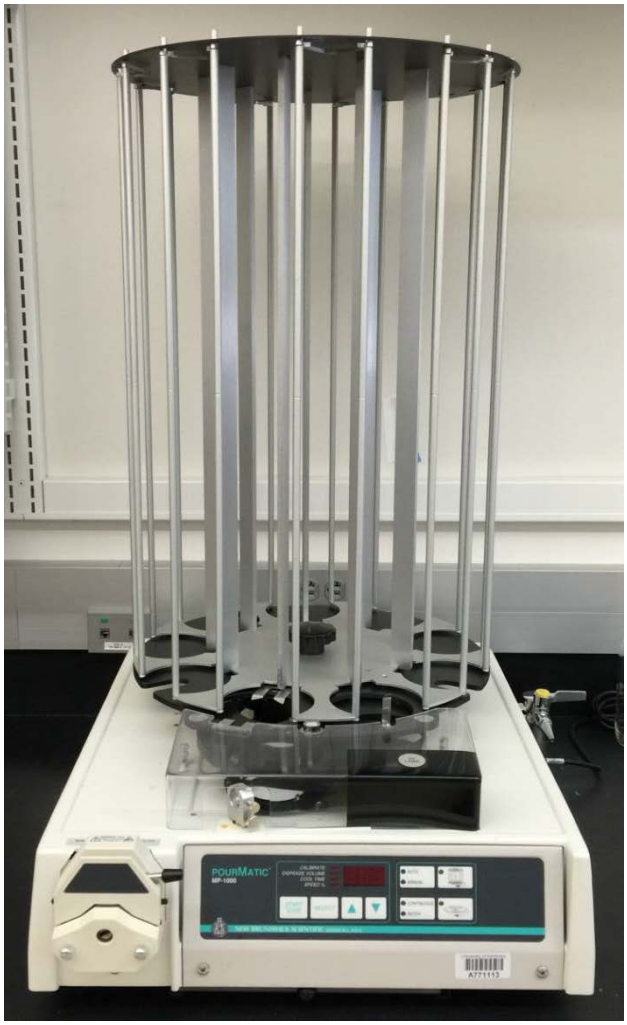


Figure 3.7: MP-1000 PourMatic® 100mm; John Morris Scientific, Chatswood, Sydney, Australia.

For subpopulation analysis experiments (see Subpopulation Analysis and Microfiltration), an extra step was added to the preparation of Mueller-Hinton agar plates. That is, after sterilization, but before the PourMatic® distributed the agar suspension onto petri dishes, antimicrobial agents were added to the agar suspension. Specifically, three unique types of antimicrobial plates were made – meropenem 16 µg/mL, meropenem 64 µg/mL, and polymyxin B 4 µg/mL – by adding the appropriate volume from the primary stock vials (see Antimicrobial Solution Preparation).

Sterilized Mueller-Hinton agar was measured in a 1000 mL graduated cylinder (Fisherbrand™, ThermoFisher Scientific, Waltham, MA) to the 1 L mark. A stir bar (Fisherbrand™, ThermoFisher Scientific, Waltham, MA) was dropped inside the cylinder and 3.13 mL of a 5.12 mg/mL meropenem stock solution (see Antimicrobial Solution Preparation) was added to the cylinder for a total concentration of 16 µg/mL meropenem. The agar was stirred on a hotplate (Fisherbrand™, ThermoFisher Scientific, Waltham, MA) for five minutes before being poured by the PourMatic® onto petri dishes which were subsequently sealed in manufacturer supplied bags and stored in a walk-in refrigerator at 4°C until needed for use. This process was repeated for the other two antimicrobial concentrations, but 12.5 mL of 5.12 mg/mL meropenem was used for the 64 µg/mL final concentration and 1.56 mL of the 2.56 mg/mL stock solution of polymyxin B was used for the 4 µg/mL final concentration plates.

Preparation of Inocula for Susceptibility Testing

Inocula were prepared by the McFarland Standard Method using 0.5 and 1 McFarland standards and a Wickerham Card (Figure 3.8; ThermoFisher Scientific, Waltham, MA). Bacterial suspensions in log-phase growth (see Bacterial Isolates) were added drop-wise using an ErgoOne micropipette (Figure 3.9; USA Scientific, Ocala, FL) to a glass test tube (Fisherbrand™ Disposable Culture Tubes 16x125mm Borosilicate Glass; ThermoFisher Scientific, Waltham, MA) containing approximately 5 mL of 0.22 micron filtered, distilled water. Using the Wickerham Card, the turbidity of the glass test tube was matched as closely as possible to the 0.5 McFarland standard which is

approximately equivalent to 1.5 x 10^8 CFU/mL. A spectrophotometer was not used, and the resulting suspension was only enumerated for Time-kill Studies, discussed later.

Figure 3.8: Test Tube with Water, McFarland Standards and Wickerham Card; ThermoFisher Scientific, Waltham, MA.

Figure 3.9: ErgoOne Micropipette; USA Scientific, Ocala, FL.

For susceptibility testing using broth microdilution, the McFarland-matched suspension was subsequently diluted 1:200 in two steps by first adding 100 µL to 9.9 mL cation-adjusted Mueller-Hinton broth, and then secondly adding 50 µL of this solution to 50 µL of broth and antimicrobial agent during the final inoculation step of the broth microdilution susceptibility testing (see Broth Microdilution Procedure). For antimicrobial plates containing inhibitor combinations, like piperacillin/tazobactam, 50 µL of the 1:100 diluted bacterial suspension was added to 75 µL of broth and antimicrobial agent with inhibitor during the final inoculation step of the broth microdilution susceptibility testing (see Broth Microdilution Procedure). The final bacterial concentration in each well was approximately 5.0 - 7.5 x 10^5 CFU/mL, which is acceptable according to CLSI guidelines.[264]

For disk diffusion and Etest®, the McFarland-matched suspension was not diluted prior to inoculating agar plates (see Inoculation of Agar Plates).

Broth Microdilution Procedure

After all necessary preparations (see Antimicrobial Solution Preparation, Media Preparation, and Preparation of Inocula for Susceptibility Testing), a stack of 96-well trays were added to the left column of the BioStack™ (Figure 3.10), sterile pipette tips were added to deck E, and the freshly prepared cation-adjusted Mueller-Hinton broth was added to each reservoir in the 4x1 reservoir. The computer that manages the Precision™ pipetting system is not shown in Figure 3.10, but the Precision Power™ software was launched and the program labeled "1 BTK Broth MIC testing (Initial 50 mcl broth only).PGM" was loaded and run. The arm then transferred a 96-well tray from the left column on the BioStack™ to deck C. The manifold picked up 12 pipette tips from deck E and aspirated broth from the 4x1 reservoir into each. The manifold then dispensed 50 µL into each well of the 96-well tray. The arm transferred the 96-well tray back to the right column of the BioStack™ and picked up a new plate from the left column of the BioStack™ to repeat this whole process for each 96-well tray.

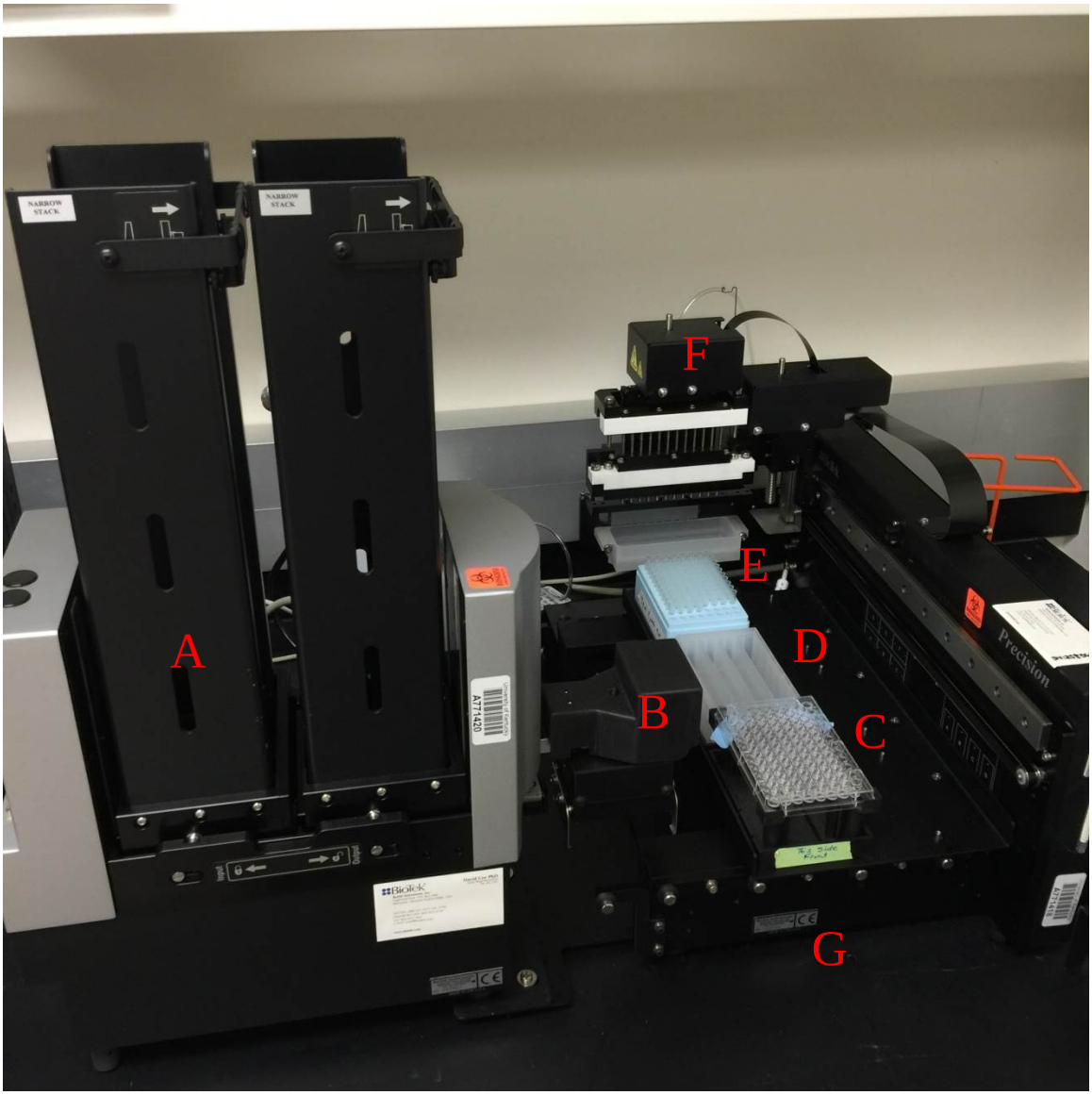


Figure 3.10: BioStack™ Attached to Precision™ Pipetting System. A) The BioStack™ consists of the two black columns (left) which hold the unfilled and filled 96-well trays. B) The arm is the mechanical device that transfers 96-well trays to and from deck C. C) This deck holds 96-well trays for broth, antimicrobial, and organism deposition. D) This deck holds the 4 row by 1 column (4x1) reservoir (shown) as well as the 1x6 reservoir (not shown). E) This deck holds the sterile pipette tips for the manifold F. F) The manifold transfers fresh broth first, antibiotic second, serially dilutes the 96-well tray third, and lastly, adds the inoculated suspension to the 96-well tray. G) The sharps disposal container is placed here to catch used pipette tips.

Once all 96-well trays contained 50µL of broth, the manifold disposed of the pipette tips into a waste container (placed at G) and the program terminated. The right column of the BioStack™ was manually exchanged with the left column, hereafter always referred to by relative position to each other (i.e. the old right column is now the left column) because each program tells the arm to pull 96-well trays from the left column. The 4x1 reservoir was exchanged with another 4x1 reservoir that contained a different antimicrobial agent in each partition of the 4x1 reservoir (see Antimicrobial Solution Preparation) which were at concentrations 4x what was needed in the first well of the 96-well trays. This would, in the next step, provide the 8x12 tray with 2 rows of each of the 4 antibiotics in the 4x1 reservoir with 11 serial dilutions (Figure 3.11).

Figure 3.11: Unique 96-well Microtiter Trays. No drug present in column 12, which served as a positive growth control.

At this point, all 96-well trays were filled with 50 µL of cation-adjusted Mueller-Hinton broth and were in the left column of the BioStack™, ready for serial dilution of antimicrobials. The program "2 BTK multiDrug MIC testing (11 dilutions).PGM" was run which instructed the arm to take a 96-well tray from the left column BioStack™ and place it on deck C. The manifold picked up 12 pipette tips, aspirated 50 µL of antimicrobial solution into each tip from the 4x1 reservoir, and dispensed the contents into the first well. Following mixing (3 full dispensing and aspiration steps), 50 µL were aspirated from the first well and added to the second well. This dilution and mixing process repeated from well to well until all remaining wells (11 total) had been serially diluted and mixed. The 12^{th} column was skipped so it could serve as a positive growth control and the remaining 50 µL of dilute drug solution and pipette tips were disposed of in the sharps disposal container (at G). The manifold next acquired 12 more sterile pipette tips while the arm exchanged the antimicrobial filled 96-well tray (at C) with a new one, placing the former into the right column BioStack™. This process repeated until all 96-well trays were filled with the set of antimicrobials. This program was repeated for each antimicrobial set as in Figure 3.11, exchanging the 4x1 reservoir for a new set of antimicrobials as needed as well as adding new pipette tips and changing the contents of the BioStack so that 96-well trays without antibiotic were on the left and the freshly serially diluted trays were set aside (removed from the right column).

For the piperacillin/tazobactam and imipenem/relebactam groups, only piperacillin or imipenem, respectively, was placed in the 4th row of the 4x1 reservoir to be serially diluted so that a constant concentration of inhibitor can be later placed into each well. Once this group of 96-well trays was finished, the columns of the BioStack™ were exchanged

and another program was loaded and run, "2.5 BTK Drug PIP TAZO MIC testing.PGM." However, prior to running this program, pipette tips were replaced if needed and a new 4x1 reservoir replaced the 4x1 reservoir with rows 1-3 being filled with broth (effectively “placebo” inhibitor) and row 4 was filled with inhibitor at 5x the needed concentration, or 20 mcg/mL (see Antimicrobial Solution Preparation). This program instructed the arm to retrieve a 96-well tray from the left column BioStack™ and place it on deck C. The manifold obtained 12 sterile pipette tips and aspirated broth from rows 1, 2, and 3, and inhibitor from row 4 of the 4x1 reservoir. 25 µL of corresponding broth or inhibitor were dispensed into each well, very similarly to the program that initially dispensed broth into each well before the serial dilutions. This program finished once all piperacillin or imipenem group 96-well trays had a total of 75 µL of volume in each well.

Once all 96-well trays had antimicrobials serially diluted across all 11 rows, each well now contained 50 µL of broth with antimicrobial agent (except the piperacillin/tazobactam or the imipenem/relebactam group trays, which contained 75 µL) and the 96-well trays were placed by group of common antimicrobials (Figure 3.11), one group at a time, in the left column of the BioStack as before. Pipette tips were replaced as necessary and the 4x1 reservoir was exchanged with a 1x6 reservoir which contained 6 unique 0.5 McFarland-matched bacterial suspensions that had been diluted 1:100 (see Preparation of Inocula for Susceptibility Testing).

Before running the final program, a checklist was used to ensure that the left column of the BioStack contained only 1 antimicrobial group of 96-well trays and that the right column of the BioStack was empty to receive the completed trays. The pipette tips were

replaced in deck E if needed and the 1x6 reservoir contained a different bacterial suspension in each of the 6 compartments.

"3 BTK multiBug MIC testing (Lay bug 50 mcl).PGM" was loaded and run in the software. This program instructed the arm to add a 96-well tray to deck C and instructed the manifold to pick up 12 sterile pipette tips, aspirate bacterial suspension from the 1st column of 6 of the 1x6 reservoir and to dispense 50 µL into each of the 96 wells, finishing the 2nd step of the 1:200 dilution and resulting in an initial bacterial concentration of approximately 7.5 x 10^5 CFU/mL in each well. Note for plates containing the extra 25 µL of either broth or inhibitor, the final concentration of bacterial was approximately 6.0 x 10^5 CFU/mL. The arm then placed the complete 96-well tray into the right column BioStack™ and retrieved another 96-well tray. The manifold disposed of the previously used pipette tips and obtained new ones. Following this, the 2nd column of 6 of the 1x6 reservoir was aspirated and 50 µL of suspension was dispensed into each well. This repeated until 6 plates were completed with a unique suspension in each. The program was repeated after replacing pipette tips if needed and changing the 1x6 reservoir for 6 new bacterial suspensions or a new group of antimicrobial plates if the same 6 isolates were needed to be plated on that set. Once all bacterial suspensions had been used for the first group of antimicrobial 96-well trays, these trays were placed in an incubator (Figure 3.12; Heratherm™ Incubator, ThermoFisher Scientific, Waltham, MA) at 35˚C and this entire processes was repeated again for the 2nd and 3rd group of a 96-well trays.

Figure 3.12: Heratherm™ Incubator; ThermoFisher Scientific, Waltham, MA.

In summary, every 96-well tray had 50 µL of cation-adjusted Mueller-Hinton broth aspirated into them, and a group of antimicrobial agents (Figure 3.11) were serially diluted across the 11 columns. Column 12 served as a growth control. 50 µL of a 1% 0.5 McFarland-matched bacterial suspension was added to each well for a total volume of 100 µL in each well. The only exception were the trays that had piperacillin or imipenem where an inhibitor was necessary. These trays which had 25 µL of 20 mg/L inhibitor or broth added and 50 µL of a 1% 0.5 McFarland-matched bacterial suspension for a total volume of 125 µL in each well. See Table 3.4 for the concentration ranges of antimicrobial agent.

Incubation

Once inoculated, all 96-well microtiter trays and Mueller-Hinton agar plates were sealed using the manufacturer supplied lids and incubated at 35˚C overnight for 16-24 hours in an incubator (Heratherm™ Incubator; ThermoFisher Scientific, Waltham, MA). Agar plates were inverted and stacked no more than six high.

Minimum Inhibitory Concentration (MIC) Determination

MIC is defined as the lowest concentration of antimicrobial agent required to completely inhibit the growth of the microorganism to the unaided eye (Figure 3.3). On the 96-well microtiter trays, this would be a complete absence of turbidity, individual colonies, and stringy growth. The resulting growth by well was depicted on data sheets for each tray (Figure 3.13). The modal minimum inhibitory concentration (MIC) was accepted. If there was no modal MIC, the higher MIC was accepted unless there was categorical disagreement in susceptibility (e.g. isolate MICs were near the susceptibility breakpoint; Table 3.10). In such a case, a third or seldom fourth experiment was performed, and the modal MIC was then accepted.

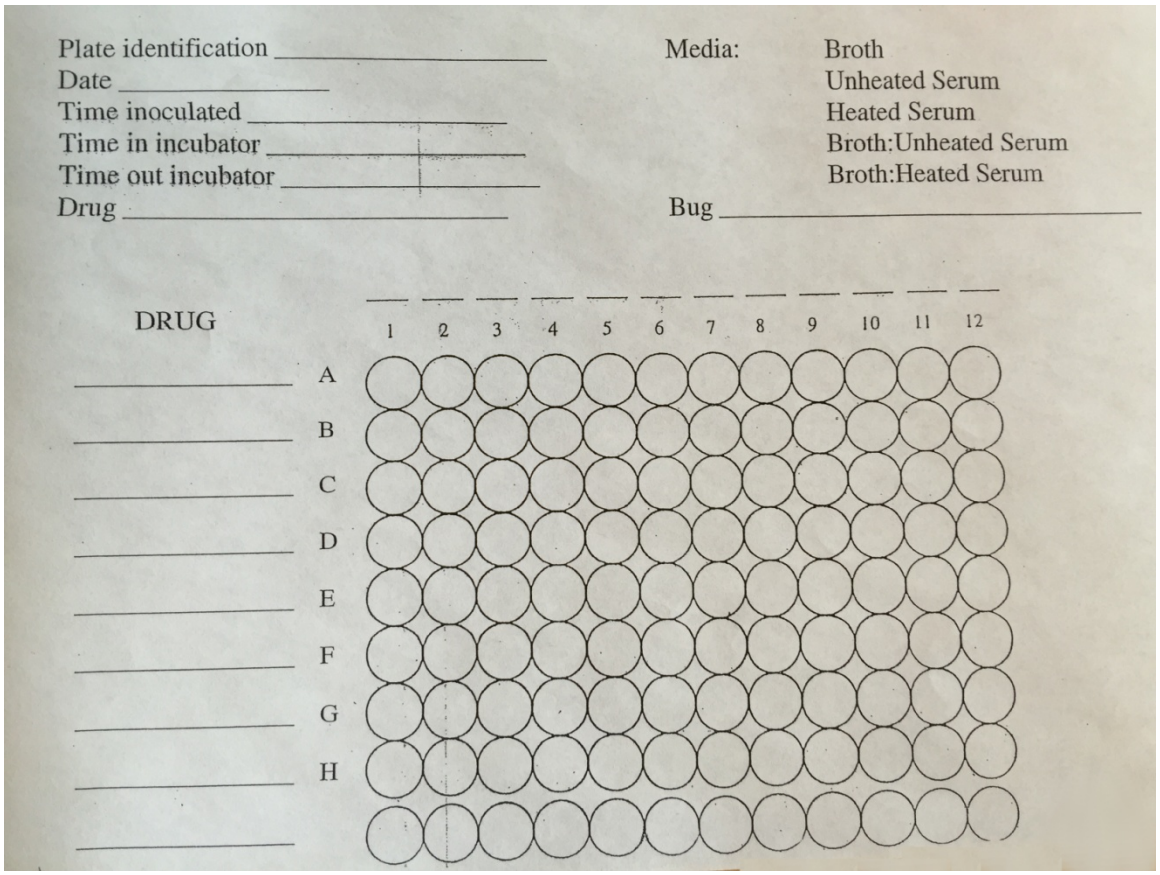


Figure 3.13: Antimicrobial Susceptibility 8x12 Microtiter Data Sheet.

Inoculation of Agar Plates

After preparing the agar plates (see Media Preparation) and bacterial suspensions for inoculation (see Preparation of Inocula for Susceptibility Testing), a wooden, sterile, cotton-tipped applicator (Fisherbrand™; ThermoFisher Scientific, Waltham, MA) was dipped into the 0.5 McFarland-matched bacterial suspension and then rolled on the side of the same glass tube to remove excess suspension. The agar plate was streaked in a back-and-forth motion as if painting the entire plate from top to bottom (Figure 3.14). The plate was rotated 90˚ and the same cotton swap was used to streak the plate again, but without dipping into the bacterial suspension a second time. This coated the agar plate with a lawn of bacteria. Note that CLSI recommends three streaking pattern with 60˚ turns between each, but a lawn was reproducible in our lab with only two passes.[272]

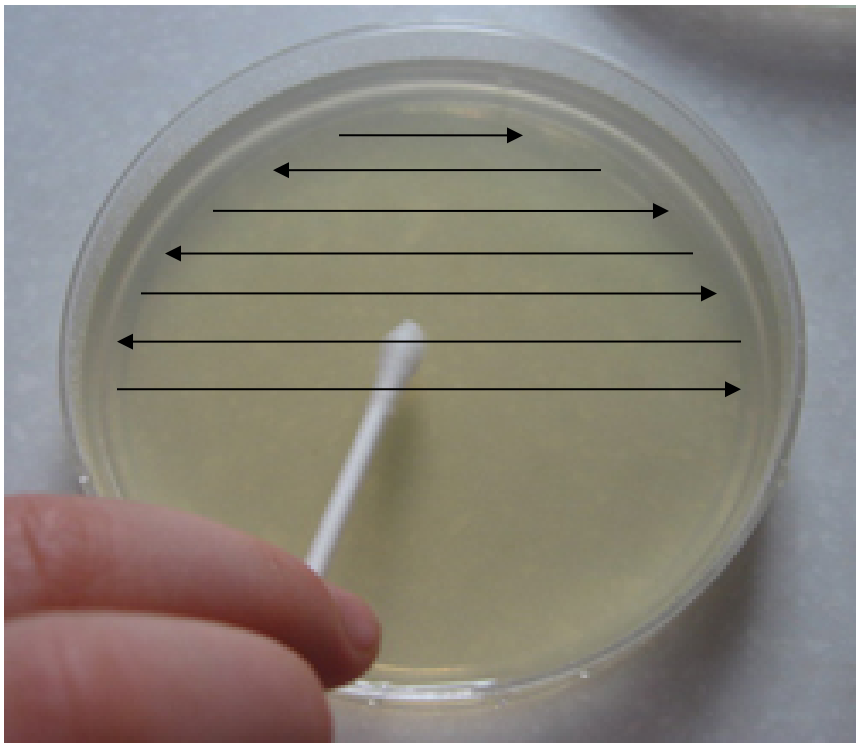


Figure 3.14: Inoculation of Mueller-Hinton Agar Plate.

Kirby-Bauer Disk Diffusion Procedure

Following inoculation of the agar plate (see Inoculation of Agar Plates), a ceftazidime/avibactam (30 µg / 20 µg) impregnated disk (Actavis; Parsippany, New Jersey) was placed on the plate using sterile forceps (Fisherbrand™, ThermoFisher Scientific, Waltham, MA). The plate was then incubated (Figure 3.12; Heratherm™ Incubator; ThermoFisher Scientific, Waltham, MA) at 35˚C for 16-24 hours and the smallest diameter of the zone of inhibition (Figure 3.5) was measured in millimeters to the nearest whole number and recorded. Susceptibility was determined based on CLSI guidelines (≥21 mm susceptible; 18-20 mm intermediate; ≤17 mm resistant).[262]

At the time of experiment, 152 unique MDR isolates had been collected, consisting of 75 CRE (*Enterobacteriaceae* with an ertapenem MIC >0.5 or a meropenem MIC >1). All 75 isolates underwent Kirby-Bauer disk diffusion testing for susceptibility to ceftazidime/avibactam. Isolates that had a zone of inhibition ≤ 21mm (borderline susceptible, intermediate, or resistant) underwent a second test to verify resistance. If a discrepancy between the results occurred, the test was repeated once more.

Modified Disk Diffusion Procedure for the Detection of MBL and KPC Enzymes

All 122 CRE isolates from the Merck study (MISP #56367), including the 75 isolates that underwent ceftazidime/avibactam screening (see Kirby-Bauer Disk Diffusion Procedure) underwent a previously published phenotypic screening assay to determine the type of carbapenem resistance (i.e. KPC, MBL, both, or neither).[266] Some small deviations from the published protocol will be discussed here.

A 5.12 mg/mL meropenem (MEM) stock solution was prepared as previously described (see Antimicrobial Solution Preparation). Phenylboronic acid (PBA; an inhibitor of serine carbapenemases like KPC) was dissolved in DMSO to a final concentration of 80 mg/mL. Anhydrous EDTA (a metal chelator that inhibits MBLs like NDM, VIM, and IMP) was dissolved in sterile distilled water to a final concentration of 0.1 M.

Mueller-Hinton agar plates were prepared (see Media Preparation), bacterial isolates were grown and standardized 0.5 McFarland inocula were prepared (see Preparation of Inocula for Susceptibility Testing), plates were streaked with the standardized suspension (see Inoculation of Agar Plates), and four blank discs were laid onto the agar plates (see Kirby-Bauer Disk Diffusion Procedure), but in a diamond pattern rather than the center of the plate (Figure 3.15). MEM was added to each disk using 1.95 µL of the 5.12 mg/mL MEM stock so that 10 µg of MEM was present on each disk. The EDTA solution was added to the left and bottom disks using 10 µL so that 292 µg would be present. The PBA solution was added to the right and bottom disks using 5 µL so that 400 µg would be present. Plates were incubated for 16-24 hours (i.e. overnight) and interpreted according to the original study criteria.[266]

Specifically, the diameters of the zones of inhibition were measured, and the zones corresponding the disks containing EDTA and/or PBA were compared to the zone corresponding to the disk containing MEM alone. Zone differences ≥5mm were considered significant changes and interpreted as follows: Plates with no zones ≥5mm larger than MEM alone were interpreted as an isolate producing neither KPC nor MBL. Plates with both EDTA-containing disks ≥5mm larger than MEM alone were interpreted as an isolate producing MBL enzymes. Plates with both PBA-containing disks ≥5mm larger than MEM alone were interpreted as an isolate producing KPC enzymes. Plates with only the EDTA- and PBA-containing disk ≥5mm larger than MEM alone were interpreted as an isolate producing both MBL and KPC enzymes. See Figure 3.15. Other results were considered indeterminate. All isolates underwent two experiments on separate occasions, and a third experiment was used to choose a modal result when the first two experiments yielded different results or both were indeterminate. All isolates producing an MBL were confirmed by patient chart review or subsequent genotyping by the University of Kentucky Clinical Microbiology Laboratory using the Verigene® system (Luminex®, Austin, TX) because KPC production is the primary mechanism of resistance for CRE in the United States.

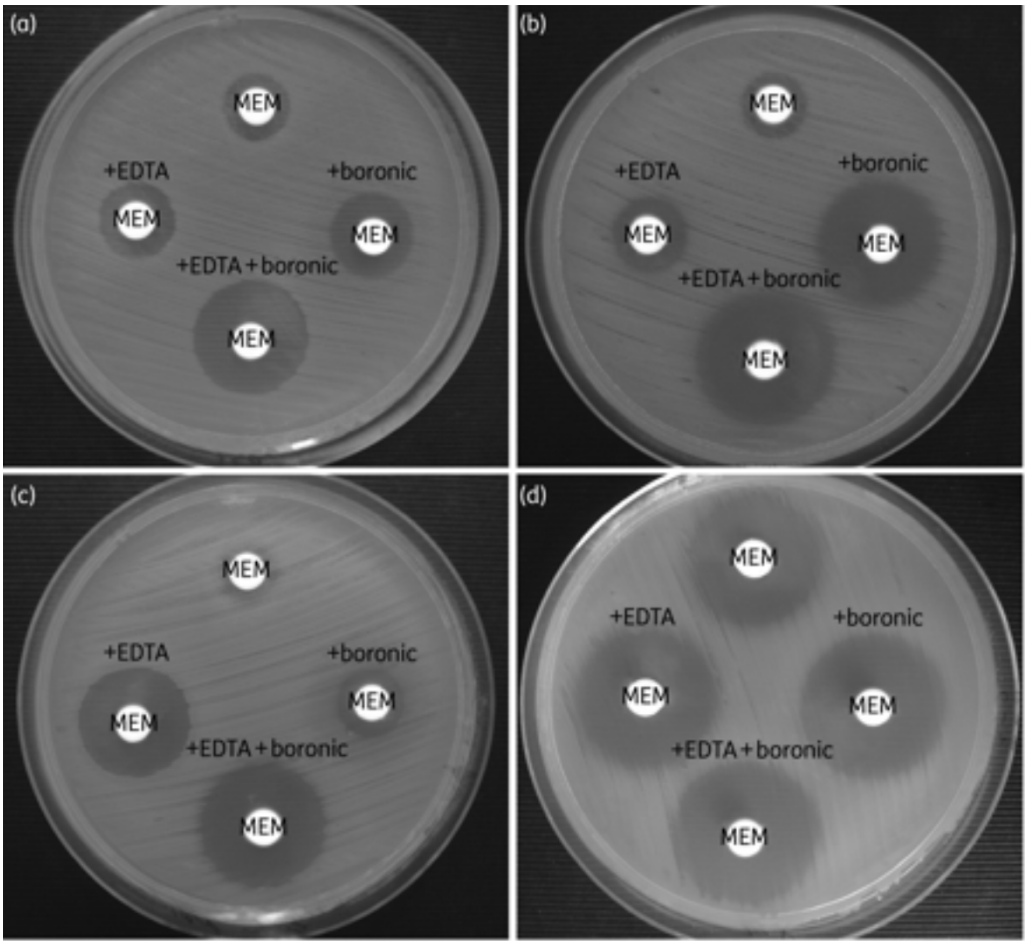


Figure 3.15: Representative Results of the Three Combined-disc Tests using discs of meropenem (MEM) alone and with EDTA, phenylboronic acid, or EDTA plus phenylboronic acid for a KPC/VIM/ESBL-possessing isolate (a), a KPC/ESBL-possessing isolate (b), a VIM-possessing isolate (c) and an AmpC/ESBL-possessing isolate (d). Reprinted.[266]

Etest® Procedure

Following typical preparation (see Bacterial Isolates, Media Preparation, Preparation of Inocula for Susceptibility Testing, and Inoculation of Agar Plates), an Etest® strip was placed on the plate using sterile forceps according to manufacturer protocol. The plate was then incubated (Heratherm™ Incubator; Figure 3.12) at 35˚C for 16-24 hours.

MBL MP/MPI Etest® strips were interpreted as positive for MBL if 1) the MIC ratio of meropenem (MP) to meropenem with EDTA (MPI) was ≥8 (see Figure 3.6 for MIC reading of Etest®), 2) if there was a phantom zone (i.e. an extra inhibition zone between the MP and MPI regions; Figure 3.16), or 3) if a deformation of the MP or MPI ellipses was present (Figure 3.16). All CRE isolates with ceftazidime/avibactam zones of inhibition measuring ≤ 21mm (borderline susceptible, intermediate, or resistant; see Kirby-Bauer Disk Diffusion Procedure) were tested for MBL production by Etest® except four isolates which were already known to produce MBL by PCR from information provided by the University of Kentucky Clinical Microbiology Laboratory.

Different growth-inhibition patterns:

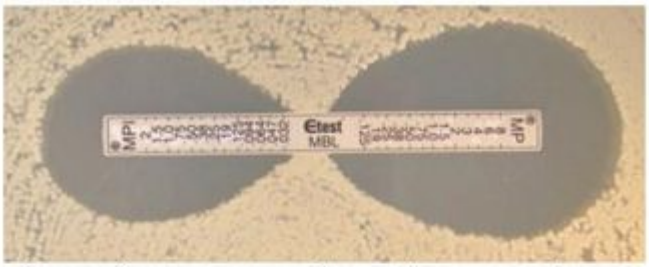


Figure 2. Clear cut MBL negative: MP/MPI IC <0.125/<0.032

Figure 3. Clear cut MBL positive: MP/MPI IC >8/0.19 = >42

Figure 4. Phantom zone between MP/MPI is indicative of MBL

Source: bioMérieux, Inc., Durham, NC

Figure 3.16: MBL MP/MPI Etest® Interpretation.

Selection of Bacterial Isolates for Time-kill Studies

Clinical isolates were selected for further testing based on the meropenem (MEM), amikacin (AMK), and polymyxin B (PMB) broth microdilution MICs (see Broth Microdilution Susceptibility Testing). For subsequent time-kill studies, four *Klebsiella pneumoniae* and four *Enterobacter cloacae* clinical isolates were chosen that showed PMB susceptibility, hereafter defined as ≤2 mg/L based on CLSI breakpoints for *A. baumannii* and *P. aeruginosa* and EUCAST colistin breakpoints for *Enterobacteriaceae* since CLSI

is without interpretive criteria for PMB against *Enterobacteriaceae*. These isolates also demonstrated varying degrees of MEM resistance (MEM MICs 4-128 mg/L).[262] In order of increasing MEM resistance, the *K. pneumoniae* isolates selected (and MEM MICs) were: 34 (4 mg/L), 22 (16 mg/L), 24 (32 mg/L), and 44 (128 mg/L).[273,274] These isolates are sometimes proceeded by "KP" to denote their genus/species. Also in order of increasing MEM resistance (and MEM MICs), the *E. cloacae* isolates were: 17 (2 mg/L), 19 (8 mg/L) 40 (16 mg/L), and 10 (32 mg/L).[275] These isolates are sometimes proceeded by "EC" to denote their genus/species. Isolates with MEM MICs of 16-32 mg/L and showing amikacin susceptibility, intermediate activity, and resistance were selected for time-kill analysis and were (with AMK MICs): 169 (8 mg/L), 32 (16 mg/L), 22 (32 mg/L), and 37 (64 mg/L).[276]

Time-kill Studies

Time-kill experiments can be used to evaluate bacteria colony counts at various time points during exposure to a fixed or variable concentration of one or more antimicrobial agents. All time kill assays were performed at least in duplicate with a positive growth control and samples collected at 0, 1, 2, 4, 8, 24, and 48 hours, diluted as necessary, and aliquots (50µL) logarithmically plated onto Mueller-Hinton agar using a spiral plater (Figure 3.17; AutoPlate® spiral plater; Advanced Instruments, Inc., Norwood, MA), which helped control for antibiotic carryover.[277] Colonies were counted using a laser colony counter (Figure 3.18; QCount Automated Colony Counter; Spiral Biotech, Advanced Instruments, Inc., Norwood, MA) with a lower limit of quantification of 10^2 CFU/mL. See Time-Kill Procedure for additional details.

Figure 3.17: AutoPlate® Spiral Plater; Advanced Instruments, Inc., Norwood, MA.

Figure 3.18: QCount Automated Colony Counter; Spiral Biotech, Advanced Instruments, Inc., Norwood, MA.

Cation-adjusted Mueller-Hinton broth was used for growth media for the selected bacterial isolates in log-growth phase (see Bacterial Isolates, Media Preparation, Preparation of Inocula for Susceptibility Testing, and Selection of Bacterial Isolates for Time-kill Studies). Meropenem, amikacin, and polymyxin B alone were evaluated at three (4, 16, and 64 mg/L), three (8, 16, and 64 mg/L), and six (0.0625, 0.125, 0.25, 1, 2, and 4 mg/L) clinically achievable concentrations, respectively.[278-280] For combination studies, polymyxin B concentrations of 0.25 and 1 mg/L were evaluated with all three concentrations of meropenem against the *K. pneumoniae* isolates 22, 24, 34, and 44 as well as the *E. cloacae* isolates 10, 17, 19, and 40. However, for the highly meropenem resistant isolate (44), polymyxin B at 4 mg/L was also evaluated in combination with the three concentrations of meropenem. Repeat MICs for polymyxin B were determined for regrowing bacteria at 24 hours (see Resistance Development Testing). Amikacin concentrations of 8 and 16 mg/L were evaluated with meropenem 4 and 16 mg/L against the *E. cloacae* isolates 10, 17, 19, 40 as well as isolates 22, 32, 37, and 169 (see Selection of Bacterial Isolates for Time-kill Studies).[275,276] All time-kill data can be found in Appendix E.

Time-Kill Procedure

Meropenem, amikacin, polymyxin B, and combinations of meropenem/polymyxin B or meropenem/amikacin were added to cation-adjusted Mueller-Hinton broth (see Media Preparation) in conical vials (50 mL polypropylene conical centrifuge tubes; USA Scientific, Ocala, FL) up to a total volume of 30 mL measured by ErgoOne micropipettes (Figure 3.9) and a Pipet-Aid® (Figure 3.19; Drummond Scientific Co., Broomall, PA). Portions of the primary stock vials of antimicrobials (see Antimicrobial Solution Preparation) were added directly to the 50 mL conical vial according to Table 3.5 and the desired concentrations.

Figure 3.19: Drummond Pipet-Aid®; Drummond Scientific Co., Broomall, PA.

Table 3.5: Time-Kill Volume Table

Meropenem (MEM) Alone									
Antimicrobial Concentration (mg/L)	**4**			**16**			**64**		
Volume of 1.02 mg/mL Stock Soln. (µL)	117			469			1875		
Volume of 0.5 McFarland Soln. (µL)	150			150			150		
Volume of Broth in 50 mL vial (mL)	29.8			29.4			28.0		
Total Volume (mL)	**30**			**30**			**30**		
Polymyxin B (PMB) Alone									
Antimicrobial Concentration (mg/L)	**0.063**	**0.125**		**0.25**	**1**		**2**	**4**	
Volume of 1.02 mg/mL Stock Soln. (µL)	2	4		7	29		59	117	
Volume of 0.5 McFarland Soln. (µL)	150	150		150	150		150	150	
Volume of Broth in 50 mL vial (mL)	29.8	29.8		29.8	29.8		29.8	29.8	
Total Volume (mL)	**30**	**30**		**30**	**30**		**30**	**30**	
Amikacin (AMK) Alone									
Antimicrobial Concentration (mg/L)	**8**			**16**			**64**		
Volume of 4.1 mg/mL Stock Soln. (µL)	59			117			469		
Volume of 0.5 McFarland Soln. (µL)	150			150			150		
Volume of Broth in 50 mL Vial (mL)	29.8			29.8			29.4		
Total Volume (mL)	**30**			**30**			**30**		
MEM + PMB Combination*									
Meropenem Concentration (mg/L)	**4**			**16**			**64**		
Volume of 1.02 mg/mL MEM Soln. (µL)	117			469			1875		
Volume of 0.5 McFarland Soln. (µL)	150			150			150		
Volume of Broth in 50 mL Vial (mL)	29.7			29.4			28.0		
Total Volume (mL)	**30**			**30**			**30**		
MEM + AMK Combination									
Meropenem Concentration (mg/L)	**4**			**16**			**64**		
Volume of 1.02 mg/mL MEM Soln. (µL)	117			469			1875		
Amikacin Concentration (mg/L)	**8**	**16**	**64**	**8**	**16**	**64**	**8**	**16**	**64**
Volume of 4.1 mg/mL AMK Soln. (µL)	59	117	469	59	117	469	59	117	469
Volume of 0.5 McFarland Soln. (µL)	150			150			150		
Volume of Broth in 50 mL Vial (mL)	29.7	29.6	29.3	29.4	29.3	28.9	27.9	27.9	27.5
Total Volume (mL)	**30**			**30**			**30**		

*Polymyxin B volume contribution ignored

After the antimicrobial agents were added to their respective conical vials, a 1:200 dilution of the 0.5 McFarland matched suspension was made by adding 150 µL of the suspension to the conical vial. Immediately following this addition, the conical vial was mixed swiftly using the pipette tip of the ErgoOne micropipette (Figure 3.9) and a 0.5 mL sample was drawn and serially diluted in 1:10 dilutions in glass test tubes (Fisherbrand™ Disposable Culture Tubes 16x125mm Borosilicate Glass; ThermoFisher Scientific, Waltham, MA) containing 4.5 mL sterile water. A dispenser (Oxford®; Cole-Parmer®, Vernon Hills, IL) or a pipette controller (Ovation® Ali-Q™; VistaLab, Brewster, NY) was calibrated and used to equally measure 4.5mL volumes. Vials were then placed in a shake incubator (Figure 3.2) at 35˚C and 220 oscillations per minute for the remainder of the experiment.

Samples were drawn by an ErgoOne micropipette (Figure 3.9) at times 0, 1, 2, 4, 8, 24, and 48 hours. Some experiments used 3 and 6 hour time points as well. Since the laser colony counter (Figure 3.18) most optimally measures 10^3 - 10^5 CFU/mL, a 1:10 and a 1:100 dilution were made at time 0 from a 0.5 mL serially diluted sample due to an initial colony count of about 7.5 x 10^5 CFU/mL. At each time point, it was noted whether the conical vial contents were clear or turbid because the unaided eye can see turbidity at approximate 10^7 CFU/mL based on previous work performed in our laboratory. If clear, an undiluted 2.5 mL sample was drawn and a 1:100 dilution sample was made from a 0.5 mL serially diluted sample, both plated via spiral plater, and both placed in an incubator (Figure 3.12). If turbid, a 1:10^4 and a 1:10^6 dilution were made from a 0.5 mL serially diluted sample, both plated via spiral plater, and both placed the aforementioned incubator.

As previously mentioned, the target measurement for the laser colony counter is 10^3-10^5 CFU/mL and dilution choices were made based on previous time-kill studies performed in the laboratory. All plates incubated for 16-24 hours and were read by a laser colony counter. The manufacturer recommended lower limit of quantification was 10^2 CFU/mL which was the colony count assumed for any time point reading less than this amount.

Staggered Administration of Antimicrobial Agents

When combination experiment antimicrobials were administered at times other than the start of the experiment (t=0), Table 3.5 was still used for the initial volumes as if only one drug were being administered – the antimicrobial present throughout the experiment (drug A). The agent administered at a later time (drug B) was added to the flask after sample was drawn and in sufficient volume so that the resulting concentration was as desired. The added drug volume (<1 mL) was less than 5% of the volume remaining in the flask after accounting for multiple sampling since a delay of 2 hours was used; therefore, no additional drug A was added to the flask.

Resistance Development Testing

Following two separate time-kill studies, MIC determination by broth microdilution was performed on colonies growing on the 24-hour time point agar plates (see Time-Kill Procedure) but were tested only for changes in polymyxin B MIC (see Broth Microdilution Procedure). Resistance development was defined as a ≥4-fold increase in MIC from colonies growing at 24 hours when compared to the baseline MIC of the organism. An MIC > 2 was considered non-susceptible according to CLSI breakpoints for *A. baumannii* and *P. aeruginosa* and EUCAST breakpoints for colistin in *Enterobacteriaceae*.[262,281]

Subpopulation Analysis and Microfiltration

A modified time-kill procedure was used to evaluate subpopulations of the four *K. pneumoniae* isolates selected for time-kill studies (see Selection of Bacterial Isolates for Time-kill Studies and Time-kill Studies). The results of this study would allow us to explain observed regrowth in the other time-kill experiments by quantifying a particular subpopulation of each isolate. These subpopulations often have different MICs than the MIC of the majority population.[140] With (only monotherapies; see Table 3.5) and without (growth controls) adding antimicrobial agents to the 50 mL conical vials, 29.8 mL of cation-adjusted Mueller-Hinton broth (see Media Preparation) with 150 µL of 0.5 McFarland matched bacterial suspension (see Preparation of Inocula for Susceptibility Testing) were used for a total of 30 mL of approximately $7.5 * 10^5$ CFU/mL of bacteria. Additionally, instead of using Mueller-Hinton agar plates, the antimicrobial-impregnated

plates were used (see Media Preparation). Finally, sampling time points were 0, 2, 3, 4, 6, and 24 hours. All other aspects of this modified study were like the Time-Kill Procedure.

It was thought that the subpopulations we wished to quantify may be below the lower limit of quantification (10^2 CFU/mL) for the laser colony counter. To address this, two comparable approaches were used. First, instead of using aliquots of 50 µL logarithmically plated by the spiral plater, a uniform 500 µL setting was used. Second, we implemented a process called microfiltration which involved taking a specific sample volume at each time point (Tables 3.5 - 3.8; Estimations of viable colony counts made by outside collaborator), passing sample through a 0.22 µm filter (Millipak® 40 filter; Merck KGaA, Darmstadt, Germany) using a vacuum filter apparatus (Figure 3.20), and then placing the filter (bacteria-side up) directly onto the antimicrobial-impregnated agar plate where nutrients could diffuse through the filter paper to the bacteria. In both cases, the plates were incubated (Figure 3.12) at 35˚C for 16-24 hours and then manually counted so that a colony count (CFU/mL) could be calculated based on the volume utilized for each sample. The lower limit of quantification associated with microfiltration ranges from 30-300 CFU/mL, decreasing as larger sample volumes are used.[282,283]

Figure 3.20: Vacuum Filter Apparatus.

Table 3.6: Microfiltration Sample Volumes at Each Time Point for isolate 34

Agar Plate	0 (hours)	2 (hours)	3 (hours)	4 (hours)	6 (hours)	24 (hours)
MEM 16 (µg/mL)	500 µL	500 µL	250 µL	100 µL	100 µL	No sample
PMB 4 (µg/mL)	1000 µL	1000 µL	500 µL	250 µL	250 µL	No sample

Table 3.7: Microfiltration Sample Volumes at Each Time Point for isolate 22

Agar Plate	0 (hours)	2 (hours)	3 (hours)	4 (hours)	6 (hours)	24 (hours)
MEM 16 (µg/mL)	1000 µL	500 µL	250 µL	250 µL	250 µL	No sample
PMB 4 (µg/mL)	1000 µL	1000 µL	500 µL	250 µL	250 µL	No sample

Table 3.8: Microfiltration Sample Volumes at Each Time Point for isolate 24

Agar Plate	0 (hours)	2 (hours)	3 (hours)	4 (hours)	6 (hours)	24 (hours)
MEM 64 (µg/mL)	1000 µL	500 µL	250 µL	100 µL	100 µL	No sample
PMB 4 (µg/mL)	1000 µL	1000 µL	500 µL	250 µL	100 µL	500 µL

Table 3.9: Microfiltration Sample Volumes at Each Time Point for isolate 44

Agar Plate	0 (hours)	2 (hours)	3 (hours)	4 (hours)	6 (hours)	24 (hours)
MEM 64 (µg/mL)	100 µL	100 µL	No sample	No sample	No sample	No sample
PMB 4 (µg/mL)	1000 µL	1000 µL	500 µL	500 µL	250 µL	No sample

Whole Genome Sequencing

Seven bacterial isolates (*E. cloacae* strains 10, 17, 19, and 40. *K. pneumoniae* strains 22, 24, and 44) and two quality control strains (*E. coli* ATCC® 25922 and *K. pneumoniae* ATCC® BAA-1705) underwent whole genome sequencing to determine genotypic resistance mechanisms present in each isolate and to subsequently compare genotype and phenotype (MIC).

Isolation, Purification, and Quantification of Bacterial DNA

Cynthia Mattingly, a senior laboratory technician, subcultured and McFarland matched bacteria as previously described (see Bacterial Isolates and Preparation of Inocula for Susceptibility Testing). The volume of subcultured suspension required to match each isolate to a 0.5 McFarland standard was used proportionally to create solutions containing approximately 2 x 10^9 CFU/mL cells which were placed in 1.5 mL Eppendorf tubes (ThermoFisher Scientific, Waltham, MA). A centrifuge (Figure 3.21; ThermoFisher Scientific, Waltham, MA) was used to spin cells to pellets at 12,000 rpm for 10 minutes. The supernatant was extracted from the pellets and Qiagen DNA Mini Kits #51304 (Qiagen, Hilden, Germany) were used according to manufacturer provided instructions for isolation of bacterial DNA.

Specifically, Buffer AL was thoroughly mixed by manual shaking, 25 mL of ethanol (96-100%) was added to Buffer AW1, and 30 mL of ethanol (96-100%) was added to Buffer AW2. Buffer ATL was added to the pellets to a total volume of 180 µL each. Gentle mixing was conducted with an ErgoOne pipet (Figure 3.9). 20 µL of proteinase K

was added, mixed by a vortexer (Maxi-Mix I Type 16700 Mixer, Barnstead International, Dubuque, IA) every 20 minutes for one hour, and incubated (Figure X; Dry Bath Incubator, Fisher Scientific, Hampton, NH) at 56ºC overnight. 4 µL of RNase A (100 mg/mL) was added to each pellet, vortexed for 15 seconds, and incubated for 30 minutes at 70ºC. 200 µL of Buffer AL was added to each tube which was vortexed every 30 minutes for 2 hours. 200 µL of ethanol (96-100%) was added to each tube which was subsequently applied to a QIAamp Mini spin column and centrifuged at 6,000 xg for 1 minute. The spine column was placed in a clean collection tube and the used tube with filtrate was discarded. 500 µL of Buffer AW1 was added and centrifuged at 8,000 xg for 1 minute, again placing the column into a clean collection tube and discarded the filtrate and old tube. 500 µL of Buffer AW2 was added and centrifuged at 20,000 xg for 3 minutes, the column was placed in a clean collection tube, and the old tube with filtrate were discarded. Nothing was added to the clean collection tube and it was centrifuged at 20,000 xg for 1 minute. The column was placed in a clean 1.5 mL Eppendorf tube and the old tube and filtrate were discarded. 200 µL of Buffer AE was added, the tube was incubated for 5 minutes at room temperature, and then the sample was centrifuged at 6,000 xg for 1 minute. DNA concentration and purity were determined using Nanodrop 2000 (ThermoFisher Scientific; Waltham, MA) with a goal 260/280 ratio between 1.7-2.0. DNA was stored at -20ºC until transport to UK Genomics Core Laboratory.

Figure 3.21: IEC Micromax RF Centrifuge. ThermoFisher Scientific, Waltham, MA.

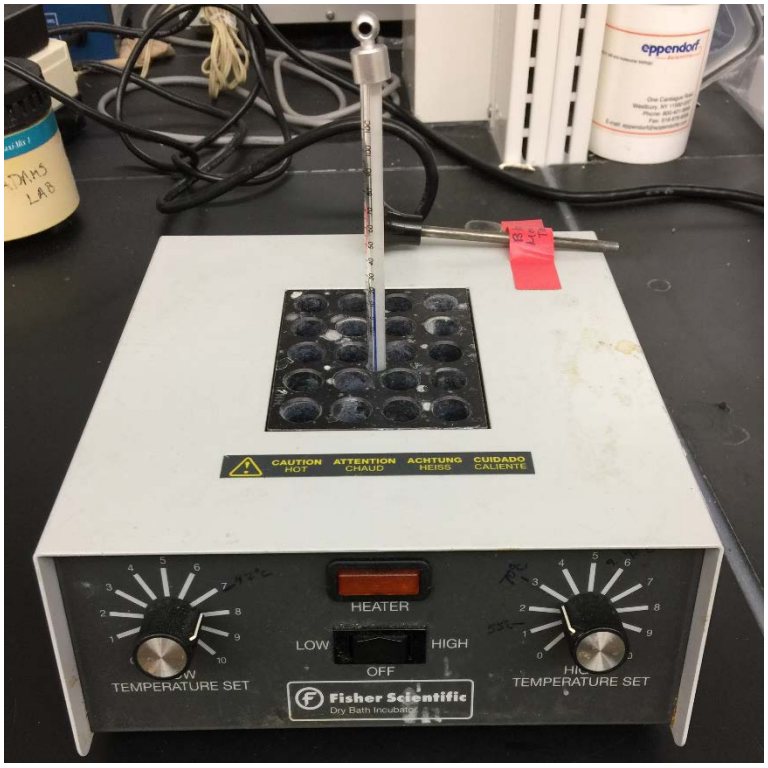


Figure 3.22: Dry Bath Incubator. Fisher Scientific, Hampton, NH.

Genome Sequencing and Resistance Mechanism Screening

Barcoded Nextera libraries were generated by UK Genomics Core Laboratory using approximately 50 ng of each bacterial DNA sample in individual tagmentation reactions, according to the manufacturer's instructions (Illumina, San Diego, CA). The tagmented DNA was purified using Zymo-Clean and Concentrator kit (Zymo Research Corp, Irvine, CA) and then used as a template in a PCR amplification using reagents from the Nextera kit. The amplified products were then purified using AMPure XP beads (Beckman Coulter, Indianapolis, IN). The concentration and sizes of the amplification products were determined using the Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA) and library quality was assessed via qPCR, using the KAPA library quantification kit (KAPA Biosystems, Wilmington, MA). Amplification conditions were as described in the manufacturer's instructions. The libraries were then pooled in equimolar fashion to a final concentration of 4 nM in a total volume of 10 µl. The libraries were denatured by adding an equal volume of 0.2N NaOH and then neutralized by adding 980 µl of Illumina hybridization buffer. Six hundred microliters of the denatured libraries were used for sequencing. Sequence data (250 bp, paired-end reads) were acquired using the MiSeq platform (Illumina, San Diego, CA). Genome assemblies were generated with Newbler v2.9, in paired-end mode and using default parameters. The presence and absence of known resistance mechanisms for each isolate were identified by Grace C. Lee, a collaborator and colleague, at the University of Texas at Austin using BLAST against two databases, ResFinder and ARG ANNOT (Antibiotic Resistance Gene ANNOTation).[275,284]

Pharmacodynamic Mathematical Modeling

The growth control and monotherapy data obtained for meropenem and polymyxin B from the time-kill experiments of *K. pneumoniae* isolates 22, 24, 34, and 44 (see Time-kill Studies) were described using a conceptual and mathematical model (Figure 3.23; Equations 1 and 2). A multi-step approach was adopted to developing the final parameters of the mathematical model using Phoenix® 8.1 Win Nonlin® (Certara; Princeton, NJ).

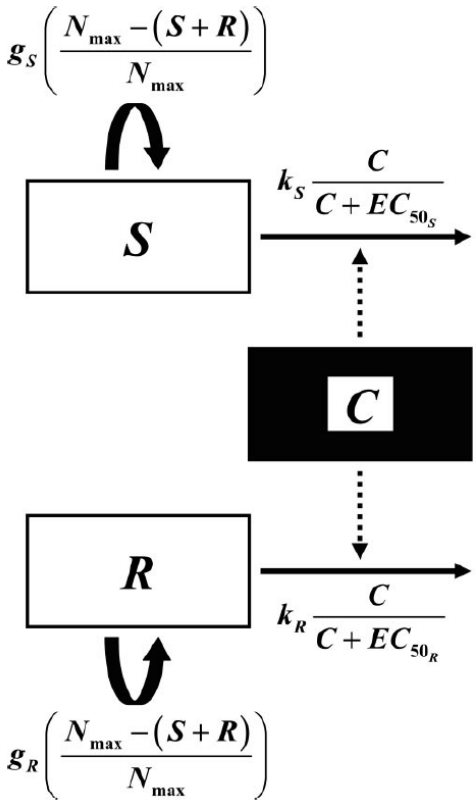


Figure 3.23: Two Population Conceptual Model of a Single Antimicrobial Agent. Each population (S and R) exhibits a unique growth rate constant (g), shares the same maximal capacity (Nmax), and compose compartment C which contains the antimicrobial concentration (C) with unique maximal killing rate constants (k) and half maximal killing rate concentrations (EC_{50}). Reprinted with modifications.[285]

$$\frac{dS}{dt} = \left(g_S\left[1 - \frac{S+R}{Nmax}\right] - k_S\frac{C}{C+EC_{50S}}\right)S \quad \textbf{(1)}$$

$$\frac{dR}{dt} = \left(g_R\left[1 - \frac{S+R}{Nmax}\right] - k_R\frac{C}{C+EC_{50R}}\right)R \quad \textbf{(2)}$$

First, model selection involved consideration of the available literature and discussion with collaborator Jeffrey J. Campion who had worked with our lab during initial time-kill characterization of the four *K. pneumoniae* isolates listed above as well as the four *E. cloacae* isolates.[274,275] A net growth equation (g; Equation 3) was chosen because our time-kill experimental design did not allow us to easily parse out bacteria growth (b; Equation 3) excluding cell death (x; Equation 3) or vice versa. Other contending antimicrobial activity models could have been growth inhibition (A*b) or death acceleration (A*x) but were not evaluated. A simple direct effect killing model (Equation 4) was chosen because it had already been used in the literature to describe antimicrobial activity against microorganisms, including gram negative organisms.[286,287] Three populations within each isolate were assumed to be present based on the subpopulation time-kill experiments (see Subpopulation Analysis). In summary, these populations were a majority population (RLS) exhibiting the phenotype measured by broth microdilution studies (see Minimum Inhibitory Concentrations (MICs)), a subpopulation exhibiting the majority polymyxin B phenotype but with much higher meropenem resistance (RHS), and a subpopulation exhibiting the majority meropenem phenotype but with polymyxin B resistance (RLR). A subpopulation exhibiting elevated meropenem resistance and polymyxin B resistance (RHR) could be considered if a poor fit was obtained under simpler assumptions, and was thought reasonable to initially exclude given the low viable colony

counts obtained for the other two subpopulations which were assumed to be of better fitness.[288,289] When parameter estimate coefficients of variation exceeded 50% (i.e. the univariate confidence interval would include 0), then simpler models were evaluated that excluded the poorly estimated parameters. Any data below the lower limit of quantification (100 CFU/mL) were omitted from the model (see Time-Kill Procedure). Alternative approaches could have included modeling these data points as zero, the average of zero and the lower limit of quantification, or the actual lower limit of quantification, but were unnecessary given the large quantity of time-kill data obtained (see Appendix E). Lag-phase data were also omitted from the model since the purpose is to understand bacterial growth, killing, and the emergence of resistance and not the experimentally-dependent delay in maximal growth rate (see Determining Initial Pharmacodynamic Model Estimates for Growth Rate Constants and Maximum Population Count).

$$\boldsymbol{g = b - x} \tag{3}$$

$$\boldsymbol{k^* = k\frac{C}{C+EC_{50}}} \tag{4}$$

Second, a net growth rate model with an assumed maximal carrying capacity of the *in vitro* system (Nmax) was initially fit to antimicrobial-free experiments stratified by isolate number (Equations 5, 6, and 7) to ascertain reasonable estimates for the growth rate parameters of the three subpopulations and the maximum experimental population size of each isolate – gRLS, gRLR, gRHS, and Nmax.

$$\boldsymbol{\frac{dRLS}{dt} = \left(g_{RLS}\left[1 - \frac{RLS+RLR+RHS}{Nmax}\right]\right)RLS} \tag{5}$$

$$\frac{dRLR}{dt} = \left(g_{RLR}\left[1 - \frac{RLS+RLR+RHS}{Nmax}\right]\right)RLR \quad \textbf{(6)}$$

$$\frac{dRHS}{dt} = \left(g_{RHS}\left[1 - \frac{RLS+RLR+RHS}{Nmax}\right]\right)RHS \quad \textbf{(7)}$$

Third, graphical analyses of the time-kill curves of each isolate established the time of linear decline of the initial polymyxin B susceptible and/or meropenem low-resistance majority population. A net effect growth model and a direct effect killing model (Equations 1) were simultaneously fit to establish parameter estimates for the maximal killing rate of the corresponding antimicrobial as well as the concentration at which there was half maximal killing – k_S and EC_{50S}. Note: polymyxin B and meropenem containing time-kill experiments were fit separately during this step. Therefore, in the case of two populations, only "S" and "R" designations were used to denote the susceptible (or least resistant in the case of meropenem) majority population and the resistant (or more resistant in the case of meropenem) minority subpopulation rather than the three letter codes used for the growth rates in step two.

Fourth, all time-kill curves, still stratified by isolate, were fit by a net effect growth and direct effect killing model for both the susceptible majority population (or less resistant in the case of meropenem) and the more resistant subpopulation which would use the previously fit parameter estimates and confidence intervals as initial estimates and bounds to ultimately fit all parameters simultaneously – g_S, g_R, Nmax, k_S, EC_{50S}, k_R, and EC_{50R}. See equations 1 and 2.

Fifth, parameter estimates of individual isolates were assessed for plausibility by calculating stationary concentrations (SC; Equation 8) and growth rate doubling times (d;

Equation 9). The stationary concentration is the theoretical antimicrobial concentration where the growth rate and the killing rate are equal so no net growth or killing would result. The bacterial doubling time is the amount of time in minutes it takes for the bacterial population to double in quantity. Parameter estimate precision was assessed by ensuring coefficients of variation (CVs) were below 50% (in order to ensure univariate confidence intervals did not include zero). In the case where the EC_{50} CV was more than 50% and studied concentrations were largely above the estimate (e.g. for the more susceptible majority population), killing was assumed to be near maximal and equation 10 was used where Z represents either R or S. In the case where EC_{50} CV was more than 50% and studied concentrations were largely below the estimate (i.e. for the more resistant subpopulation), the Emax equation collapses into equation 11 where Z represents either R or S, and k' represents the merged parameter k/EC_{50}. Overall model fit and predictability was assessed by evaluating the uniformity and R^2 values of the observed vs. predicted plot as well as the Akaike Information Criterion (AIC) for minimizing information loss when considering alternative non-nested models.[290]

$$\boldsymbol{SC = \frac{g}{k-g} EC_{50}} \quad \textbf{(8)}$$

$$\boldsymbol{d = \frac{ln(2)}{g}} \quad \textbf{(9)}$$

$$\boldsymbol{\frac{dZ}{dt} = \left(g_Z \left[1 - \frac{S+R}{Nmax}\right] - k_Z\right) Z} \quad \textbf{(10)}$$

$$\boldsymbol{\frac{dZ}{dt} = \left(g_Z \left[1 - \frac{S+R}{Nmax}\right] - k'_Z * C\right) Z} \quad \textbf{(11)}$$

Sixth, an overall "composite" model was fit to all growth control and polymyxin B monotherapy experiments to generalize the model parameters to isolates other than the

individual isolates. Since all isolates had similar MICs to polymyxin B, the composite was fit with and without MIC normalization of the drug concentrations and EC_{50} parameter. Furthermore, all growth control and meropenem monotherapy experiments were fit to a composite model, but only the MIC normalized model was considered because these isolates exhibited drastically different MICs to meropenem and therefore expected to behave differently at similar concentrations of meropenem. In essence, individual isolates were assumed to have similar parameter estimates so all experiments were fit simultaneously (no longer stratified) to equations 1 and 2. The AIC values were compared and differences in AIC >10 (and corresponding relative likelihood) were considered significant enough to lend tremendous support of one model over the comparator.[290]

Determining Initial Pharmacodynamic Model Estimates for Growth Rate Constants and Maximum Population Count

Graphical analysis of each growth control time-kill experiment of isolates 22, 24, 34, and 44 was performed to identify regions of lag phase, log phase, stationary phase, and death phase (Figure 3.24). All time points identified to be within log phase were stratified by isolate number and by experiment type (antibiotic free agar, meropenem containing agar, and polymyxin B containing agar) and natural log transformed. Linear regression was performed on each stratum using Office 365 Excel (Microsoft, Redmond, WA) to ascertain initial estimates for gRLS, gRLR, and gRHS, using 95% confidence intervals as boundary conditions. All time points identified to be within stationary phase were stratified by isolate number and descriptive statistics (geometric mean with 95% confidence intervals) were

generated using Office 365 Excel to use as initial estimates and bounds for Nmax for each isolate. Only antibiotic free agar experiments were utilized for Nmax estimates since resistant subpopulation experiments performed on antibiotic-containing agar were inhibited by the abundance of susceptible majority population and would yield biased estimates of the experimental viable colony count capacity. In the case where resistant subpopulation data was too scarce to generate meaningful confidence intervals, the lower boundary was set to 0 and the upper boundary was set to match the upper boundary of the corresponding isolate gRLS since it is unlikely for the more resistant isolate to grow at a faster rate than the more susceptible majority population.

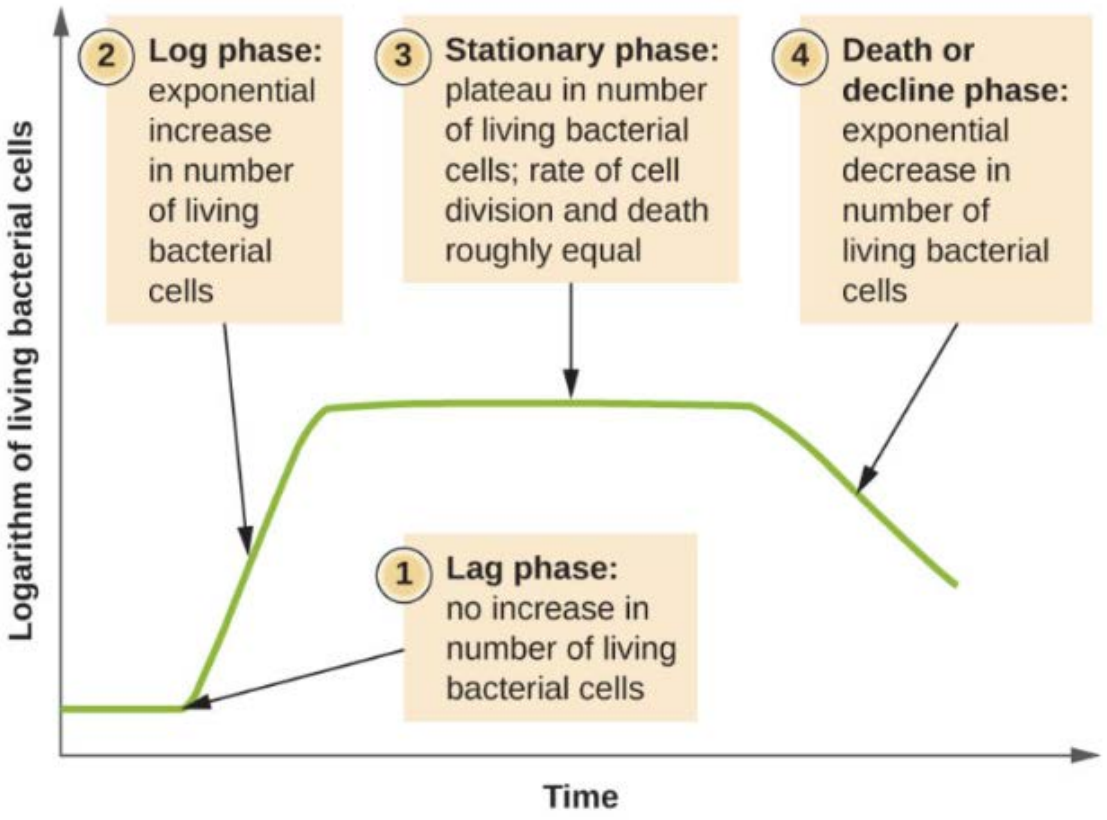


Figure 3.24: Phases of Bacterial Growth over Time. Reprinted.[291]

Determining Initial Pharmacodynamic Model Estimates for Susceptible Population Killing Rate Constants

Graphical analysis of each meropenem or polymyxin B containing time-kill experiment of isolates 22, 24, 34, and 44 was performed to identify regions of initial killing phase, regrowth phase, stationary phase, and death phase (Figure 3.25).

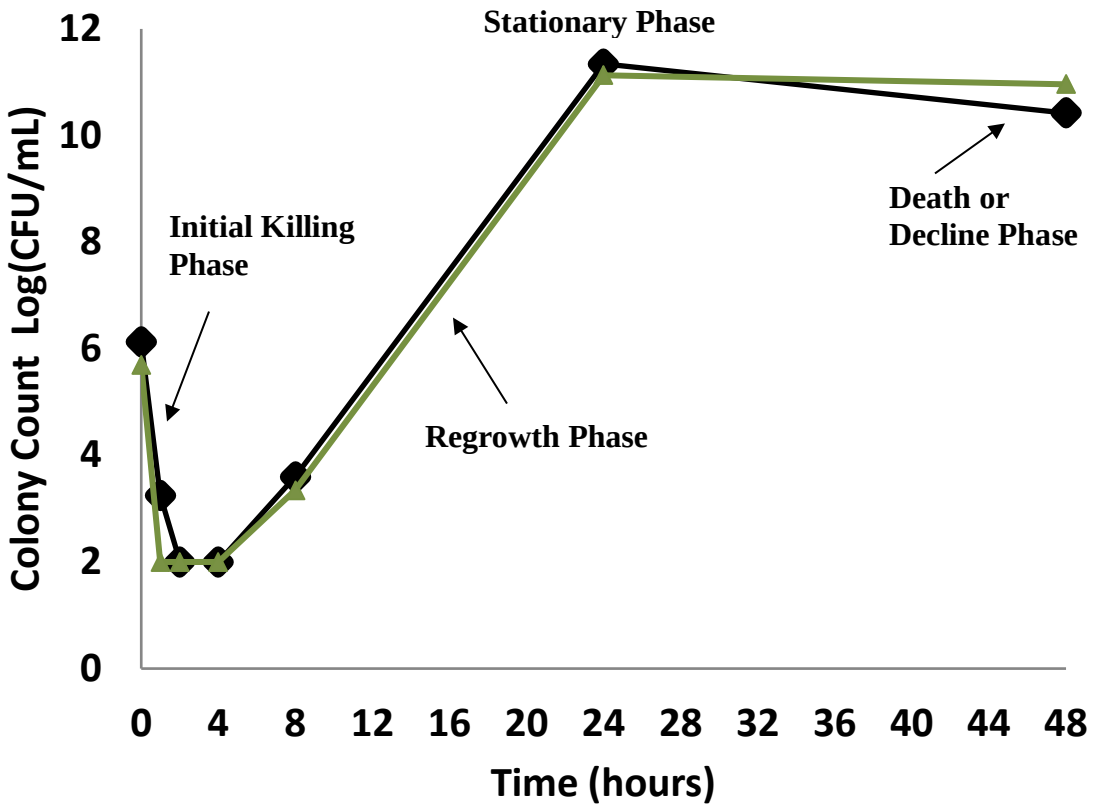


Figure 3.25: Phases of Bacterial Killing over Time. Two time-kill experiments with polymyxin B 0.25 mg/L against isolate 22.

All time points identified to be within the initial killing phase were stratified by isolate number and drug concentration, and subsequently natural log transformed. Linear regression was performed on each pooled stratum using Office 365 Excel (Microsoft, Redmond, WA) to ascertain pairings of drug concentration and the regression slope (m_S; Equation 12). Note that the observed initial decline in colony count is growth rate minus apparent killing rate (k^*; Equation 13). Therefore, the gRLS parameter estimate obtained from modeling growth control experiments (see Pharmacodynamic Mathematical Modeling) was used to transform linear regression slopes into apparent killing rates (Equation 12).

$$\boldsymbol{m_S = g_S - k^*_S} \quad \textbf{(12)}$$

$$\boldsymbol{k^*_S = k_S \frac{C}{C+EC_{50S}}} \quad \textbf{(13)}$$

All possible pairwise systems of equations for concentration-k^* were subsequently solved (Equations 14 and 15), generating a data set of k_S and EC_{50S} estimates.

$$\boldsymbol{k^*_{1S} = k_S \frac{C_1}{C_1+EC_{50S}}} \quad \textbf{(14)}$$

$$\boldsymbol{k^*_{2S} = k_S \frac{C_2}{C_2+EC_{50S}}} \quad \textbf{(15)}$$

Descriptive statistics (mean with 95% confidence intervals) were generated using Office 365 Excel to use as initial estimates and bounds for k_S and EC_{50S} for each isolate. Stationary concentrations (Equation 8) were calculated to assess the plausibility of these initial parameter estimates.

Determining Initial Pharmacodynamic Model Estimates for Resistant Population Killing Rate Constants

Graphical analysis of each meropenem or polymyxin B containing time-kill experiment of isolates 22, 24, 34, and 44 was performed to identify regions of initial killing phase, regrowth phase, stationary phase, and death phase (Figure 3.25), as in Determining Initial Pharmacodynamic Model Estimates for Susceptible Population Killing Rate Constants.

All time points identified to be within the regrowth phase were stratified by isolate number and drug concentration, and subsequently natural log transformed. Linear regression was performed on each pooled stratum using Office 365 Excel (Microsoft, Redmond, WA) to ascertain pairings of drug concentration and the regression slope (m_R; Equation 16). Note that the observed regrowth in colony count is growth rate minus apparent killing rate (k^*; Equation 17). Therefore, the gRLR (polymyxin B experiments) or gRHS (meropenem experiments) parameter estimates obtained from modeling growth control experiments (see Pharmacodynamic Mathematical Modeling) were used to transform linear regression slopes into apparent killing rates (Equation 16).

$$\boldsymbol{m_R = g_R - k^*_R} \quad \textbf{(16)}$$

$$\boldsymbol{k^*_R = k_R \frac{C}{C+EC_{50R}}} \quad \textbf{(17)}$$

All possible pairwise systems of equations for concentration-k^* were subsequently solved (Equations 18 and 19), generating a data set of k_R and EC_{50R} estimates.

$$\boldsymbol{k^*_{1_R} = k_R \frac{C_1}{C_1 + EC_{50R}}} \quad \textbf{(18)}$$

$$\boldsymbol{k^*_{2_R} = k_R \frac{C_2}{C_2 + EC_{50R}}} \quad \textbf{(19)}$$

Descriptive statistics (mean with 95% confidence intervals) were generated using Office 365 Excel to use as initial estimates and bounds for k_R and EC_{50R} for each isolate. Stationary concentrations (Equation 8) were calculated to assess the plausibility of these initial parameter estimates. When calculated stationary concentrations were not reasonable (i.e. SC < C | regrowth or SC > C | killing), then a different approach was used (see below).

Determining Initial Pharmacodynamic Model Estimates for Resistant Population Killing Rate Constants Given Unreasonable Stationary Concentrations

In summary, this approach assumed that stationary concentrations fell within a given range and then estimated lowest and highest possible k and EC_{50} values that could give such a range in stationary concentrations. The lowest stationary concentration was assumed to be the highest concentration of antimicrobial tested where regrowth was observed (i.e. the true stationary concentration must be more than this value). The highest stationary concentration was assumed to be the lowest concentration of antimicrobial tested where regrowth was not observed (i.e. the true stationary concentration of antimicrobial tested must be less than this value). When no concentration of antimicrobial prevented regrowth, then the highest observed MIC of the regrowing subpopulation was used as the stationary concentration (see Resistance Development to Polymyxin B) following selection for this isolate at concentrations 2-4x the MIC of the majority population. Higher concentrations may elicit an adaptive response and explain the higher MICs observed in Table 4.19.

Additionally, the lower and upper 95% univariate confidence interval values for gRLR or gRHS were considered rather than just the parameter estimate as in Determining Initial Pharmacodynamic Model Estimates for Resistant Population Killing Rate Constants. Next, k_R was assumed to be no greater than the upper planar 95% confidence interval of k_S since it is unlikely that the more resistant subpopulation could be killed faster than the more susceptible subpopulation. Finally, EC_{50R} was assumed to be at least the highest antimicrobial concentration tested since the best explanation for poor stationary

concentrations is that the concentrations tested were well below the EC_{50R}. Otherwise, the original analysis should have yielded plausible k_R and EC_{50R} combinations.

To summarize, all possible combinations of the assumptions above were used to calculate new k_R and EC_{50R} estimates. In other words, the stationary concentration was assumed either low or high (bounds discussed previously), the growth rate constant was assumed either low or high (bounds discussed previously), and either the maximum k_R assumption was used or the minimum EC_{50R} assumption was used generating 8 possible parameter estimates for k_R and EC_{50R} that generate stationary concentrations between the bounds assumed. The average of the lowest and highest k_R and EC_{50R} was used as an initial estimate with the lowest and highest values also being used as boundary conditions for the model.

Fixed Parameters

Initial viable colony counts for the model were fixed as opposed to being fit as parameters themselves. For the susceptible population, the initial counts were measured in all experiments and were fixed to the measured viable colony counts at time=0. For the resistant subpopulation, only experiments utilizing antimicrobial containing agar measured the starting concentration of the resistant subpopulation. Therefore, this quantity was assumed constant for all other experiments. In reality, this value likely varied but because counts were so small (usually <50 CFU/mL), estimating this value as another parameter in the model yielded very high CVs and was not useful without additional experimental data to improve the precision of starting resistant population colony count. When MICs were

utilized in the model to normalize parameters between isolates, they were fixed according to the broth microdilution MIC results (see Minimum Inhibitory Concentrations (MICs)). However, given the intrinsic lack of precision of antimicrobial susceptibility testing (within one two-fold dilution of MIC result), MICs were also fit as parameters for the meropenem composite model using the MICs measured by BMD as initial estimates and using one two-fold dilution as lower and upper limits.

Full Model Initial Estimates of Parameters

Initial parameter estimates for the full monotherapy model (Equations 1 and 2) utilized the fit growth parameters (gRLS, gRHS, and RLR) of the growth control experiments, the fit k_S and EC_{50S} parameters of the initial monotherapy killing phase, and the initial parameter estimates of the resistant subpopulation (k_R and EC_{50R}) of the regrowth phase. Univariate 95% confidence intervals were utilized for bounds except in the cases of anticipated great uncertainty where planar 95% confidence intervals were used to account for the variability of the other parameter estimates. Specifically, the growth rates of gRHS and gRLR were anticipated to be uncertain since only few experiments followed this growth rate over time, so planar confidence intervals were utilized as bounds. Since most polymyxin B monotherapy concentrations utilized were well above the MIC of the organisms, planar confidence intervals were utilized for the maximal killing rate constant k_S and the EC_{50S} parameters. However, for meropenem monotherapy, the concentrations utilized were likely not as close to the k_S parameter, so a better fitting estimate of the k_S and EC_{50S} relationship was anticipated. Therefore, univariate confidence intervals were

used for k_S bounds and planar confidence intervals were utilized for the less certain EC_{50S} parameter.

Data Analysis

In Vitro Susceptibility Testing

For each isolate, MIC results from within the same experiment as well as from at least two replicate experiments were compared and evaluated for essential agreement (within one two-fold dilution).[262] If MIC results did not agree, broth microdilution was repeated for these strains. When MIC results were not the same, but at least two replicate experiments were in agreement, the most common (modal) MIC was accepted. If there was no mode, the greater of the two results was accepted as the MIC. Additionally, MIC range, MIC_{50}, and MIC_{90} were determined. Percent susceptible was calculated based on breakpoints established by CLSI (Table 3.8).[268]

Table 3.10: Susceptibility Breakpoints for *Enterobacteriaceae*

	Susceptible	Intermediate	Resistant
Amikacin	≤16	32	≥64
Ampicillin	≤8	16	≥32
Ampicillin/Sulbactam	≤8/4	16/8	≥32/16
Aztreonam	≤4	8	≥16
Cefazolin	≤2	4	≥8
Cefepime	≤2	4-8*	≥16
Cefoxitin	≤8	16	≥32
Ceftazidime	≤4	8	≥16
Ceftazidime / avibactam	≤8/4		≥16/4
Ceftriaxone	≤1	2	4
Cefuroxime	≤4	8-16	≥32
Ciprofloxacin	≤1	2	≥4
Colistin[†]	≤2	4	≥8
Ertapenem	≤0.5	1	≥2
Imipenem	≤1	2	≥4
Imipenem / Relebactam[#]	≤1	2	≥4
Gentamicin	≤4	8	≥16
Levofloxacin	≤2	4	≥8
Meropenem	≤1	2	≥4
Minocycline	≤4	8	≥16
Polymyxin B[†]	≤2	4	≥8
Nitrofurantoin	≤32	64	≥128
Piperacillin / tazobactam	≤16/4	32/4 - 64/4	≥128/4
Tetracycline	≤4	8	≥16
Tigecycline[§]	≤2	4	≥8
Tobramycin	≤4	8	≥16
Sulfamethoxazole / trimethoprim	≤2/38	-	≥4/76

*Cefepime does not have an intermediate susceptibility but instead has a susceptible dose-dependent designation

[†]Colistin and polymyxin B do not have CLSI breakpoints for *Enterobacteriaceae*. The breakpoints for *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were used instead[262]

[#]Imipenem/relebactam does not have CLSI breakpoints for *Enterobacteriaceae*. Imipenem breakpoints were utilized according to literature and manufacturer recommendations

[§]Tigecycline does not have CLSI breakpoints for gram-negative organisms. An FDA-approved breakpoint of ≤ 2 μg/mL was considered susceptible[168]

Time-Kill Studies

Plots of colony count (log_{10} CFU/mL) versus time were constructed for each isolate and antimicrobial(s) studied. Furthermore, combined plots across multiple experiments were generated using geometric means of the colony counts and standard deviations for each time point. Additionally, plots of 24-hour change in log_{10} CFU/mL were constructed using the logarithm of the geometric mean of the initial (time = 0) colony count subtracted from the logarithm of the geometric mean of the 24-hour colony count with un-pooled standard deviations. Activity was evaluated as bactericidal, bacteriostatic, or growth where bactericidal activity was defined as a $\geq 10^3$ decrease in colony count at 24 hours, bacteriostatic was defined as a $< 10^3$ decrease in colony count at 24 hours, and growth was any positive change at 24 hours. Synergy was also evaluated for combinations, being defined as a $\geq 10^2$ CFU/mL lower colony count at 24 hours when compared to the most active agent used alone.

Subpopulation Analysis

A table of colony count (log_{10} CFU/mL) at time 0 was constructed which included each isolate studied in time-kill assays. Measurements from microfiltration were preferentially used when either microfiltration data were below the lower limit of quantification (10^2 CFU/mL) for the laser colony counter or when the laser colony counter data were below the lower limit of quantification. When the laser colony counter and microfiltration data were above 10^2 CFU/mL or if the microfiltration method produced too many colonies to count, the laser colony counter value was used. Additionally, reported

values were not rounded to the lower limit of quantification of microfiltration because higher error was accepted as a limitation for comparing colony counts that were expected to be so low.

Whole Genome Sequencing

Multilocus sequence typing (MLST) on the *K. pneumoniae* isolates 22, 24, and 44, and the *E. cloacae* isolates 10, 17, 19, and 40 was performed by Dr. Grace C. Lee.[274,275] When coverage of all housekeeping genes was insufficient to verify novel MLST, the closest match was reported. Antimicrobial resistance genes were stratified by class of antibiotic. For β-lactam antimicrobials, the Ambler classification system (see Classification of β-lactamases) further stratified resistance mechanisms. Likewise, for the aminoglycoside modifying enzymes, acetyltransferases, phosphotransferases, and nucleotidyltransferases were indicated separated. Furthermore, amikacin resistance mechanisms were uniquely identified among the aminoglycoside stratum according to structural analysis of amikacin. For example, some aminoglycoside resistance mechanisms may not apply to amikacin due to its structure not matching the active site of the aminoglycoside modifying enzyme.

Pharmacodynamic Mathematical Model Simulations

The final model parameters were used to simulate viable colony counts over time for every experiment. The geometric average of these simulations were plotted with the

geometric average of the observed data for each concentration of antimicrobial to verify visual goodness of fit. Plots of observed vs. predicted viable colony counts were generated and linear regression performed to evaluate any residual bias in the model. Stationary concentrations and bacterial doubling rates were also calculated. Models were compared using the Akaike Information Criterion (AIC) and ensuring that parameter CVs yielded confidence intervals that exclude zero.[290] All parameter estimates, CVs, and confidence intervals were reported.

Statistical Analyses

Fisher's exact or Chi-squared analyses with a Holm-Bonferroni correction, when appropriate, were used to compare non-parametric antimicrobial susceptibility data such as broth microdilution, BD Phoenix™, Etest® and nationally reported data.[292,293] However, statistical inferences are limited in that high sample sizes may confer statistical significance with a lack of clinical significance. For example, a difference in susceptibility of 62% compared to 60% may be statistically significant, depending on the sample size, but a difference in susceptibility of 100% to 98% indicating first appearance of resistance may be more clinically significant, regardless of statistical significance. For paired data, McNemar's test was used instead of Fisher's exact or Chi-squared.

Geometric means and standard deviations are most meaningful regarding time-kill and log-change studies due to the very high inter-experiment variability (heterogeneity) observed across studies. Therefore, statistical parameters describing intra-experiment variability (e.g. standard deviation and coefficients of variance) are a better indicator of

valid results in the face of dynamically growing organisms where external factors are difficult to control without many samples. This has been a limitation and described by numerous meta-analyses and review articles[14,201,205,212] leading to standardized definitions for describing and comparing data (e.g. bactericidal activity and synergy), primarily by CLSI.[262,264]

Akaike Information Criterion (AIC) was determine by the Phoenix® Win Nonlin® software and was used to compare models. Since none of the comparator models were considered nested (i.e. no model contained all of the parameters of another model), the relative likelihood rather than the likelihood ratio test was appropriate.[290] Parameter CVs were also considered in choosing the best model in that if the univariate confidence interval of a parameter contained zero, then the parameter could not be concluded significant enough to the model to warrant inclusion. However, it was acknowledged when this occurred and an explanation as to why this parameter had such a poor estimate was provided.

Chapter Four:

Publications, Results, and Discussion

Part of the research contained within this chapter has been published as Kulengowski, B. *In vitro* activity of polymyxin B and meropenem alone and in combination against carbapenem-resistant *Enterobacteriaceae*. 2016. *Theses and Dissertations—Pharmacy*. 57. Other parts of this chapter have also either been published, are in press, or are currently under review and the reader will be informed when this is the case by a lead-in paragraph disclosure. Due to the volume of this dissertation, the relevant background and methods were kept with the results and discussion of the manuscripts so that readers do not need to go back and forth between the various sections within this manuscript.

In Vitro Susceptibility Testing

Bacterial Isolates

Clinical isolates of 612 non-duplicate, MDR, gram-negative organisms were collected between November 9, 2008 and October 1, 2018 from the Clinical Microbiology Laboratory at the University of Kentucky Chandler Medical Center in Lexington, Kentucky.[166] The most common gram-negative MDR species in descending order were *Escherichia coli* (27%), *Pseudomonas aeruginosa* (25%), *Klebsiella pneumoniae* (19%), and *Enterobacter cloacae* (10%). CRE numbered 164 (27%) of the MDR isolates obtained, and *Klebsiella pneumoniae* (41%), *Enterobacter cloacae* (30%), and *Citrobacter freundii* (8%) composed most of this group. These isolates are described in Table 4.1 and Table 4.2.

Table 4.1: All MDR Clinical Isolates

Organism	Number of Isolates	Percentage
A. baumannii/ calcoaceticus complex	38	6.2%
A. xylosoxidans	1	0.2%
Achromobacter sp.	4	0.7%
B. cepacia	1	0.2%
C. amalonaticus	3	0.5%
C. farmeri	2	0.3%
C. freundii	14	2.3%
C. youngae	2	0.3%
E. aerogenes	5	0.8%
E. cloacae	62	10.1%
E. coli	166	27.1%
E. gergoviae	2	0.3%
E. hormaechei	2	0.3%
E. vulneris	1	0.2%
Enterobacter sp.	2	0.3%
K. oxytoca	14	2.3%
K. ozaenae	2	0.3%
K. pneumoniae	116	19.0%
P. aeruginosa	153	25.0%
P. agglomerans	4	0.7%
P. mirabilis	5	0.8%
P. putida	4	0.7%
P. rettgeri	1	0.2%
P. vulgaris	1	0.2%
P. vulgaris/penneri	2	0.3%
S. aureus	1	0.2%
S. marcescens	3	0.5%
S. paucimobilis	1	0.2%
TOTAL	612	

Table 4.2: Carbapenem-resistant *Enterobacteriaceae* Clinical Isolates

Organism	Number of Isolates	Percentage
C. amalonaticus	2	1.2%
C. freundii	13	7.9%
C. youngae	2	1.2%
E. aerogenes	4	2.4%
E. cloacae	50	30.5%
E. coli	9	5.5%
E. gergoviae	2	1.2%
E. hormaechei	2	1.2%
Enterobacter sp.	2	1.2%
K. oxytoca	5	3.0%
K. ozaenae	2	1.2%
K. pneumoniae	68	41.5%
P. rettgeri	1	0.6%
S. marcescens	2	1.2%
TOTAL	164	

Minimum Inhibitory Concentrations (MICs)

All isolates underwent identification and antimicrobial susceptibility testing for clinical purposes through the University of Kentucky Clinical Microbiology Laboratory using BD Phoenix™ prior to collection by our lab. This data was provided to our lab and the specific CRE data were subsequently verified using antimicrobial susceptibility testing by broth microdilution methodology, the gold standard for determination of MICs.[264] The primary objective of antimicrobial susceptibility testing through broth microdilution was to characterize CRE observed in a tertiary referral U.S. academic medical center. Carbapenem resistance was described by the measured MIC value, and these isolates exhibited low to high levels of resistance (MICs ranging 4 - >128) to meropenem– the most commonly used carbapenem antimicrobial at the University of Kentucky Chandler Medical Center.[262]

The MICs for all 164 CRE isolates are shown for the 20 antimicrobials tested by BD Phoenix™ in Appendix A. The MIC_{50}, MIC_{90}, and percentage susceptible across CRE isolates numbering at least 30 according to CLSI guidelines for cumulative susceptibility reporting are shown in Appendix B.[292] Since clinical isolates were continually being sent throughout the study from the University of Kentucky Clinical Microbiology Laboratory, only 125 of ultimately 164 CRE organisms underwent antimicrobial susceptibility testing by broth microdilution (BMD). Only 122 of these 125 met the CDC definition of CRE when BMD was used (see Bacterial Isolates). MICs of the antimicrobials evaluated against these CRE are shown in Appendix C. The MIC_{50}, MIC_{90}, and percentage susceptible for these isolates are presented in Appendix D and stratified into various comparator groups as manuscripts submitted for publication. Specifically, these manuscripts are: 1) Evaluation of an Automated System for Determining Antimicrobial Susceptibility against Carbapenem-resistant *Enterobacteriaceae* Compared to Broth Microdilution, 2) Antimicrobial Activity against Carbapenem-resistant *Enterobacteriaceae* that Produce Verona Integron-encoded Metallo-beta-lactamase or *Klebsiella pneumoniae* Carbapenemase, and 3) Imipenem/relebactam Activity Compared to Commonly Utilized Antimicrobials against Carbapenem-resistant *Enterobacteriaceae* from an Academic Medical Center as part of an investigator initiated research proposal for Merck & Co. (MISP #56367).

Evaluation of an Automated System for Determining Antimicrobial Susceptibility against Carbapenem-resistant *Enterobacteriaceae* Compared to Broth Microdilution

To be submitted: *International Journal of Antimicrobial Agents* as an original article

Authors: Zachary J. Haffler, Brandon Kulengowski, David S. Burgess

Kulengowski contribution: Experimental design, execution, data analysis, revisions and editing of final manuscript

Special acknowledgements: I thank former pharmacy student, Zachary Haffler for the tremendous amount of effort he has put forth as the primary author of this manuscript submission and his words are reproduced here with his permission.

Abstract

Carbapenem-resistant *Enterobacteriaceae* (CRE) are increasingly common and result in infections associated with significant mortality. The aim of this study is to evaluate the categorical agreement between the automated system BD Phoenix™ and broth microdilution in determining minimum inhibitory concentrations for CRE. We evaluated the activity of amikacin, aztreonam, cefepime, ceftazidime, ertapenem, gentamicin, levofloxacin, meropenem, nitrofurantoin, piperacillin-tazobactam, and tobramycin on 125 CRE isolates collected from an academic medical center. We determined categorical agreement between BD Phoenix™ and broth microdilution. BD Phoenix™ significantly overestimated susceptibility of CRE isolates for 9 of 11 tested antimicrobials compared to gold-standard broth microdilution. All antimicrobials exhibited increased error rates compared to previous literature. BD Phoenix™ overestimates antimicrobial susceptibility of CRE isolates, regardless of carbapenem mechanism of resistance. A secondary testing method should be utilized to confirm antimicrobial susceptibility results. Further studies are warranted in order to validate BD Phoenix™ susceptibility testing in highly resistant CRE isolates.

Introduction

Carbapenem-resistant *Enterobacteriaceae* (CRE) infections are associated with a mortality rate of approximately 50% and have become an increasingly prevalent global problem.[294] As carbapenem agents are considered to be among the most potent antimicrobials for combating gram-negative infections, the emergence and spread of carbapenemases that hydrolyze these agents is a cause for alarm. According to the Center for Disease Control (CDC), CRE infections pose an immediate public threat that requires urgent and aggressive action. Though historically uncommon, CRE have been identified in healthcare facilities in all 50 states as of 2018.[295] Nationally, it has been reported that approximately 10% of *Klebsiella* spp. bloodstream infections are caused by CRE.[120]

Resistance to carbapenem agents primarily results from the horizontal transfer of plasmid-encoded carbapenem hydrolyzing enzymes. Molecular class A, group 2f β-lactamases include the *Klebsiella pneumoniae* Carbapenemase (KPC), which has been associated with outbreaks of multidrug-resistant infections worldwide.[43,294,296,297] Molecular class B, groups 3a and 3b β-lactamases include the metallo-β-lactamases (MBL) IMP and VIM which are becoming increasingly common in the United States.[43] As of February 2018, New Delhi metallo-β-lactamase-producing (NDM) CRE are the most commonly reported CRE infections.[295] Plasmids which carry the genes containing carbapenem hydrolyzing enzymes also carry genes which confer resistance to many other antimicrobial drug classes, including fluoroquinolones and aminoglycosides.[298-300]

Many laboratories utilize automated identification and antimicrobial susceptibility systems for clinical isolates to decrease the time and labor required to determine

susceptibility results for hospitalized patients. Inaccuracies in these systems could result in potentially devastating treatment failures for patients with CRE infections. Due to the significant morbidity and mortality associated with CRE infections, it is vital that healthcare practitioners are able to assess which medications are treatment options when patients present with CRE infections. Previous studies have demonstrated the accuracy of automated systems for identification and susceptibility testing of gram-negative organisms when compared to the agar dilution method, broth dilution, and Etests®.[301-304] Conversely, there is little information on the validation of automated systems to appropriately determine susceptibility data for highly resistant CRE isolates. The purpose of this study was to assess the level of categorical agreement (CA) between the BD Phoenix™ automated system and broth microdilution (BMD) in determining minimum inhibitory concentrations (MICs) of CRE at an academic medical center.

Methods

Bacterial Isolates. 125 isolates of carbapenem-resistant *Enterobacteriaceae* (CRE) were obtained from UK HealthCare Albert. B Chandler Hospital from January 1, 2012 to December 31, 2016. We identified organisms by their resistance to either ertapenem or meropenem on BD Phoenix™. Isolates were frozen and stored at -80°C for use in our microbiology laboratory.

Susceptibility Testing. The MICs were obtained from BD Phoenix™ automated susceptibility testing (versions V6.01, V6.01A, and V6.21A) and by broth microdilution for all CRE isolates against amikacin, aztreonam, cefepime, ceftazidime, ertapenem,

gentamicin, levofloxacin, meropenem, nitrofurantoin, piperacillin-tazobactam, and tobramycin. This panel was selected for drugs used at our institution during routine clinical practice. Stock antimicrobials were prepared for microdilution according to Clinical and Laboratory Standards Institute (CLSI) guidelines. MICs from broth microdilution were determined in accordance with CLSI standardized laboratory practices utilizing the Biotek Instruments, Inc. BioStack™ Microplate Stacker and Precision Pipetting Systems.[305] Dilution of clinical isolates to a standard inoculum of $1.5x10^8$ CFU/ml was performed via McFarland standard matching. An additional 1:200 dilution was created with cation-adjusted Mueller Hinton broth to make a final inocula of $6x10^5$ CFU/ml. Isolates were then cultured and incubated at 37°C overnight. Broth microdilution experiments were performed in duplicate on two separate days; the modal MIC was documented. We utilized *Pseudomonas aeruginosa* ATCC® 27853™ and *Escherichia coli* ATCC® 25922™ as quality control strains. CLSI susceptibility, intermediate, and resistant breakpoints were utilized.[268]

Subgroup Analysis. Further analysis was performed on subgroups divided according to isolate resistance mechanism: KPC-producing (n=71), MBL-producing (n=21), and non-carbapenemase-producing CRE (n=27). Isolates that expressed both KPC and MBL enzymes were excluded from subgroup analysis. Resistance phenotypes were determined using a combined-disc test utilizing meropenem alone, as well as in combination with phenylboronic acid or ethylenediaminetetraacetic acid. Tsakris et al. evaluated phenotypic testing method for the detection of carbapenemase production and differentiation of KPC and MBL enzymes.[266]

Susceptibility Data Analysis. All data from BMD and BD Phoenix™ were reported and transcribed into Microsoft Excel spreadsheets for analysis. We determined categorical agreement between the two testing methods. Essential agreement was not evaluated because BD Phoenix™ has a very limited reportable MIC range for each antimicrobial compared to BMD. Categorical agreement was defined as identical susceptibility testing categories reported for both testing methods. McNemar's test was used to assess statistical significance for paired, binominal data. Two-sided *P*-values of 0.05 or less indicated statistical significance.

Error Rates and CLSI Acceptable Discrepancy Rates. We determined the rates of minor error (MiE), major error (ME), and very major error (VME) between broth microdilution and the BD Phoenix™ automated system. MiE was when one testing method categorized an isolate as intermediate and the other did not. ME was when BD Phoenix™ categorized an isolate as resistant and broth microdilution categorized the same isolate was susceptible. VME was when BD Phoenix™ categorized an isolate as susceptible and broth microdilution categorized the same isolate to be resistant. The count of MiEs, MEs, and VMEs were used as numerators in their respective equations to determine the error rates for BD Phoenix™. The denominator was established using the appropriate population of isolates outlined in CLSI's guidance on susceptibility testing criteria based on the isolates' MIC value.[306] Therefore, isolates for each antimicrobial were divided into three populations: those with MIC values greater than or equal to two dilutions above the intermediate MIC (≥I+2), those with MIC values between one dilution above the intermediate MIC and one dilution below the intermediate MIC (I+1 to I-1), and those with MIC values less than or equal to the two dilutions below the intermediate MIC (≤I-2).[306]

Results

Of the 125 CRE clinical isolates collected, 14 distinct species were identified by the automated system BD Phoenix™: *Klebsiella pneumoniae* (n=58), *Enterobacter cloacae* (n=31), *Citrobacter freundii* (n=9), *Escherichia coli* (n=7), *Enterobacter aerogenes* (n=4), *Klebsiella oxytoca* (n=3), *Enterobacter* sp. (n=2), *Citrobacter amalonaticus* (n=2), *Citrobacter youngae* (n=2), *Enterobacter hormaechei* (n=2), *Klebsiella ozaenae* (n=2), *Enterobacter gergoviae* (n=1), *Providencia rettgeri* (n=1), and *Serratia marcescens* (n=1). Table 4.3 summarizes the *in vitro* activity of all antimicrobials tested via BMD and BD Phoenix™ as percent susceptibility. BD Phoenix™ overestimated susceptibility for CRE isolates for 82% (9/11) of tested antimicrobials – amikacin, gentamicin, tobramycin, levofloxacin, nitrofurantoin, aztreonam, cefepime, ceftazidime, and meropenem. High levels of resistance to ertapenem and piperacillin-tazobactam precluded detection of a statistically significant difference between broth microdilution and BD Phoenix™ – 2% vs. 1% and 1% vs. 3% susceptibility, respectively (Table 4.3).

Overall categorical agreement was calculated as 76% for all organisms and antimicrobials tested. Categorical agreement varied greatly, even between antimicrobial agents of the same class, ranging from 50-95%. The highest rate of categorical agreement was seen in beta-lactam agents, at 79%, followed by 76%, 63%, and 61% with aminoglycosides, nitrofurans, and fluoroquinolones, respectively. The antimicrobials with the highest categorical agreement values (aztreonam, ertapenem, and piperacillin-tazobactam) were the agents with the lowest percent susceptibility by both BD Phoenix™ and broth microdilution (Table 4.3). MIC values determined from both testing methods for all isolates and antimicrobials are listed in the Appendix A and Appendix C.

The susceptibility results for KPC-producing isolates, MBL-producing isolates, and non-carbapenemase-producing CRE isolates are summarized in Table 4.4, Table 4.5, and Table 4.6. In isolates producing KPC, susceptibility was overestimated by BD Phoenix™ for cefepime, levofloxacin, aztreonam, and nitrofurantoin with an average categorical agreement of 75% [range 44-99%] (Table 4.4). BD Phoenix™ also significantly overestimated susceptibility for MBL-producing isolates for meropenem, aztreonam, and nitrofurantoin (Table 4.5). In isolates where carbapenemase enzymes were not detected, a significant overestimation of susceptibility by BD Phoenix™ was detected only for levofloxacin (Table 4.6).

All tested antimicrobials showed increased error rates in at least one category – VME, ME, or MiE. Only ertapenem exhibited VME rates as low as previously observed evaluations of automated susceptibility testing methods. Only amikacin, cefepime, nitrofurantoin, and tobramycin exhibited low ME error rates. Finally, only 4 of 11 antimicrobials demonstrated relatively low MiE rates – aztreonam, ertapenem, gentamicin, and piperacillin-tazobactam. Table 4.7 reports the calculated error rates for broth microdilution vs. BD Phoenix™. No agent exhibited consistently low rates of error per BD Phoenix™ when compared against broth microdilution for CRE isolates.

Discussion

Our study demonstrated that BD Phoenix™ overestimates susceptibility for CRE compared to gold-standard broth microdilution for 9/11 (82%) tested antimicrobials. The differences in susceptibility were found to be statistically significant, despite previous

literature validating the utilization of the BD Phoenix™ automated system for gram-negative bacilli by repeated testing.[301-304,307,308] In 2002, Endimiani *et al*. evaluated BD Phoenix™ against broth microdilution for the identification and susceptibility testing of 136 non-fermenting gram-negative isolates. They concluded the automated system correctly measured the susceptibility of antipseudomonal drugs with a reported categorical agreement of 93.1%.[303] The present study reports an overall categorical agreement of 76%, but there were antimicrobials with categorical agreement considerably higher than the average: aztreonam, ertapenem, and piperacillin-tazobactam. However, this observation may best be explained by observed high levels of resistance to these drugs (98-99%).

For all drugs tested, we observed increased error rates when compared to rates previously described for automated susceptibility testing, with 10/11 (91%) drugs yielding an elevated rate of VME within at least a single intermediate range-demarcated subpopulation. The rates of error found for each antimicrobial agent stratified by population are outlined in Table 5. According to CLSI *in vitro* susceptibility testing criteria, of greatest concern are the discrepancies that occur with MICs greater than or equal to twofold concentrations above (≥I+2) or below (≤I-2) the intermediate MIC.[306] Our study found rates of MiE, ME, and VME considerably greater than observed previous studies. In 2002, Steward *et al*. assessed the accuracy of five antimicrobial testing methods – agar dilution, disk diffusion, Etest®, MicroScan WalkAway, and Vitek – in *Enterobacteriaceae* and *Pseudomonas* isolates using imipenem and meropenem. For *Enterobacteriaceae*, the number of MEs across all testing methods ranged from 0 to 1 for imipenem and 0 to 2 for meropenem, and a ME rate ranging from 0 to 2.3%. Despite these comparatively lower error rates, the authors concluded testing susceptibilities of carbapenems with automated

systems resulted in high rates of ME and variability, requiring a second verification testing method for these agents.[309] The present study supports this conclusion.

There is a paucity of data on the validation of automated systems to appropriately determine susceptibility data for highly resistant isolates. Endimiani *et al*. included a small number of resistant isolates producing extended spectrum β-lactamases (n=5) and MBLs (n=4) in their study evaluating BD Phoenix™, though separate susceptibility data for resistant isolates was not reported.[303] Ours is the largest study evaluating the BD Phoenix™ automated susceptibility testing method for multiple drug classes against highly-resistant CRE isolates. Our results are consistent with a previous study by Zhao *et al*. in 2017 which evaluated three automated susceptibility testing methods against their reference method, agar dilution. Seventy-five CRE isolates were run against four agents: amikacin, ciprofloxacin, gentamicin, and levofloxacin. The resulting rates of minor, major, and VME were 3.11%, 2.44%, and 4.33%, respectively.[308] Our rates of error are markedly greater than that observed by Zhao and colleagues. Even with comparatively lower error rates, the authors arrived at the conclusion that clinical laboratories should seek a second, independent method for determining susceptibility data for CRE isolates when using aminoglycosides and fluoroquinolones. Our study arrives at the same conclusion given the greater rates of MiE, ME, and VME.

Subgroup analyses demonstrated similar patterns of elevated error rates associated with BD Phoenix™, despite smaller population sizes in the subgroups resulting in fewer statistically significant results (see Table 4.4, Table 4.5, and Table 4.6). Furthermore, for some agents, it was not possible to conclude that the results were different due to the high levels of resistance, or in the case of amikacin, susceptibility.

Limitations of the present study include that our study isolates were taken from a single referral medical center. Therefore, data from other centers should be reported to determine external validity. Furthermore, because the susceptibility results from BD Phoenix™ were provided to the research laboratory independent of this project, there were a small number of isolates and agents for which BD Phoenix™ susceptibility data did not exist (0.51% of susceptibility data). In the event of these absences, the MIC value was omitted from analysis. Additionally, isolates collected were tested via BD Phoenix™ during the course of routine clinical practice. The isolates were then frozen and broth microdilution was performed at a later date, potentially providing an explanation for differentiation in MIC results. A limitation is also the absence of repeat susceptibility testing using the BD Phoenix™ system. However, in routine patient care, BD Phoenix™ susceptibility results are typically not repeated before being reported to clinicians. Repeated testing is performed using Etest® if an organism is ertapenem resistant and meropenem susceptible to verify the result, which is then reported. If CREs are identified, all carbapenem susceptibility reporting changes to document resistance. All MIC values are still reported. Therefore, the study design remains applicable to current clinical practice, with repeated testing performed by broth microdilution permitting more accurate reference MIC determination for each organism. Lastly, the BD Phoenix™ system was used to identify which isolates were carbapenem resistant for study inclusion. This was almost entirely determined by resistance to ertapenem, the most sensitive marker for carbapenemase production. This may have excluded isolates susceptible to ertapenem by BD Phoenix™ and resistant by BMD. This may further explain why ertapenem exhibited lower very major error rates relative to the other study antimicrobials.

The automated system BD Phoenix™ significantly overestimated susceptibility for CRE isolates compared to the gold-standard, BMD. This phenomenon was still observed when stratifying isolates by mechanism of carbapenem resistance. When compared to previous evaluations of automated susceptibility testing, BD Phoenix™ was associated with comparably elevated error rates for all tested antimicrobials, and only ertapenem exhibited low rates of VME. We agree with previous conclusions that secondary susceptibility testing methods should be utilized to verify antimicrobial activity against CRE. Further data is needed to assess the ability of BD Phoenix™ to accurately determine the MIC of highly resistant CRE isolates.

Table 4.3: Antimicrobial Susceptibility for CRE Isolates Using BMD and Phoenix by Drug Class (n=125)

Antimicrobial	Broth Microdilution Susceptibility	BD Phoenix™ Susceptibility	p-value	Categorical Agreement
Aminoglycosides				
Amikacin	84%	93%	**0.021**	91%
Gentamicin	35%	42%	**0.002**	66%
Tobramycin	16%	31%	**0.001**	71%
β-Lactam/ β-lactamase inhibitors				
Piperacillin-tazobactam	1%	3%	0.125	95%
Carbapenems				
Ertapenem	2%	1%	1	94%
Meropenem	18%	37%	**0.002**	50%
Cephalosporins				
Cefepime	5%	20%	**0.0001**	68%
Ceftazidime	1%	6%	**0.03**	85%
Fluoroquinolones				
Levofloxacin	15%	33%	**0.001**	61%
Monobactams				
Aztreonam	2%	12%	**0.003**	94%
Nitrofurans				
Nitrofurantoin	16%	36%	**0.0002**	63%

Susceptibility breakpoints determined by CLSI.[268]

Table 4.4: Antimicrobial Susceptibility for KPC-producing CRE Isolates Using BMD and Phoenix by Drug Class (n=71)

Antimicrobial	Broth Microdilution Susceptibility	BD Phoenix™ Susceptibility	p-value	Categorical Agreement
Aminoglycosides				
Amikacin	82%	89%	0.1306	89%
Gentamicin	25%	39%	0.5023	56%
Tobramycin	15%	30%	0.9609	73%
β-Lactam/ β-lactamase inhibitors				
Piperacillin-tazobactam	0%	0%	1	99%
Carbapenems				
Ertapenem	0%	0%	1	94%
Meropenem	6%	46%	0.0817	44%
Cephalosporins				
Cefepime	25%	5%	**0.0005**	65%
Ceftazidime	0%	0%	0.1310	85%
Fluoroquinolones				
Levofloxacin	6%	32%	**0.0002**	62%
Monobactams				
Aztreonam	0%	14%	**0.0094**	96%
Nitrofurans				
Nitrofurantoin	18%	35%	**0.0095**	61%

Susceptibility breakpoints determined by CLSI.[268]

Table 4.5: Antimicrobial Susceptibility for MBL-producing CRE Isolates Using BMD and Phoenix by Drug Class (n=21)

Antimicrobial	Broth Microdilution Susceptibility	BD Phoenix™ Susceptibility	p-value	Categorical Agreement
Aminoglycosides				
Amikacin	90%	100%	0.4795	90%
Gentamicin	14%	33%	0.1336	71%
Tobramycin	0%	5%	1	86%
β-Lactam/ β-lactamase inhibitors				
Piperacillin-tazobactam	0%	0%	1	100%
Carbapenems				
Ertapenem	0%	0%	1	100%
Meropenem	0%	19%	**0.0455**	71%
Cephalosporins				
Cefepime	0%	0%	1	95%
Ceftazidime	0%	0%	1	100%
Fluoroquinolones				
Levofloxacin	57%	33%	0.1820	48%
Monobactams				
Aztreonam	0%	48%	**0.0044**	52%
Nitrofurans				
Nitrofurantoin	10%	43%	**0.0233**	57%

Susceptibility breakpoints determined by CLSI.[268]

Table 4.6: Antimicrobial Susceptibility for Non-carbapenemase-producing CRE Isolates Using BMD and Phoenix by Drug Class (n=27)

Antimicrobial	Broth Microdilution Susceptibility	BD Phoenix™ Susceptibility	p-value	Categorical Agreement
Aminoglycosides				
Amikacin	100%	100%	1	100%
Gentamicin	33%	52%	0.0736	81%
Tobramycin	33%	52%	0.0771	56%
β-Lactam/ β-lactamase inhibitors				
Piperacillin-tazobactam	0%	15%	0.3711	74%
Carbapenems				
Ertapenem	7%	0%	1	85%
Meropenem	67%	22%	0.0817	44%
Cephalosporins				
Cefepime	4%	15%	0.0736	78%
Ceftazidime	4%	7%	0.1336	89%
Fluoroquinolones				
Levofloxacin	7%	33%	**0.0455**	63%
Monobactams				
Aztreonam	7%	11%	0.2482	81%
Nitrofurans				
Nitrofurantoin	19%	30%	1	81%

Susceptibility breakpoints determined by CLSI.[268]

Table 4.7: Error Rates for BMD vs. Phoenix™

Antimicrobial	MIC Range Category	Minor Errors	Major Errors	Very Major Errors
Amikacin	≥I+2	0%		100%
	I+1 to I-1	44%	0%	2%
	≤I-2	0%	13%	
Gentamicin	≥I+2	2%		20%
	I+1 to I-1	35%	11%	16%
	≤I-2	4%	4%	
Tobramycin	≥I+2	4%		11%
	I+1 to I-1	29%	0%	14%
	≤I-2	6%	11%	
Levofloxacin	≥I+2	4%		27%
	I+1 to I-1	22%	11%	30%
	≤I-2	15%	31%	
Nitrofurantoin	≥I+2	13%		10%
	I+1 to I-1	30%	2%	13%
	≤I-2	13%	0%	
Aztreonam	≥I+2	1%		10%
	I+1 to I-1	0%	0%	67%
	≤I-2	0%	100%	
Cefepime	≥I+2	8%		14%
	I+1 to I-1	83%	0%	0%
	≤I-2	0%	0%	
Ceftazidime	≥I+2	6%		5%
	I+1 to I-1	0%	33%	33%
	≤I-2	0%	0%	
Piperacillin-tazobactam*	$\geq I_{High}+2$	2%		2%
	$I_{High}+1$ to $I_{Low}-1$	25%	25%	25%
	$\leq I_{Low}-2$	0%	0%	
Ertapenem	≥I+2	3%		1%
	I+1 to I-1	33%	17%	0%
	≤I-2	0%	100%	
Meropenem	≥I+2	7%		39%
	I+1 to I-1	31%	12%	12%
	≤I-2	6%	63%	

I – Intermediate MIC

*Piperacillin-tazobactam has a lower and higher intermediate MIC range

Numbers in MIC range category column correspond to number of 2-fold serial dilutions

Polymyxin B Etest® Compared to Gold-standard Broth Microdilution in Carbapenem-resistant *Enterobacteriaceae* Exhibiting a Wide Range of Polymyxin B MICs

Published: *Clinical Microbiology and Infection.* 2019. 25(1): 92-95

Authors: Brandon Kulengowski, Julie A. Ribes, David S. Burgess

Kulengowski contribution: Experimental design, execution, data analysis, revisions and editing of final manuscript

Abstract

Objectives: Polymyxins have been revitalized to combat carbapenem-resistant *Enterobacteriaceae* (CRE). However, evaluating the activity of these agents by traditional broth dilution methods is not practical for busy clinical laboratories. We compared polymyxin B (PMB) activity utilizing two quantitative susceptibility testing methods, Etest® and broth microdilution (BMD), against CRE isolates from patients at an academic medical center.

Methods: PMB activity against 70 CRE clinical isolates was determined by Etest® according to manufacturer and by BMD according to CLSI guidelines. *P. aeruginosa* ATCC® 27853 and *E. coli* NCTC 13846 served as quality control strains. The EUCAST colistin susceptibility breakpoint of *Enterobacteriaceae* (≤2 mg/L) was used. Essential agreement was isolates with an MIC within 1 $\log_2$ dilution over total isolates. Categorical agreement was number of isolates in the same susceptibility category (susceptible or resistant) over total isolates. Major and very major error rates were calculated using number of susceptible and number of resistant isolates, respectively, as the denominator. McNemar's test was used for determining a difference in susceptibility between methods.

Results: CRE isolates were primarily *Klebsiella spp.* (49%) and *Enterobacter spp.* (36%). PMB susceptibility was significantly higher by Etest® compared to BMD (97% vs. 77%; P=0.0001). Categorical agreement was 80%, but essential agreement was low (10%). False non-susceptibility was never observed by Etest® (BMD reference), but the very major errors were high (88%).

Conclusions: Etest® reporting of false susceptibility may result in inappropriate antibiotic utilization and treatment failure clinically. We do not recommend using Etest® for PMB susceptibility testing for routine patient care.

Introduction

The polymyxins are an older class of antimicrobials from the 1950s reemerging for the treatment of multidrug resistant infections such as those caused by carbapenem-resistant *Enterobacteriaceae* (CRE).[131] Infections caused by CRE are reported worldwide and led to significant mortality, often requiring antimicrobial combinations for effective treatment.[274] Polymyxin B (PMB) and colistin (CST) cause significant neurotoxicity and nephrotoxicity, and so their use was sparse until the era of CRE infections.[310] As a result, these older agents are usually not available on standard susceptibility testing panels of automated systems such as Vitek®, MicroScan®, and Phoenix™. Hence, many clinical laboratories have utilized Etest® strips as a rapid alternative given traditional laboratory-prepared broth dilution methods are too laborious for routine use in clinical laboratories.

There exist, however, several concerns with *in vitro* susceptibility testing of PMB and CST. Principally, there are conflicting data reported in the literature regarding proper antimicrobial susceptibility testing procedures.[267] There have also been disputes regarding the most appropriate reference standard for comparison as well as many limitations in modern studies around testing populations that fully represent the spectrum of polymyxin MICs (very susceptible to very resistant).[311] Heteroresistance within bacterial isolates renders obtaining reproducible susceptibility information difficult as well.[140] Lastly, even

if MIC information is obtained, one value may not appropriately describe populations that exhibits heteroresistance.

CLSI and EUCAST have jointly released a recommendation regarding the use of broth microdilution (BMD) as the reference standard, but these organizations have been unable to recommend other methods (e.g. agar dilution, disk diffusion, and gradient diffusion) until new study data have been generated.[267] The aim of this study was to compare Etest® to broth microdilution using the new CLSI/EUCAST standards in a CRE population.[267]

Methods

Bacterial isolates. 70 non-duplicate clinical isolates that were non-susceptible to any of the carbapenems were collected from a tertiary academic medical center during 2010 to 2016. Isolates were stored at -80˚C in 10% glycerol stocks and subcultured twice before testing. *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* NCTC 13846 served as susceptible (0.5 – 2 mg/L) and non-susceptible (4 – 8 mg/L) controls, respectively.

Antimicrobial Powder. Polymyxin B sulfate was obtained from Sigma-Aldrich (St. Louis, MO). Certificates of analysis were used to determine potency of antimicrobials.

Broth microdilution. BMD was conducted in duplicate on at least 2 separate occasions according to CLSI guidelines using cation-adjusted Mueller-Hinton broth (BBL-Becton Dickinson, Sparks, MD).[267,312] The modal MIC was accepted, or a third replicate experiment was conducted for discordant MICs near the breakpoint (e.g. 1, 2, and 4 mg/L) or for MICs greater than 1 $\log_2$ dilution apart. The higher MIC was accepted for other cases

where there was no mode (e.g. one experiment measuring 0.25 mg/L and a second measuring 0.5 mg/L would be reported as 0.5 mg/L).

Etest®. Polymyxin B Etest® (bioMérieux Inc., Durham, NC) on Mueller-Hinton agar (BBL-Becton Dickinson, Sparks, MD) was performed according to manufacturer's protocol on at least 2 separate occasions. A similar approach as BMD MIC interpretation was taken for Etest® results.

Analysis. All Etest® intermediate MICs were first rounded up to the nearest MIC measurable by BMD (e.g. 0.75 was rounded to 1, 3 was rounded to 4, etc.). Descriptive statistics were calculated for BMD and Etest® – percentage susceptible (%S) using EUCAST susceptibility breakpoints for colistin in *Enterobacteriaceae*, MIC_{50}, MIC_{90}, and MIC range. Comparative statistics were calculated for BMD and Etest®. The rate of essential agreement (EA) was defined as the ratio of the percentage of isolates with a BMD and Etest® MIC within 1 $\log_2$ dilution to the total number of isolates (n=70). The rate of categorical agreement (CA) for the test systems was defined as the ratio percentage of isolates with the same susceptibility category (susceptible vs. resistant) reported by both methods to the total number of isolates (n=70). The rate of major errors (MEs) was defined as the ratio percentage of isolates reported resistant by Etest® but susceptible by BMD to the number of isolates susceptible by BMD. Very major error (VME) rate was defined as the ratio percentage of isolates reported susceptible by Etest® but resistant by BMD to the number of resistant isolates by BMD.[270] MIC distributions were determined for each test method (Figure 4.1). Etest® MIC vs. BMD MIC data were plotted (Figure 4.2). McNemar's test was utilized to compare paired susceptibility data between testing methods.

Results

Isolates were predominately *Klebsiella spp.* (34/70; 49%), followed by *Enterobacter spp.* (25/70; 36%), *Escherichia coli* (5/70; 7%), *Citrobacter spp.* (5/70; 7%), and *Providencia rettgeri* (1/70; 1%). PMB susceptibility, MIC_{50}/MIC_{90}, MIC range, EA, CA, and error rates are presented in Table 4.8. Overall susceptibility measured by Etest® was significantly higher than susceptibility measured by BMD (P=0.0001). BMD isolates generally exhibited a wider range of MICs (Figure 4.1 and Figure 4.2), supported also by comparing the MIC_{50}/MIC_{90} ratios (Table 4.8). The categorical discordance between BMD and Etest® was exclusively very major errors. Roughly 90% isolates with PMB MICs >2 mg/L by BMD were reported as ≤2 mg/L by Etest® (Table 4.8). Etest® correlated poorly with BMD, generally overestimating the MIC when BMD MICs were <0.5 mg/L, but underestimating the MIC when BMD MICs were >1 mg/L (Figure 4.2). For BMD, *P. aeruginosa* 27853 ranged 0.5 – 1 mg/L. *E. coli* NCTC 13846 ranged 4 – 8 mg/L. For Etest®, *P. aeruginosa* 27853 measured 0.5 mg/L. *E. coli* NCTC 13846 measured 4 mg/L.

Discussion

Summary of principal findings

In a sizeable sampling (n=70) of CRE clinical isolates consisting primarily of *Klebsiella spp.* and *Enterobacter spp.*, we found considerable discordance between Etest® and BMD (EA 10%, CA 80%, VME 88%). Most importantly, Etest® poorly predicted the polymyxin B MIC for isolates exhibiting elevated PMB MICs by BMD when utilizing the recommendations of CLSI/EUCAST for BMD susceptibility testing.[267]

Findings of the present study relative to previous studies

A recent review of polymyxin susceptibility testing has analyzed susceptibility studies for PMB and CST.[313] Although CST has been studied frequently, only two other studies have evaluated PMB in a CRE population comparing Etest® to BMD as a reference, but with different conclusions regarding which method was more sensitive to polymyxin resistance.[314,315] Other insights have come from Rojas et al. who used colistin Etest® to compare with polymyxin B broth microdilution.[316] Lat et al. found PMB Etest® overestimates resistance relative to BMD whereas the other two studies were consistent with the present findings – PMB Etest® underestimates resistance. Percent susceptible was grossly similar, ranging 64-87% vs. present study 77%. Interestingly, very major error rates for PMB have ranged from 14 to 35% whereas we report the highest VME to date of 88%. The highest reported CST VME rate was 46%.[317] A Chi-squared comparison between Rojas et al. VME rate (35%, n=25) vs. the present study (88%, n=16) was P=0.001.[316] Discrepancies between our data and previous findings could be explained by the rigor with which PMB MICs were ascertained or interpreted because skip wells are frequent.[318] Other explanations include differences in regional resistance mechanisms (e.g. presence of mcr-1). Chew et al. found mcr-1 in a majority of resistant isolates whereas Rojas et al. found none. We did not test for mcr-1, and other polymyxin resistance mechanisms are not as easy understood, but they are considered to involve the PhoPQ regulatory system.[314]

Strengths and limitations of the study

Strengths of the present study include the use of clinical isolates from a tertiary academic medical center where patients come from all over the state, a varied representation of MICs across the susceptible-resistant spectrum, use of standardized laboratory methods performed in duplicate on at least two different days, and literature support of findings from other laboratory groups. Limitations of the present study include that the population is exclusively CRE organisms, limited quantities of isolates with polymyxin B MICs >2 mg/L (n=16), single-center, lack of genetic data regarding the mechanisms of resistance to carbapenems or polymyxin B, and unavailability of clinical outcomes data. Additionally, this study is limited in its ability to ascertain assay variability with only duplicate, seldom triplicate MIC measurements. The dearth of pharmacokinetic/pharmacodynamic and clinical outcomes data for establishing a polymyxin B susceptibility breakpoint limited this study to using the susceptibility breakpoint for colistin. Finally, this study lacked sufficient quantities of individual species of CRE to perform sufficiently powered species subgroup analyses.

Understanding possible mechanism

We propose that the mechanism behind the discrepancy we and others have observed between PMB Etest® and BMD may be explained if BMD is more sensitive to heteroresistant subpopulation growth than Etest®. For example, if a few colonies are growing inside the Etest® zone of inhibition but are not close enough to the Etest® strip to confound the interpretation, then this may suggest the presence of heteroresistance but at too small of a quantity to reliably alter the Etest® interpretation. Consider also when these

heteroresistant colonies are instead in one of the microtiter wells. They may grow in such an environment to turn the wells turbid and result in an elevated PMB MIC. Differences in heteroresistance rate would then explain very major error differences between our study and previous studies. To date, no study has been published that describes such a mechanism, and so additional data are needed.

Implications for practice and future research

Although BMD is considered the standard by CLSI/EUCAST,[267] alternative antimicrobial susceptibility testing methodologies conducive to clinical laboratory workflow are and should continue to be explored such as automated systems, Sensititre®, and others.[313] However, our data do not support the continued use of Etest® strips for determining PMB MICs in CRE populations.

Table 4.8: Comparison of BMD and Etest® for Polymyxin B against 70 CRE Clinical Isolates

	Polymyxin B BMD	Polymyxin B Etest®	P-value
%S	77%	97%	0.0001
MICR_{50}	0.125	0.5	
MICR_{90}	32	1	
MIC range	≤0.06 – >64	0.125 – 1024	
EA	10%		
CA	80%		
VME	88%		
ME	0%		

%S – susceptible percentage; MIC_x – The lowest concentration of antimicrobial at which X% of isolates were inhibited; EA – Essential Agreement; CA – Categorical Agreement; VME – Very Major Error; ME – Major Error

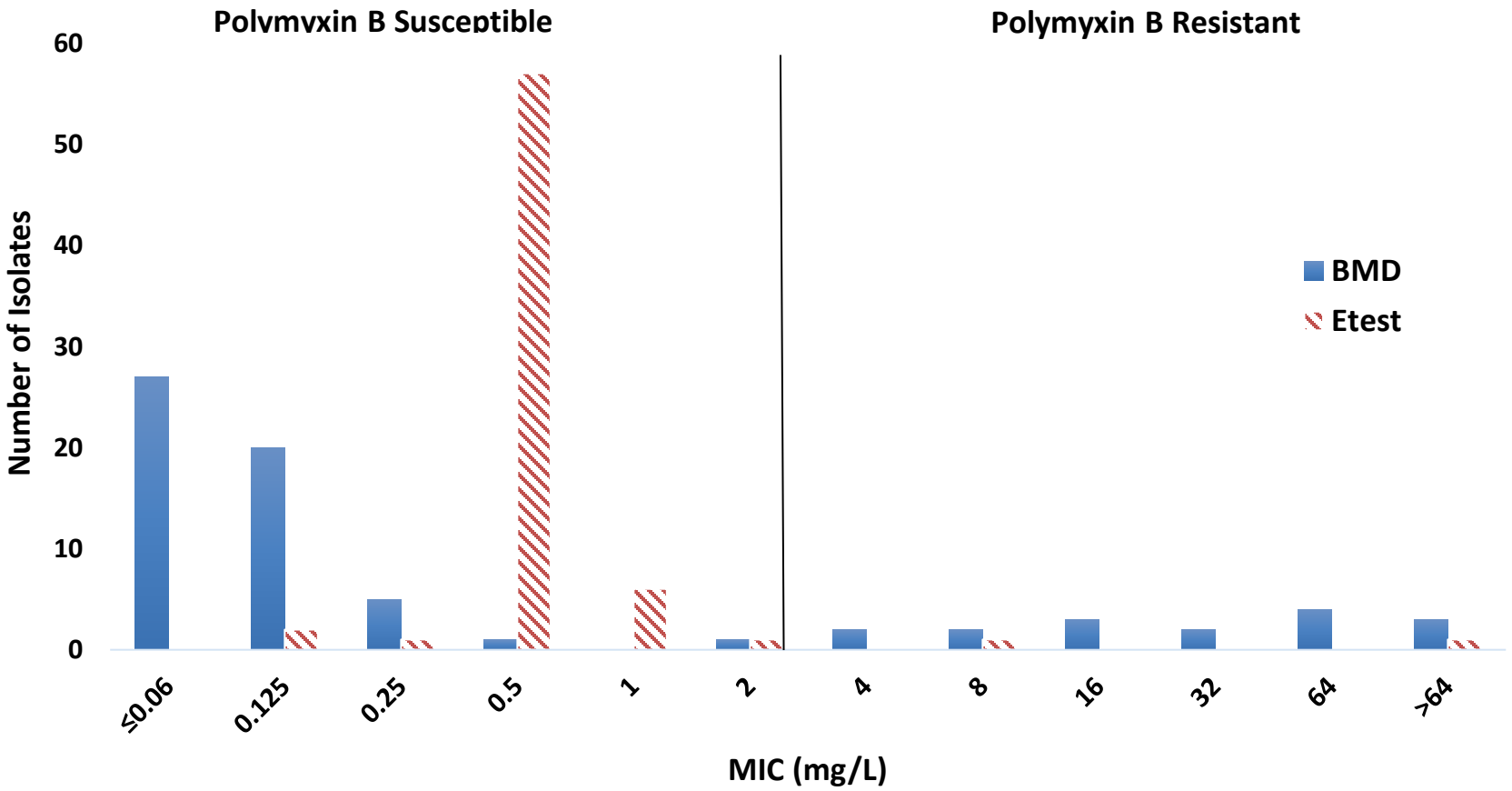


Figure 4.1: Comparison of Broth Microdilution and Etest® MIC Distributions of Polymyxin B against 70 Carbapenem-resistant *Enterobacteriaceae*. MIC – minimum inhibitory concentration. BMD – Broth microdilution. BMD measurable range 0.125 – 64. Etest® MIC values rounded up to nearest measurable MIC by BMD.

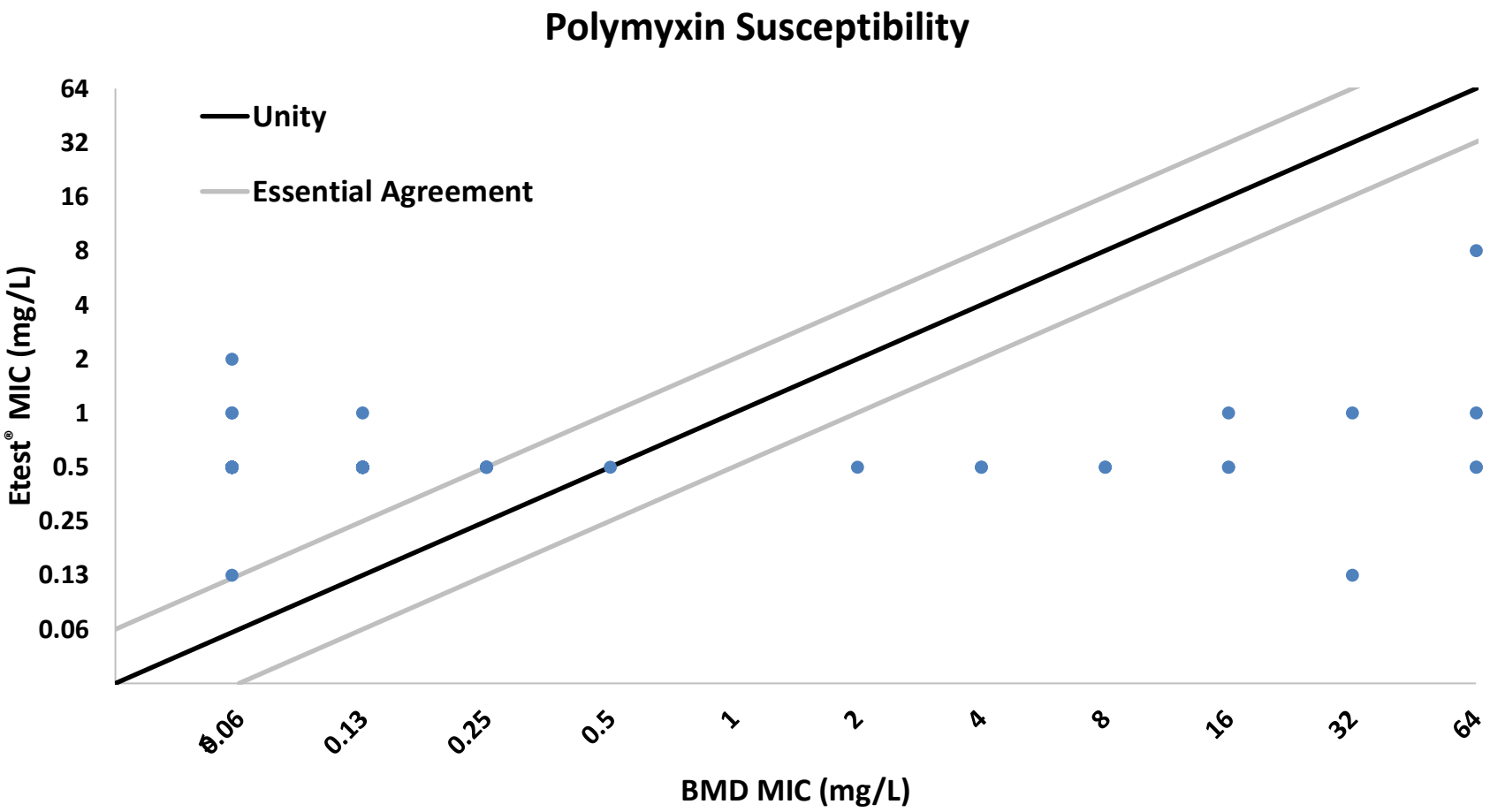


Figure 4.2: Minimum Inhibitory Concentrations by Etest® and Gold-standard Broth Microdilution. BMD – broth microdilution. N=70 CRE clinical isolates. Black line indicates equivalent MIC measurements between testing methods. Grey line indicates ±1 $\log_2$ dilution agreement between testing methods. Note that any one circle may represent multiple overlapping data points.

Antimicrobial Activity against Carbapenem-resistant *Enterobacteriaceae* that Produce Verona Integron-encoded Metallo-beta-lactamase or *Klebsiella pneumoniae* Carbapenemase

Submitted: *Journal of Antimicrobial Chemotherapy* as a brief report

Authors: Brandon Kulengowski, Justin A. Clark, David S. Burgess

Kulengowski contribution: Experimental design, execution, data analysis, drafting, revisions and editing of final manuscript

Abstract

Background: Metallo-beta-lactamase- (MBL-) producing carbapenem-resistant *Enterobacteriaceae* (CRE) are becoming more prevalent in the United States. Identifying differences in resistance phenotypes will help clinicians determine appropriate antimicrobial therapy against organisms that produce MBLs. With genotypic rapid diagnostics, carbapenemase phenotype is often known before susceptibility data.

Objectives: To ascertain differences in antimicrobial activity among KPC- and VIM-producing CRE.

Methods: Commonly utilized antimicrobials (n=16) were tested by broth microdilution according to CLSI guidelines against 92 carbapenemase-producing CRE. Antimicrobial activity was compared between VIM- (n=20) and KPC-producing (n=72) CRE using Fisher's exact test.

Results: Polymyxin B, colistin, and levofloxacin exhibited significantly higher susceptibility against VIM-producing CRE than KPC-producing CRE. Gentamicin and tobramycin, but not amikacin, exhibited significantly higher susceptibility against KPC-producing CRE than VIM-producing CRE.

Conclusions: Carbapenemase phenotype among CRE should direct clinician choice of antimicrobial agents. VIM-producing CRE in the United States were more susceptible to polymyxins and fluoroquinolones than KPC-producing CRE.

Introduction

Carbapenem-resistant *Enterobacteriaceae* (CRE) are a growing problem in the setting of few active antimicrobial options and relatively high mortality (50%).[14] A one-size-fits-all treatment objective for CRE is naively optimistic, so an approach focused instead on the nuanced resistance phenotypes among CRE is ideal, particularly given the variability associated with CRE susceptibility profiles.[205] In the U.S., *Klebsiella pneumoniae* carbapenemases (KPCs) represent the majority of CRE resistance phenotypes followed by New Delhi metallo-beta-lactamase (NDM) and then other metallo-beta-lactamases (MBLs) according to the Centers for Disease Control and Prevention.[79] However, other antimicrobial resistance genes are frequently transferred on the same plasmids carrying carbapenemase genes which confer resistance to other antimicrobial agents.[14] Furthermore, interpretation of antimicrobial resistance genotyping is confounded by inactive but present genes, since constitutive production of enzymes or other resistance mechanisms is often associated with a fitness cost to the organism.[289] Therefore, phenotypic antimicrobial activity profiles are necessary to evaluate differences in CRE sub-groups, such as CRE that produce KPCs compared to CRE that produce MBLs.

Since a majority of CRE in the United States produce only KPC, most U.S. *in vitro* studies have focused on KPC-producing CRE. In fact, the FDA recently approved ceftazidime/avibactam and meropenem-vaborbactam for use against CRE, but neither exhibit activity against MBL producing CRE.[14] Plazomicin is a novel aminoglycoside with activity against CRE, but methyltransferases are associated with reduced activity and were subsequently found to be associated with MBL production.[319] In response to a call for additional *in vitro* data with MBL-producing CRE, this study aimed to evaluate differences

in susceptibility of commonly utilized antimicrobial against carbapenem-resistant *Enterobacteriaceae* that produce VIM.[214]

Materials and Methods

CRE isolates (n=92), defined as *Enterobacteriaceae* resistant to at least one carbapenem, from January 1, 2012 through January 1, 2017 were obtained from the University of Kentucky HealthCare System, a tertiary referral academic medical center. Susceptibility testing for ertapenem and meropenem were performed by the automated testing system, BD Phoenix™, as part of routine patient care and used to identify study isolates. MBL genotyping was also performed by the Verigene® system as part of routine patient care which identified 20 VIM producing isolates. All 92 isolates also underwent duplicate separate day phenotyping experiments for KPC and/or MBL production according to a previously published EDTA/phenylboronic acid disk diffusion protocol.[266] Subsequent susceptibility testing was performed on CRE by broth microdilution according to CLSI guidelines.[268] Susceptibility testing was performed in duplicate on at least two separate days, accepting the modal MIC. If no modal MIC was identified, the higher of the MICs was accepted. If the accepted higher MIC was within one two-fold dilution of an interpretive boundary, a third experiment was performed. Antimicrobial agents (n=16) tested include: ertapenem, meropenem, imipenem, amikacin, gentamicin, tobramycin, colistin, polymyxin B, piperacillin-tazobactam, aztreonam, cefepime, ceftazidime, minocycline, tigecycline, levofloxacin, and nitrofurantoin. Tazobactam was tested at a fixed dose of 4 mg/L. All powders were obtained from Sigma-Aldrich (St. Louis, MO,

USA). Because susceptibility breakpoints for polymyxin B and colistin are not available for *Enterobacteriaceae*, the EUCAST susceptibility breakpoint for colistin of 2 mg/L was used for both antimicrobials.

Results and Discussion

A total of 72 KPC-producing CRE and 20 VIM-producing CRE were obtained as clinical isolates from UK HealthCare and were confirmed by the previously mentioned EDTA/phenylboronic acid diffusion assay. *Klebsiella pneumoniae* composed most of the isolates (n=44; 48%), followed by *Enterobacter spp.* (n=32; 35%), *Citrobacter spp.* (n=8; 9%), other *Klebsiella spp.* (n=4; 4%), *Escherichia coli* (n=3; 3%), and *Serratia marcescens* (n=1; 1%). As displayed in Table 4.9, KPC-producing isolates were most susceptible to tigecycline (83%), amikacin (81%), and the polymyxins (64%). VIM-producing isolates exhibited improved susceptibility to the polymyxins (95%), tigecycline (90%), and amikacin (90%), but only the polymyxin class was statistically significant (P=0.003). Levofloxacin was also significantly more active against VIM-producing CRE than KPC-producing CRE (60% vs. 6%; $p < 0.0001$; Table 4.9). Gentamicin and tobramycin, but not amikacin, were significantly more active against KPC-producing CRE than VIM-producing CRE (Table 4.9), but the clinical relevance of this observation is diminished by the overall relatively low activity of either agent (<40%).

Differences in antimicrobial activity may be explained by differences in the plasmids carrying VIM compared to those carrying KPC since neither VIM nor KPC confer resistance to aminoglycosides, polymyxins, glycylcyclines, or fluoroquinolones on their

own. However, phylogenetic analysis and multilocus sequence typing was not possible since complete genomic data were not available on this collection of isolates. These data, in conjunction with clinical data, support the use of alternative agents like ceftazidime/avibactam and meropenem-vaborbactam whose activity against KPC-producing isolates is reportedly higher and associated with superior clinical outcomes.[257,320,321] However, as mentioned previously, neither of these newer agents are active against MBL-producing isolates like the CRE in the present study that produce VIM. Instead, our data suggest that amikacin, tigecycline, and the polymyxins are most active against these isolates, but we would recommend their use in combination, most likely with a carbapenem, based on other studies.[322] A limitation of the present study is the data comes from a single center. However, the isolates in this study come from the surrounding Kentucky communities, and other U.S. centers combined have not reported as many VIM-producing CRE as Kentucky according to the CDC.[79] Additional data are also warranted with plazomicin and eravacycline, whose CRE activity is not directly impacted by MBL-production, but other resistance genes may have been transferred on plasmids. Additionally, aztreonam/avibactam offers promise against MBL-producing isolates because aztreonam is not inhibited by MBLs and avibactam inhibits the other serine based carbapenemases like KPCs and some OXA-like carbapenemases.[323]

Antimicrobial susceptibility patterns vary considerably by region, so clinicians should be familiar with their local antibiograms.[14] Furthermore, genotypic/phenotypic information should direct clinical decision-making as to which antimicrobials are most appropriate to combat infections caused by organisms like CRE, especially given the present findings of susceptibility differences with antimicrobials like the aminoglycosides

and polymyxins against VIM- and KPC-producing CRE. Specifically, the polymyxins and levofloxacin exhibited superior activity whereas gentamicin and tobramycin exhibited reduced activity against VIM-producing CRE when compared to KPC-producing CRE.

Table 4.9: Cumulative Antimicrobial Susceptibility Data for Carbapenem-resistant *Enterobacteriaceae* that produce KPC or VIM

Antimicrobial	KPC (n=72) MIC_{50} / MIC_{90} (mg/L)	VIM (n=20) MIC_{50} / MIC_{90} (mg/L)	KPC (n=72) Susceptible (%)	VIM (n=20) Susceptible (%)	P-value
AMINOGLYCOSIDES					
Amikacin	4 / 32	8 / 16	81	90	0.18
Gentamicin	32 / 128	16 / 128	35	15	**0.05**
Tobramycin	32 / 128	16 / 64	19	0	**0.02**
β-LACTAM/β-LACTAMASE INHIBITOR					
Piperacillin/tazobactam	>512/4 / >512/4	>512/4 / >512/4	0	0	1
β -LACTAMS					
Imipenem	16 / 128	32 / 64	6	0	0.37
Meropenem	32 / 128	16 / 32	6	0	0.37
Ertapenem	64 / >128	8 / 64	0	0	1
Cefepime	256 / >256	128 / 256	3	0	0.61
Ceftazidime	512 / >512	>512 / >512	0	0	1
CYCLINES					
Tigecycline	2 / 4	1 / 2	83	90	0.23
Minocycline	16 / 64	16 / 32	17	25	0.17
FLUOROQUINOLONES					
Levofloxacin	32 / >32	2 / 8	6	60	**<0.0001**
MONOBACTAMS					
Aztreonam	512 / >512	128 / 256	0	0	1
NITROFURANS					
Nitrofurantoin	128 / 256	64 / 256	18	10	0.2
POLYMYXINS					
Colistin[#]	0.125 / >64	≤0.06 / 0.125	64	95	**0.004**
Polymyxin B[#]	0.25 / >64	≤0.06 / 0.25	64	95	**0.004**

Susceptibility breakpoints were determined by CLSI 2018 criteria[268]
[#]The EUCAST 2018 colistin susceptibility breakpoint of ≤2 mg/L was used for polymyxin B and colistin because there is not a CLSI breakpoint established for *Enterobacteriaceae*[281]

Imipenem/relebactam Activity Compared to Commonly Utilized Antimicrobials against Carbapenem-resistant *Enterobacteriaceae* from an Academic Medical Center

Submitted: *Journal of Global Antimicrobial Resistance* as a short communication

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Kulengowski contribution: Experimental design, execution, data analysis, drafting, revisions and editing of final manuscript

Abstract

Objectives: Carbapenem-resistant *Enterobacteriaceae* cause significant mortality (50%) and are resistant to nearly all known antimicrobial agents. Imipenem/relebactam, a novel beta-lactam/beta-lactamase inhibitor combination, and 16 other antimicrobials were evaluated against non-MBL-producing carbapenem-resistant *Enterobacteriaceae* (CRE) clinical isolates from a United States tertiary academic medical center.

Methods: Clinical isolates (n=96) resistant to ertapenem or meropenem by BD Phoenix™ and negative for metallo-beta-lactamase- (MBL-) production by an EDTA/phenylboronic acid disk diffusion assay were identified and collected from January 2012 to January 2017. *In vitro* susceptibility by broth microdilution was performed according to CLSI guidelines for 17 antimicrobials.

Results: CRE consisted primarily of *K. pneumoniae* (55%) and *Enterobacter spp.* (25%), followed by *Citrobacter spp.* (10%), *E. coli* (5%), and others (5%). CRE were most susceptible to imipenem/relebactam (100%), followed by amikacin (85%), tigecycline (82%), and polymyxin B/colistin (65%). The median reduction of imipenem MICs of non-MBL-producing CRE was 16-fold, but ranged from 0.5 to >512-fold. The MIC_{50}, MIC_{90}, and MIC range of imipenem/relebactam was 0.5 mg/L, 1 mg/L, and 0.06 – 1 mg/L, respectively.

Conclusions: Imipenem/relebactam exhibits excellent activity against non-MBL-producing CRE.

Keywords: Carbapenem-resistant *Enterobacteriaceae*; imipenem/relebactam; antimicrobial susceptibility testing; KPC; MBL

Introduction

The optimal treatment for infections caused by carbapenem-resistant *Enterobacteriaceae* (CRE) is not yet established. The greatest factor contributing to this gap in clinical knowledge is the lack of randomized controlled trials concerning CRE, but there are also other confounding variables that render meta-analyses and case studies ineffectual for generalization such as complex and myriad possible antimicrobial combinations. Heterogeneity among treatment regimens and differences among CRE populations, even among institutions within similar geographical regions, impact clinical outcomes.[205] As a result, *in vitro* and animal studies have become critically important for evaluating CRE treatment, for which a carbapenem in combination with a polymyxin have, generally, been the front-runner in the United States. However, analysis of certain subgroups within CRE have suggested that even this combination has pitfalls, specifically when carbapenem MICs exceed 8 mg/L.[204] Furthermore, in an age where the importance of precision medicine is realized, perhaps a generalized solution to a diverse problem will always be unsatisfactory.

The concern for CRE is justifiably growing as antimicrobial resistance rates continue to rise.[11] KPC enzymes comprise the major carbapenem resistance mechanism in the United States, followed by NDM, OXA-48, VIM, and IMP in order of decreasing prevalence.[324] Other resistance mechanisms such as a cephalosporinase in combination with a porin channel mutation or efflux gene also affect the United States.[14] Therefore, it is critical that reports of local and regional resistance patterns continue to be published to best track the epidemiology of CRE and to develop optimized treatment strategies to combat these organisms. Most literature from the United States has been from the New

England area where CRE have had the greatest impact, with resistance rates reported up to 40% in some hospitals.[122] At our institution, we observe CRE rates <5%. The objective of this study was to first describe the CRE population reflective of a tertiary referral academic medical center located in southeastern United States, and then to compare the activity of a novel antimicrobial, imipenem/relebactam (IMI/REL), with 16 other antimicrobials. Relebactam is a non-beta-lactam, bicyclic diazabicyclooctanase, beta-lactamase inhibitor with activity against Ambler class A and C beta-lactamases. Relebactam recently completed phase 3 clinical studies combined with imipenem/cilastatin (ClinicalTrials.gov. NCT02452047). Other than Class B (i.e., metallo-beta-lactamases,) and Class D (i.e., OXA) inactivation of the OmpK36 porin protein has been reported to confer resistance to imipenem/relebactam.[325]

Material and Methods

CRE isolates from January 1, 2012 through January 1, 2017 were obtained from the University of Kentucky HealthCare System. Susceptibility testing for commonly utilized antimicrobials was performed by the automated testing system, BD Phoenix™, as part of routine patient care. CRE isolates also underwent duplicate separate day phenotypic experiments for *Klebsiella pneumoniae* carbapenemase (KPC) and/or metallo-β-lactamase (MBL) production according to a previously published EDTA/phenylboronic acid disk diffusion protocol.[266] Subsequent susceptibility testing was performed on non-MBL producing CRE by broth microdilution according to CLSI guidelines.[268] Susceptibility testing was performed in duplicate on at least two separate days, accepting the modal MIC.

If no modal MIC was identified, the higher of the MICs was accepted. If the accepted higher MIC was within one two-fold dilution of an interpretive boundary, a third experiment was performed. Antimicrobial agents tested include: imipenem/relebactam, ertapenem, meropenem, imipenem, amikacin, gentamicin, tobramycin, colistin, polymyxin B, piperacillin-tazobactam, aztreonam, cefepime, ceftazidime, minocycline, tigecycline, levofloxacin, and nitrofurantoin. All inhibitors were tested at a fixed dose of 4 mg/L. Imipenem and relebactam powders were obtained from Merck & Co., Inc (Kenilworth, NJ, USA). All other powders were obtained from Sigma-Aldrich (St. Louis, MO, USA). Because susceptibility breakpoints have yet to be determined for IMI/REL, the imipenem susceptibility breakpoint (1 mg/L) was utilized.

Results

In total, 500 multidrug resistant gram-negative bacteria were collected from UK HealthCare, with 96 *Enterobacteriaceae* being resistant to at least one carbapenem tested (meropenem or ertapenem) and lacking production of MBL (Class B) enzymes. We found *K. pneumoniae* (55%) and *Enterobacter spp.* (25%) to be the predominant CRE, followed by *Citrobacter spp.* (10%), *E. coli* (5%), other *Klebsiella* spp. (4%), and *S. marcescens* (1%). Patients were predominantly white, above the age of 50, and in the ICU at the time of culture (Table 4.10). The most active antimicrobials against CRE were IMI/REL (100%), amikacin (85%), tigecycline (84%), and the polymyxins (65%) (Table 4.11). For most isolates, relebactam significantly enhanced the activity of imipenem, but the degree of change in activity varied (0.5 to >512-fold reduction in MIC). The distribution of

imipenem/relebactam MICs tightly clustered around 0.25-1 mg/L whereas imipenem alone was approximately evenly distributed from 1-64 mg/L (Table 4.12).

Discussion

In a large population of non-MBL CRE clinical isolates (n=96) primarily composed of *Klebsiella* spp. and *Enterobacter* spp., we found excellent restored activity of imipenem when relebactam (at a fixed concentration of 4 mg/L) was added against non-MBL producing CRE (from 23% to 100%; n=96). Importantly, imipenem/relebactam exhibited better activity than other commonly utilized antimicrobials to treat CRE, including amikacin (85%), tigecycline (82%), and the polymyxins (65%) (Table 4.11). Our findings are consistent with other reports of improved imipenem activity when relebactam is added.[326-329] Strengths of the present study include analyzing a sizeable population of carbapenem-resistant *Enterobacteriaceae*, collecting clinical isolates from a large referral academic medical center, and utilizing a robust design of gold-standard broth microdilution for susceptibility testing in double replicate sometimes triple replicate. Limitations, like other reports, include that isolates primarily come from nearby communities rather than a multi-center or international collection of isolates and that genetic information is unavailable on all of these isolates.

Carbapenem-resistant *Enterobacteriaceae* populations are not sufficiently described by nationally or internationally observed antimicrobial activity, but rather, regional susceptibility data needs to be reported to improve epidemiological study and rational therapeutic decision-making in the clinical setting. For example, we have found a

population of non-MBL producing CRE in the southeastern United States exhibiting relatively high polymyxin MICs, but for which IMI/REL activity is excellent (100%).

Conclusions

Imipenem/relebactam exhibits excellent activity against non-MBL producing CRE. Our data support the continued development of imipenem/relebactam and its use against carbapenem-resistant *Enterobacteriaceae*.

Table 4.10: Patient Demographics for Non-MBL-producing Carbapenem-resistant *Enterobacteriaceae* (n=96) Collected from January 2012 – January 2017

Patient Demographics	
Age	
Mean	56 yrs
Median	57.4 yrs
Range	3 wks – 96 yrs
Sex	
Male	56%
Female	44%
Ethnicity	
White	95%
African-American	4%
Asian	1%
ICU Status	
Any ICU visit during stay	74%
Culture drawn in ICU	72%

Table 4.11: Cumulative Antimicrobial Susceptibility for Non-MBL-producing Carbapenem-resistant *Enterobacteriaceae* (n=96)

Antimicrobial	Susceptibility Breakpoint (mg/L)	Susceptible (%)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Range (mg/L)
AMINOGLYCOSIDES					
Amikacin	≤ 16	85	4	32	≤0.5-64
Gentamicin	≤ 4	34	32	128	≤0.125->128
Tobramycin	≤ 4	23	16	128	≤0.125->128
β-LACTAM/ β-LACTAMASE INHIBITOR					
Imipenem/relebactam	≤ 1/4*	100	0.5	1	0.06-1
Piperacillin/tazobactam	≤ 16/4	0	>512	>512	32->512
β -LACTAMS					
Imipenem	≤ 1	23	8	128	0.25->128
Meropenem	≤ 1	21	16	128	≤0.125->128
Ertapenem	≤ 0.5	0	64	128	1->128
Cefepime	≤ 2	2	256	>256	1->256
Ceftazidime	≤ 4	0	512	>512	8->512
CYCLINES					
Tigecycline	≤ 2	82	2	4	0.06-16
Minocycline	≤ 4	16	16	>64	0.25->64
FLUOROQUINOLONES					
Levofloxacin	≤ 2	5	32	>32	0.06->32
MONOBACTAMS					
Aztreonam	≤ 4	1	512	>512	4->512
NITROFURANS					
Nitrofurantoin	≤ 32	18	256	256	2-512
POLYMYXINS					
Colistin	≤ 2[#]	65	0.25	>64	≤0.06->64
Polymyxin B	≤ 2[#]	65	0.125	>64	≤0.06->64

Susceptibility breakpoints were determined by CLSI 2018 criteria except for imipenem/relebactam where the imipenem breakpoint of ≤1 mg/L was utilized

*CLSI *Enterobacteriaceae* breakpoints for imipenem were utilized (susceptible, 1 mg/L; intermediate, 2 mg/L; resistant, 4 mg/L)[268]

[#]The EUCAST 2018 colistin susceptibility breakpoint of ≤2 mg/L was used for polymyxin B and colistin because there is not a CLSI breakpoint established for *Enterobacteriaceae*.

Table 4.12: MIC Frequency Distribution of Carbapenem-resistant *Enterobacteriaceae* (n=96)

MIC (mg/L)	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>*
AMINOGLYCOSIDES													
Amikacin	-	-	-	3	19	22	18	4	16	11	3	0	0
Gentamicin	-	6	8	3	0	6	10	6	6	19	19	10	3
Tobramycin	-	3	7	5	2	1	4	10	19	20	11	6	8
BL/BLI													
Imipenem/relebactam	2	7	28	28	31	0	0	0	0	0	-	-	0
Piperacillin/tazobactam	-	-	-	0	0	0	0	0	0	1	1	0	94
β -LACTAMS													
Imipenem	-	0	4	6	12	7	12	11	14	9	11	6	4
Meropenem	-	1	7	6	6	6	8	5	10	17	18	10	2
Ertapenem	-	0	0	0	1	2	9	13	7	13	19	23	9
Cefepime	-	-	0	0	1	1	2	0	5	10	11	14	52
Ceftazidime	-	-	-	0	0	0	0	1	1	1	1	7	85
CYCLINES													
Tigecycline	1	0	1	8	28	41	10	4	3	0	-	-	0
Minocycline	0	0	1	0	0	1	13	27	19	10	15	-	10
FLUOROQUINOLONES													
Levofloxacin	2	0	1	0	1	1	4	11	15	18	-	-	43
MONOBACTAMS													
Aztreonam	-	-	-	0	0	0	1	0	0	1	3	7	84
NITROFURANS													
Nitrofurantoin	-	-	-	0	0	1	0	1	5	10	14	15	50
POLYMYXINS													
Colistin	22	24	11	1	1	3	1	4	3	7	5	-	14
Polymyxin B	27	26	5	2	0	2	4	3	5	4	7	-	11

BL/BLI – β-lactam/ β-lactamase inhibitor; The first number of isolates for each drug is the number of isolates less than or equal to the corresponding MIC. ">*" indicates the number of isolates with MICs greater than the MIC of the last column number.

Time-kill Studies of Meropenem and Polymyxin B against *Klebsiella pneumoniae* Isolates 22, 24, 34, and 44

Research contained within this subchapter was published as Kulengowski, B. *In vitro* activity of polymyxin B and meropenem alone and in combination against carbapenem-resistant *Enterobacteriaceae*. 2016. *Theses and Dissertations—Pharmacy*. 57. Furthermore, this research was subsequently published in a refereed journal as Kulengowski B, Campion JJ, Feola DJ, Burgess DS. 2017. Effect of the meropenem MIC on the killing activity of meropenem and polymyxin B in combination against KPC-producing *Klebsiella pneumoniae*. J Antibiot (Tokyo) **70:**974-978.

All time-kill data of 22, 24, 34, and 44 are located in Appendix E. However, in order to best compare antimicrobial agents within the same organism, the average time-kill results are displayed for each isolate at all concentrations tested in combination as well as the corresponding concentrations of agents used alone. Using these graphs, the killing activity of each antimicrobial alone and their combination can be described as growth – any increase in colony count (CFU/mL) from the previous time point, bacteriostatic – any decrease in colony count (CFU/mL) from starting inoculum that is $<10^3$ CFU/mL, and bactericidal – any decrease in colony count (CFU/mL) from starting inoculum that is $\geq 10^3$ CFU/mL. It is important to distinguish between describing antimicrobial activity as bactericidal (or bacteriostatic) overall (which implies $\geq 10^3$ CFU/mL killing compared to starting inoculum that persisted up to 24 hours) and describing an antimicrobial as exhibiting bactericidal activity for a small window of time, which is a more detailed description of killing activity over time.

Additionally, the interaction of the two antimicrobial agents can be described as synergistic – a $\geq 10^2$ CFU/mL lower colony count of the combination at 24 hours compared to the more active agent (the agent with a lower colony count) alone, additive/indifferent – an absolute difference in colony count of $< 10^2$ CFU/mL between the combination and the more active agent alone, or antagonistic – a $\geq 10^2$ CFU/mL higher colony count of the combination at 24 hours compared to the more active agent alone. In some cases, the interaction may not be determinable if one of the antimicrobial agents alone exhibits enough killing to be $<10^4$ CFU/mL at 24 hours because this is within 10^2 CFU/mL (unable to determine synergy) of the lower limit of quantification (10^2 CFU/mL). A plot of log change in colony count from 0 to 24 hours facilitates evaluation of the interaction of meropenem and polymyxin B, which is described later.

Antimicrobial Activity in KP 34

Figure 4.3 describes KP 34 (MICs: MEM 4 µg/mL, PMB 0.125 µg/mL) and the activity of polymyxin B (0.25 and 1 µg/mL), meropenem (4 and 16 µg/mL), and their combination at clinically relevant concentrations. Specifically, both concentrations of polymyxin B exhibited bactericidal activity within 1 hour, but growth was observed by 4 hours.

Meropenem 4 µg/mL (1 x MIC) displayed bacteriostatic activity with growth observed by 8 hours. Meropenem 16 µg/mL (4 x MIC) displayed bactericidal activity within 2 hours, but growth was observed by 24 hours. Meropenem 64 µg/mL (16 x MIC)

displayed bactericidal activity by 2 hours and maintained this activity throughout the 48-hour time period of testing (Appendix E).

All combinations tested were bactericidal by 2 hours and maintained this activity throughout the 48-hour time period of testing.

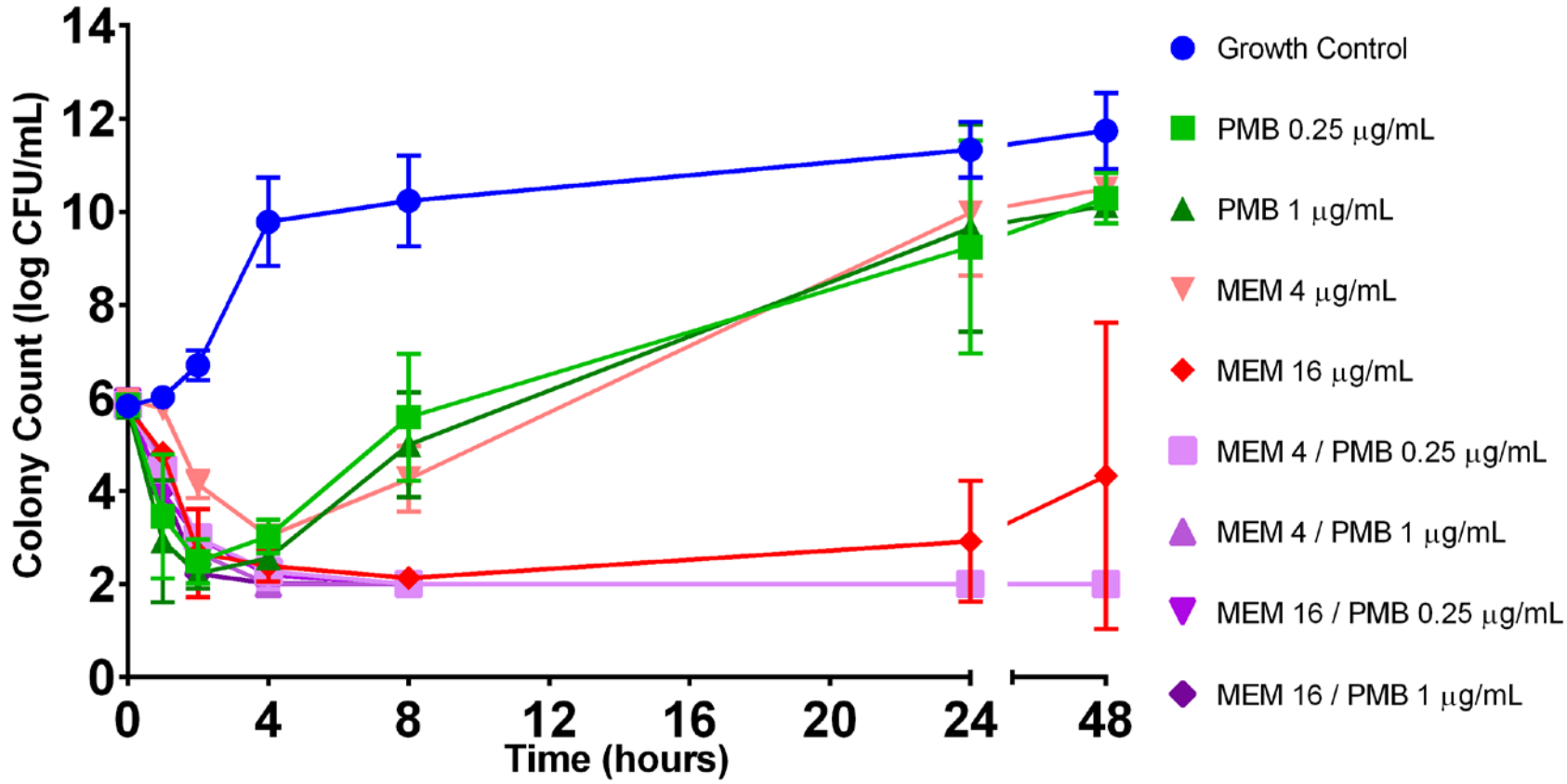

Figure 4.3: Time-kill Curves against KP 34.
Time-kill curve of meropenem (MEM) and polymyxin B (PMB) alone and in combination against KP 34 (MICs: MEM 4 μg/mL, PMB 0.125 μg/mL). Data points are geometric means with error bars being one standard deviation of replicate experiments (n = 2). The lower limit of quantification was 10^2 CFU/mL. Note: all combinations resulted in killing throughout 48-hour study period.

Meropenem and Polymyxin B Interaction in KP 34

In addition to synergy, additivity/indifference, and antagonism, the definitions of growth, bacteriostatic, and bactericidal can be applied to further characterize the interaction of polymyxin B and meropenem at 24 hours. The activity of combinations with meropenem 4 µg/mL (1 x MIC) were all bactericidal and synergistic. Combinations with meropenem 16 µg/mL (4 x MIC) were all bactericidal, but the interaction was indeterminate because the activity of meropenem 16 µg/mL alone was too close to the lower limit of quantification to evaluate synergy among the corresponding combinations. Table 4.13 summarizes these results. Figure 4.4 describes the change in colony count from 0 to 24 hours for each antimicrobial tested alone and in combination.

Table 4.13: Meropenem and Polymyxin B Interaction for KP 34

	Meropenem 4 (µg/mL)	**Meropenem 16 (µg/mL)**	**Meropenem 64 (µg/mL)**
Polymyxin B 0.25 (µg/mL)	S / B	I / B	Not Tested
Polymyxin B 1 (µg/mL)	S / B	I / B	Not Tested
Polymyxin B 4 (µg/mL)	Not Tested	Not Tested	Not Tested

B - Bactericidal
I - Indeterminate
S - Synergistic

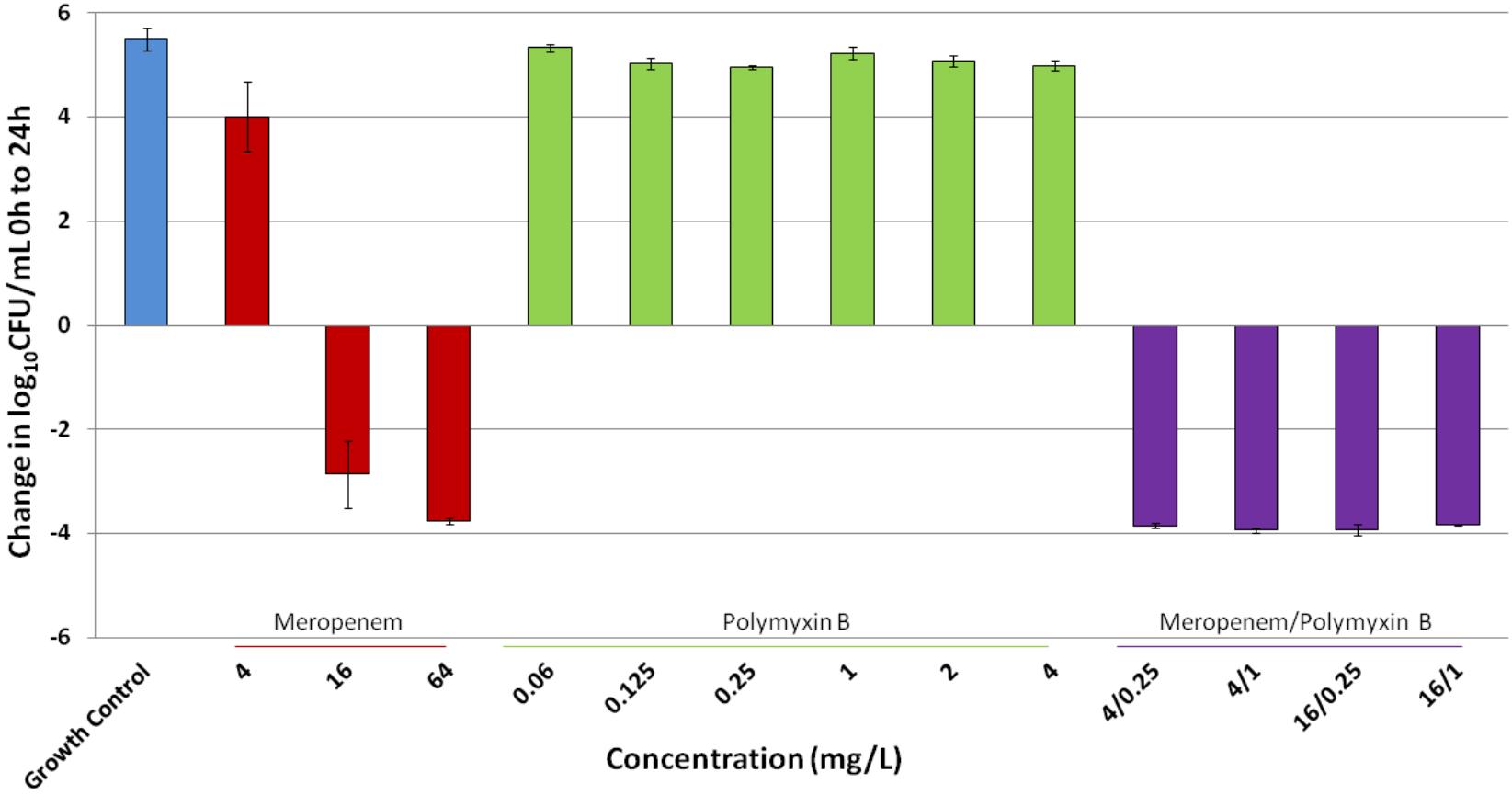

Figure 4.4: Twenty-four Hour Change in Colony Count against KP 34.
24-hour change in colony count for meropenem (MEM) and polymyxin B (PMB) alone and in combination against KP 34 (MICs: MEM 4 µg/mL, PMB 0.125 µg/mL). Data are geometric means with un-pooled standard deviations of replicate experiments (n = 2).

Antimicrobial Activity in KP 22

Figure 4.5 describes KP 22 (MICs: MEM 16 µg/mL, PMB 0.06 µg/mL) and the activity of polymyxin B (0.25 and 1 µg/mL), meropenem (4, 16, and 64 µg/mL), and their combination at clinically relevant concentrations. Specifically, both concentrations of polymyxin B exhibited bactericidal activity within 1 hour, but growth was observed by 8 hours instead of 4 hours as seen in KP 34.

Meropenem 4 µg/mL (1/4 x MIC) displayed bacteriostatic activity with growth observed by 8 hours. Meropenem 16 µg/mL (1 x MIC) displayed bactericidal activity within 4 hours, but growth was observed by 8 hours. Meropenem 64 µg/mL (4 x MIC) displayed bactericidal activity by 2 hours and maintained this activity throughout the 48-hour time period of testing.

All combinations tested were bactericidal by 1 hour (compared to 2 hours observed in KP 34) and maintained this activity throughout the 48-hour time period of testing.

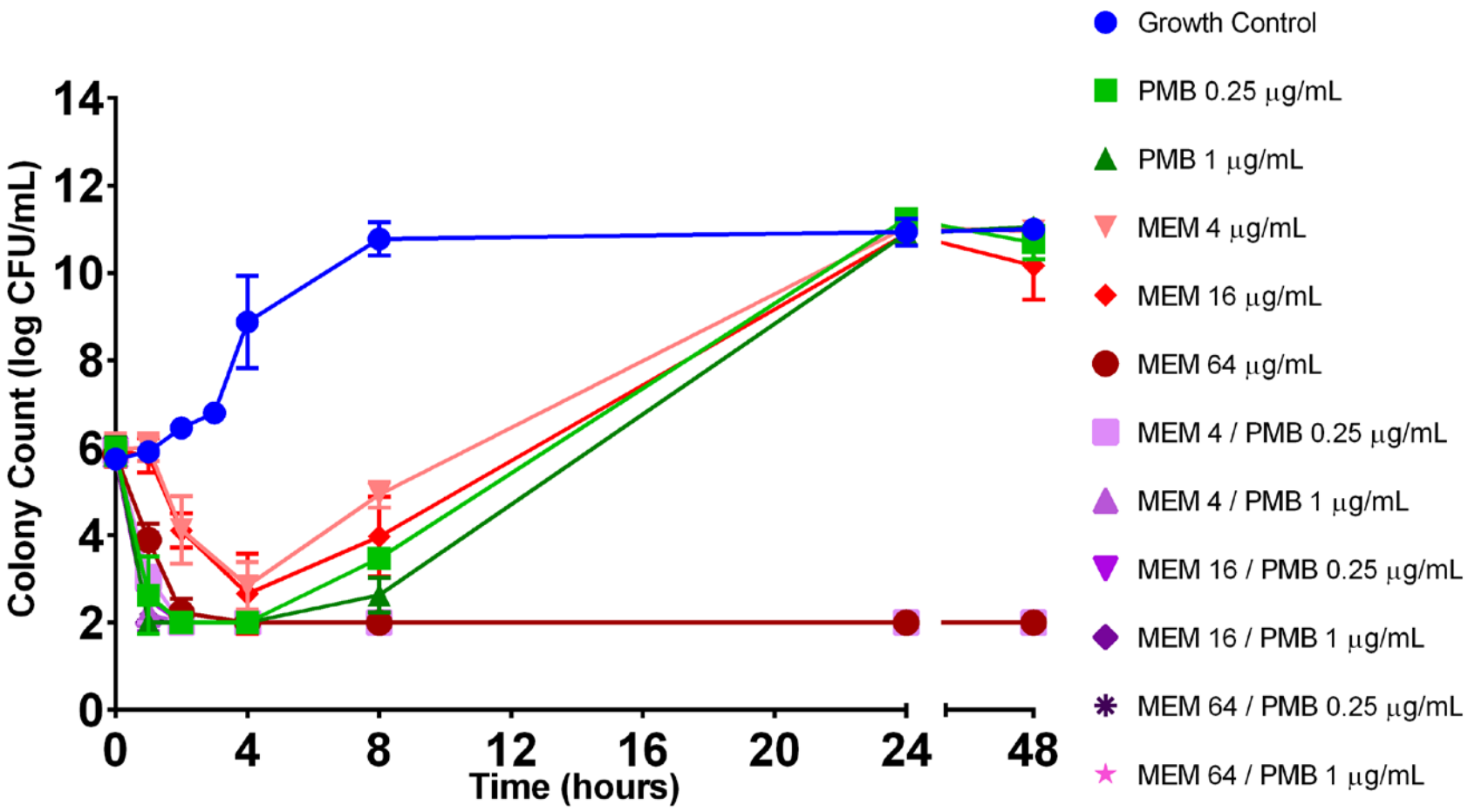

Figure 4.5: Time-kill Curves against KP 22.
Time-kill curve of meropenem (MEM) and polymyxin B (PMB) alone and in combination against KP 22 (MICs: MEM 16 μg/mL, PMB 0.06 μg/mL). Data points are geometric means with error bars being one standard deviation of replicate experiments (n = 2 to 3). The lower limit of quantification was 10^2 CFU/mL. Note: all combinations resulted in killing throughout 48-hour study period.

Meropenem and Polymyxin B Interaction in KP 22

The activity of combinations with meropenem 4 µg/mL and 16 µg/mL (1/4 x MIC and 1 x MIC, respectively) were all bactericidal and synergistic. Combinations with meropenem 64 µg/mL (4 x MIC) were all bactericidal, but the interaction was indeterminate because the activity of meropenem 64 µg/mL alone was too close to the lower limit of quantification to evaluate synergy among the corresponding combinations. Table 4.14 summarizes these results. Figure 4.6 describes the change in colony count from 0 to 24 hours for each antimicrobial tested alone and in combination.

Table 4.14: Meropenem and Polymyxin B Interaction for KP 22

	Meropenem 4 (µg/mL)	**Meropenem 16 (µg/mL)**	**Meropenem 64 (µg/mL)**
Polymyxin B 0.25 (µg/mL)	S / B	S / B	I / B
Polymyxin B 1 (µg/mL)	S / B	S / B	I / B
Polymyxin B 4 (µg/mL)	Not Tested	Not Tested	Not Tested

B - Bactericidal
I - Indeterminate
S - Synergistic

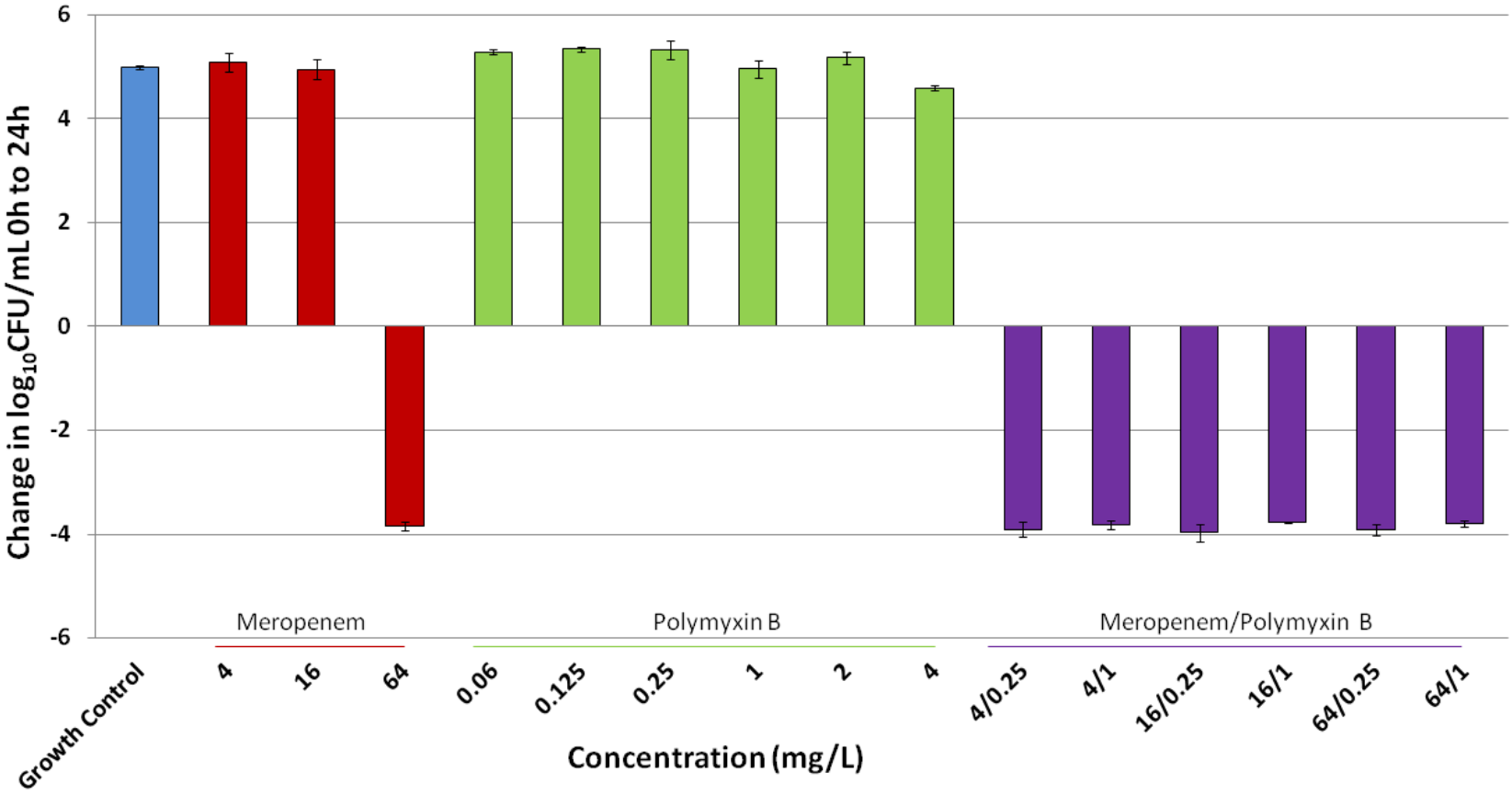

Figure 4.6: Twenty-four Hour Change in Colony Count against KP 22.
24-hour change in colony count for meropenem (MEM) and polymyxin B (PMB) alone and in combination against KP 22 (MICs: MEM 16 µg/mL, PMB 0.06 µg/mL). Data are geometric means with un-pooled standard deviations of replicate experiments (n = 2 to 3).

Antimicrobial Activity in KP 24

Figure 4.7 describes KP 24 (MICs: MEM 32 µg/mL, PMB 0.125 µg/mL) and the activity of polymyxin B (0.25 and 1 µg/mL), meropenem (4, 16, and 64 µg/mL), and their combination at clinically relevant concentrations. Specifically, polymyxin B 0.25 µg/mL (2 x MIC) exhibited bactericidal activity within 2 hours whereas polymyxin B 1 µg/mL (8 x MIC) exhibited bactericidal activity within 1 hour, but growth was observed for both concentrations by 8 hours, more similar to KP 22 than KP 34.

Meropenem 4 µg/mL (1/8 x MIC), 16 µg/mL (1/2 x MIC), and 64 µg/mL (2 x MIC) displayed bacteriostatic activity with growth observed by 8 hours.

All combinations with meropenem concentrations ≥16 µg/mL (≥1/2 x MIC) were bactericidal by 1 hour and maintained this activity throughout the 48-hour time period of testing. However, both combinations with meropenem concentrations 4 µg/mL (1/8 x MIC) were bactericidal by 1 hour with growth observed by 8 hours.

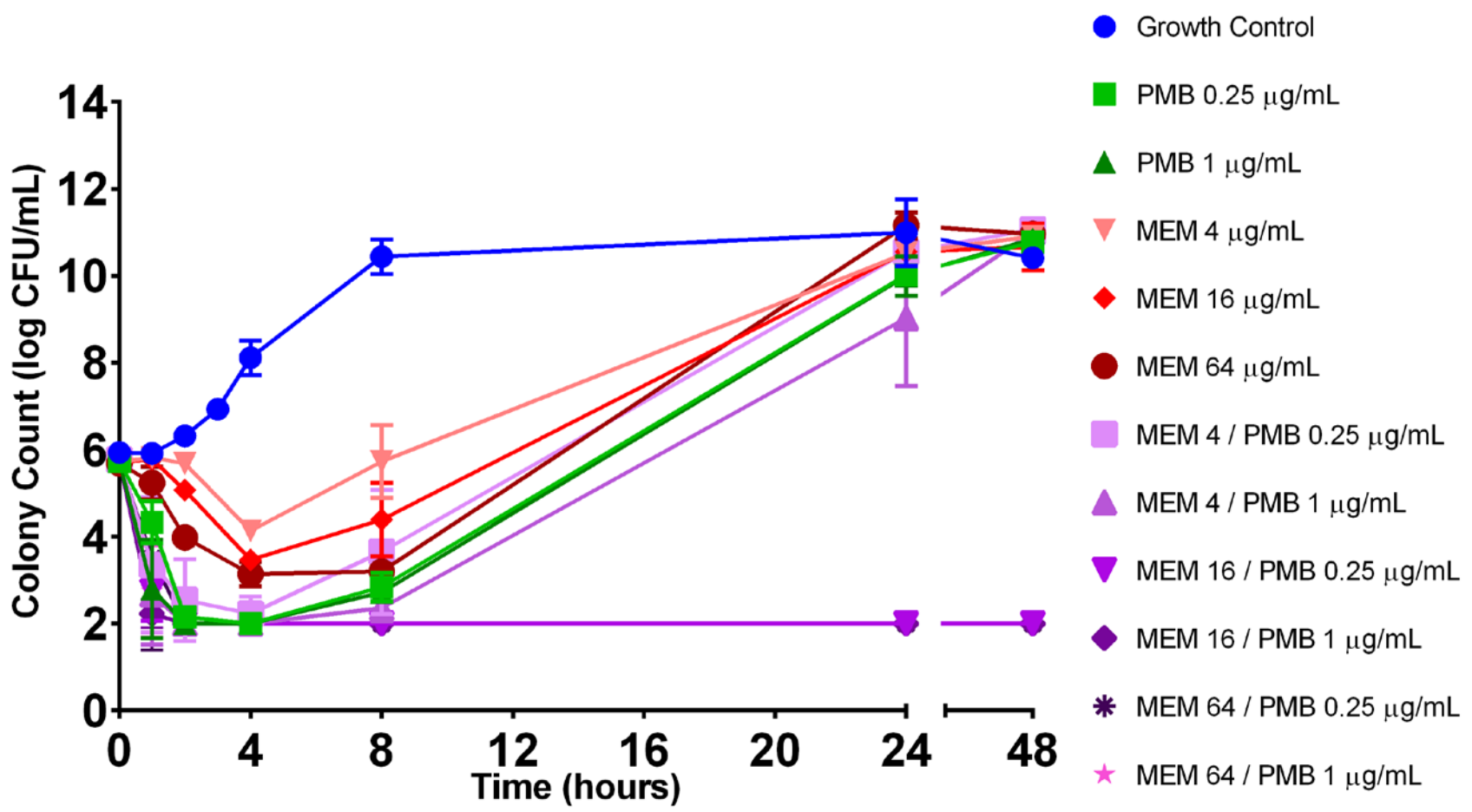

Figure 4.7: Time-kill Curves against KP 24.
Time-kill curve of meropenem (MEM) and polymyxin B (PMB) alone and in combination against KP 24 (MICs: MEM 32 µg/mL, PMB 0.125 µg/mL). Data points are geometric means with error bars being one standard deviation of replicate experiments (n = 2 to 3). The lower limit of quantification was 10^2 CFU/mL. Note: some combinations resulted in killing throughout 48-hour study period.

Meropenem and Polymyxin B Interaction in KP 24

The activity of combinations with meropenem 4 µg/mL (1/8 x MIC) were all additive/indifferent and growth was observed at 24 hours. In contrast, combinations with meropenem at 16 and 64 µg/mL (1/2 x MIC and 2 x MIC, respectively) were all bactericidal and synergistic. Table 4.15 summarizes these results. Figure 4.8 describes the change in colony count from 0 to 24 hours for each antimicrobial tested alone and in combination.

Table 4.15: Meropenem and Polymyxin B Interaction for KP 24

	Meropenem 4 (µg/mL)	**Meropenem 16 (µg/mL)**	**Meropenem 64 (µg/mL)**
Polymyxin B 0.25 (µg/mL)	A / G	S / B	S / B
Polymyxin B 1 (µg/mL)	A / G	S / B	S / B
Polymyxin B 4 (µg/mL)	Not Tested	Not Tested	Not Tested

A - Additive/Indifferent
B - Bactericidal
G - Growth
S - Synergistic

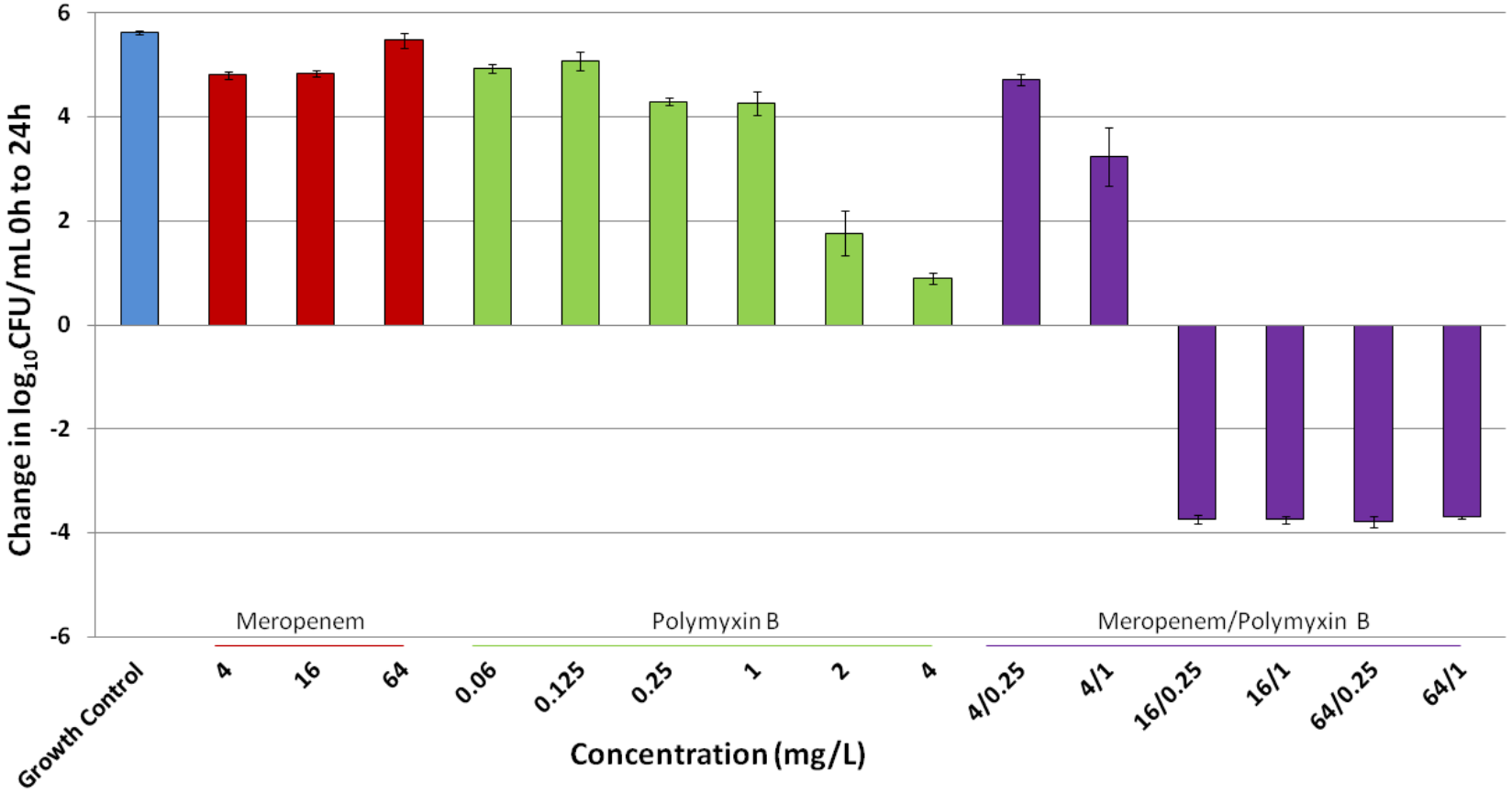

Figure 4.8: Twenty-four Hour Change in Colony Count against KP 24.
24-hour change in colony count for meropenem (MEM) and polymyxin B (PMB) alone and in combination against KP 24 (MICs: MEM 32 µg/mL, PMB 0.125 µg/mL). Data are geometric means with un-pooled standard deviations of replicate experiments (n = 2 to 3).

Antimicrobial Activity in KP 44

Figure 4.9 describes KP 44 (MICs: MEM 128 µg/mL, PMB 0.06 µg/mL) and the activity of polymyxin B (0.25, 1, and 4 µg/mL), meropenem (4, 16, and 64 µg/mL), and their combination at clinically relevant concentrations. Specifically, all concentrations of polymyxin B exhibited bactericidal activity within 1 hour, but growth was observed by 8 hours for polymyxin B at 0.25 and 1 µg/mL (4 x MIC and 16 x MIC, respectively) and 24 hours for polymyxin B 4 µg/mL (64 x MIC), more similar to KP 22 and KP 24 than KP 34.

Meropenem 4 µg/mL (1/32 x MIC) displayed no activity, with growth observed by 1 hour. Meropenem 16 µg/mL (1/8 x MIC) exhibited bacteriostatic activity with growth observed by 8 hours. Meropenem 64 µg/mL (1/2 x MIC) displayed bacteriostatic activity with growth observed by 24 hours.

Combinations with polymyxin B concentrations ≥1 µg/mL (≥16 x MIC) were bactericidal by 1 hour whereas combinations with polymyxin B concentrations of 0.25 µg/mL (4 x MIC) were bactericidal by 2 hours. Growth was observed by 8 hours for combinations with meropenem 4 µg/mL (1/32 x MIC) whereas growth was observed by 24 hours for combinations with meropenem ≥16 µg/mL (≥1/8 x MIC). The only combination that maintained bactericidal activity throughout the 48-hour time period of testing was the combination with the highest concentrations of both antimicrobial agents – meropenem 64 µg/mL (1/2 x MIC) in combination with polymyxin B 4 µg/mL (64 x MIC).

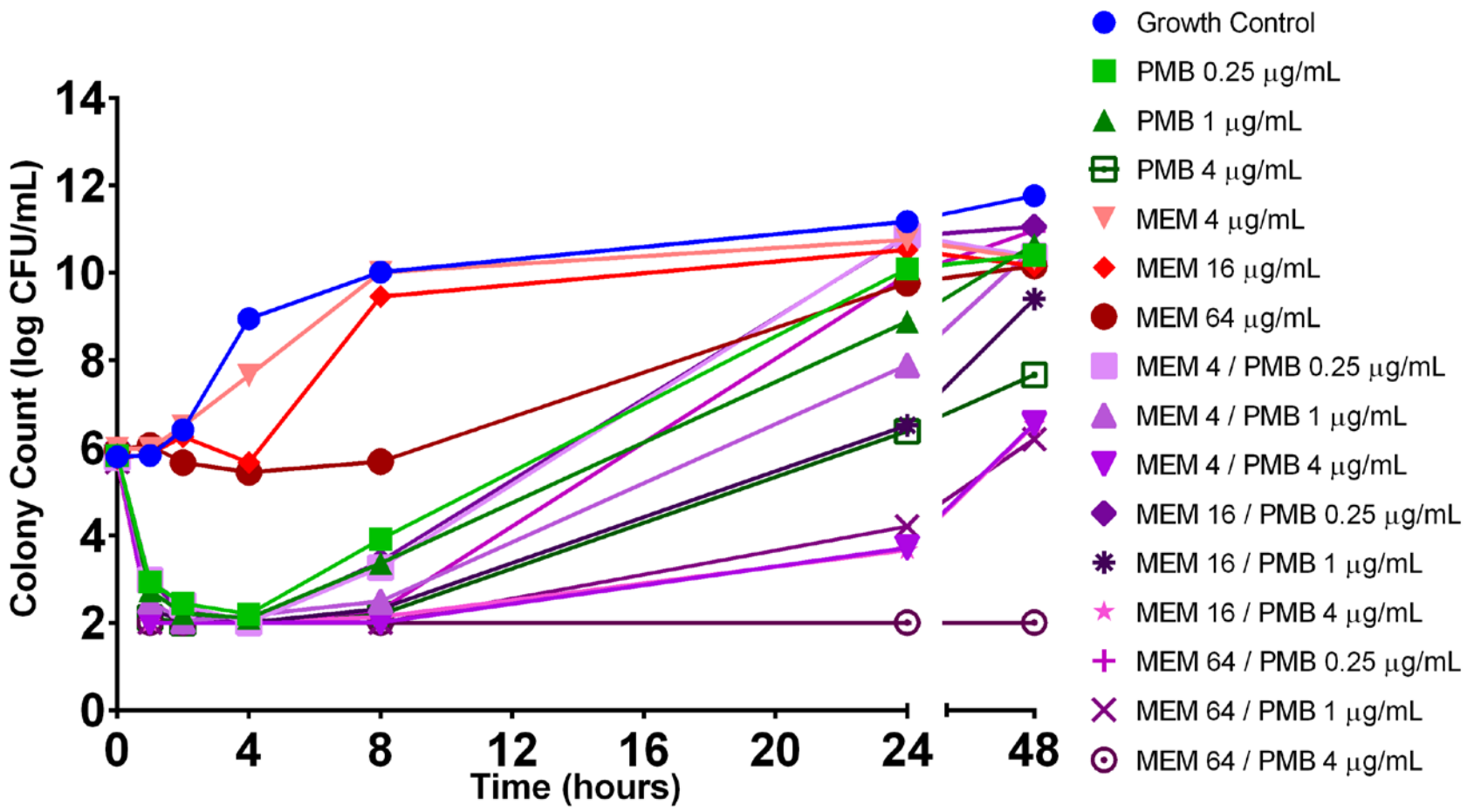

Figure 4.9: Time-kill Curves against KP 44. Time-kill curve of meropenem (MEM) and polymyxin B (PMB) alone and in combination against KP 44 (MICs: MEM 128 μg/mL, PMB 0.06 μg/mL). Data points are geometric means of replicate experiments (*n* = 2 to 4). The lower limit of quantification was 10^2 CFU/mL.

Meropenem and Polymyxin B Interaction in KP 44

The activity of meropenem 4 µg/mL (1/32 x MIC) in combination with polymyxin B at 0.25 or 1 µg/mL (2 x MIC and 8 x MIC, respectively) was additive/indifferent and growth was observed at 24 hours. However, meropenem 4 µg/mL in combination with polymyxin B 4 µg/mL (32 x MIC) was synergistic with bacteriostatic activity observed at 24 hours.

The activity of meropenem 16 µg/mL (1/8 x MIC) in combination with polymyxin B 0.25 µg/mL was additive/indifferent whereas in combination with polymyxin B at 1 or 4 µg/mL was synergistic. However, at 24 hours, growth was observed for the lower two polymyxin B combinations and bacteriostatic activity was observed for the combination with polymyxin 4 µg/mL.

The activity of meropenem 64 µg/mL (1/2 x MIC) in combination with polymyxin B 0.25 was additive/indifferent with growth observed. The combination with polymyxin B 1 µg/mL was synergistic with bacteriostatic activity. The only combination to produce synergistic, bactericidal activity was meropenem 64 µg/mL in combination with polymyxin B 4 µg/mL.

Table 4.16 summarizes these results. Figure 4.10 describes the change in colony count from 0 to 24 hours for each antimicrobial tested alone and in combination.

Table 4.16: Meropenem and Polymyxin B Interaction for KP 44

	Meropenem 4 (µg/mL)	**Meropenem 16 (µg/mL)**	**Meropenem 64 (µg/mL)**
Polymyxin B 0.25 (µg/mL)	A / G	A / G	A / G
Polymyxin B 1 (µg/mL)	A / G	S / G	S / BS
Polymyxin B 4 (µg/mL)	S / BS	S / BS	S / BC

A - Additive/Indifferent
BC - Bactericidal
BS - Bacteriostatic
G - Growth
S - Synergistic

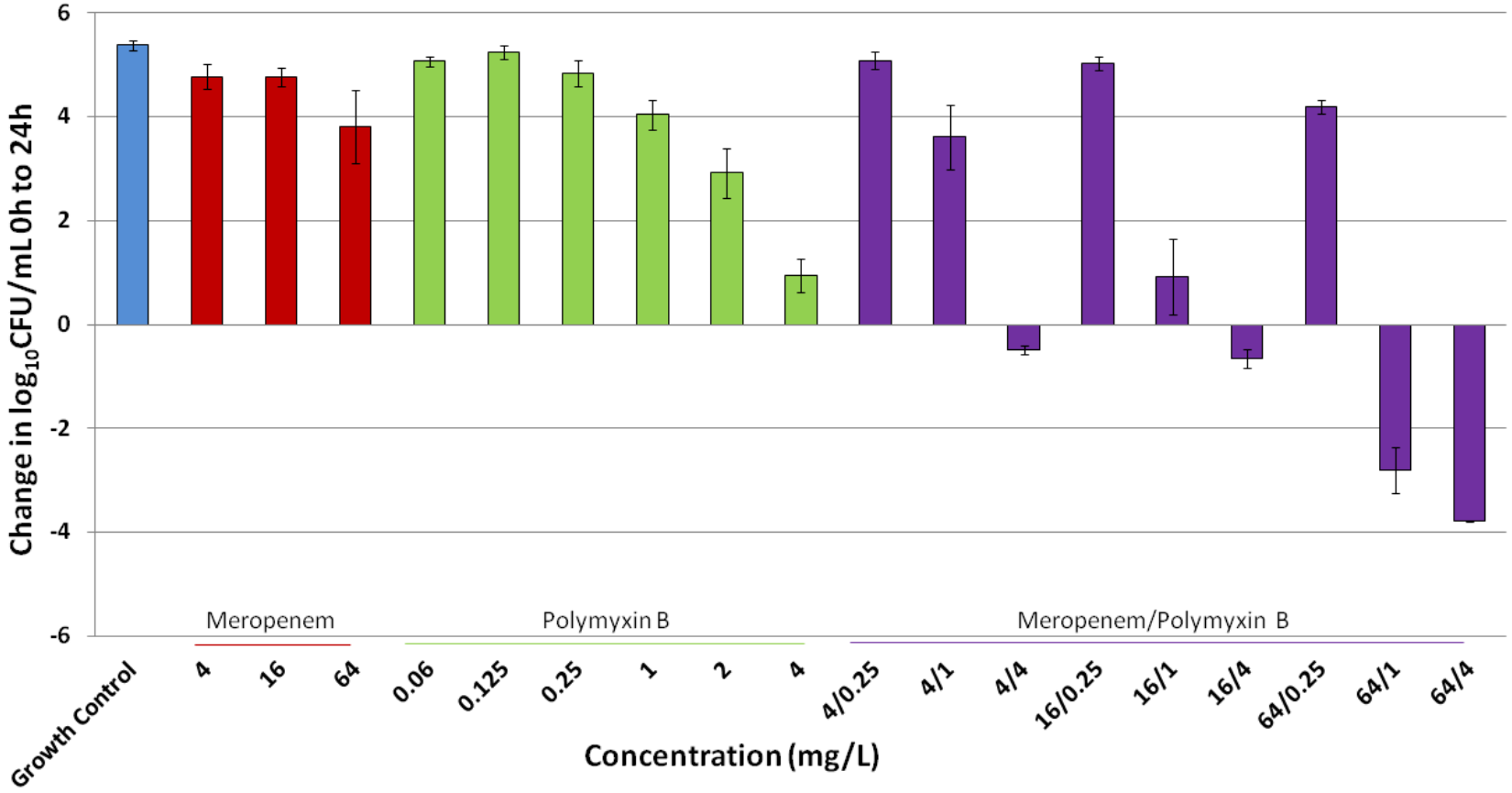

Figure 4.10: Twenty-four Hour Change in Colony Count against KP 44.
24-hour change in colony count for meropenem (MEM) and polymyxin B (PMB) alone and in combination against KP 44 (MICs: MEM 128 µg/mL, PMB 0.06 µg/mL). Data are geometric means with un-pooled standard deviations of replicate experiments (n = 2 to 4).

Summary of Meropenem and Polymyxin B Alone against Carbapenem-resistant *K. pneumoniae*

Meropenem alone, at all concentrations tested, achieved bactericidal activity ($\geq 10^3$ decrease in CFU/mL) within four hours for KP 34 (MEM MIC 4 µg/mL) and KP 22 (MEM MIC 16 µg/mL). Regrowth in these strains was observed for the two lowest (4 and 16 µg/mL) but not the highest (64 µg/mL) meropenem exposures (Figures 4.1 and 4.2). In contrast, meropenem alone produced only bacteriostatic activity ($< 10^3$ decline in CFU/mL) in KP 24 (MEM MIC 32 µg/mL) and KP 44 (MEM MIC 128 µg/mL; Figures 4.3 and 4.4). Regrowth for these two isolates began by 8 hours.

Polymyxin B alone produced bactericidal activity at all concentrations tested against all strains within 2 hours, but regrowth occurred within 8 hours in all instances (Figure 4.3, Figure 4.5, Figure 4.7, and Figure 4.9).

Summary of Meropenem and Polymyxin B in Combination against Carbapenem-resistant *K. pneumoniae*

The interaction of meropenem with polymyxin B in combination was characterized by synergism and 24-hour bactericidality as described in Time-Kill Studies. Both combinations of meropenem 4 µg/mL and polymyxin B (0.25 or 1 µg/mL) concentrations achieved synergistic activity ($\geq 10^2$ decrease in CFU/mL at 24 hours compared to the most active agent alone) against KP 34 (MEM MIC 4 µg/mL; Figure 4.4), with no regrowth over 48 hours (Figure 4.3). Higher concentrations of meropenem alone (16 or 64 µg/mL) eradicated KP 34 and so synergism was indeterminate for these combinations.

All combinations of meropenem (4 or 16 µg/mL) and polymyxin B (0.25 or 1 µg/mL) concentrations achieved synergistic activity against KP 22 (MEM MIC 16 µg/mL; Figure 4.6) with no regrowth over 48 hours (Figure 4.5), but higher concentrations of meropenem alone (64 µg/mL) eradicated KP 22 which rendered synergism assessment indeterminate.

Meropenem 4 µg/mL in combination with polymyxin B 0.25 or 1 µg/mL produced additive/indifferent activity ($<10^2$ change in CFU/mL at 24 hours compared to the most active agent alone) against KP 24 (MEM MIC 32 µg/mL; Figure 4.8) with regrowth occurring by 8 hours (Figure 4.7), but all remaining combinations of meropenem 16 or 64 µg/mL with polymyxin B 0.25 or 1 µg/mL achieved synergistic activity with no regrowth over 48 hours (Figure 4.7 and Figure 4.8).

Combinations of meropenem 4, 16, or 64 µg/mL with polymyxin B 0.25 µg/mL displayed additive/indifferent activity against KP 44 (MEM MIC 128 µg/mL; Figure 4.10) with variable regrowth (Figure 4.9). Combinations with polymyxin B at 1 or 4 µg/mL displayed synergy, but only the highest tested concentration of meropenem (64 µg/mL) and polymyxin B (4 µg/mL) also prevented regrowth against KP 44 (Figure 4.9 and Figure 4.10).

Discussion

Polymyxin B alone against polymyxin-susceptible, KPC-producing *Klebsiella pneumoniae* generally exhibited quick bactericidal activity, with rapid regrowth observed whereas meropenem alone generally exhibited bacteriostatic activity initially with growth observed as well. These results are consistent with *in vitro* data from other groups.[212,330] When used in combination, results were often bactericidal, synergistic, and maintained this activity throughout 48 hours unless resistance to meropenem was high (≥32 μg/mL) in which case higher levels of antimicrobial agents were shown to overcome the strains with elevated MICs, but such regimens may have limited feasibility in a patient where antimicrobial concentrations are not static but change as drug is eliminated. Therefore, additional *in vitro* or animal (or even human) models are needed to elucidate the impact of pharmacokinetics and the degree of meropenem resistance on the activity of meropenem and polymyxin B in combination against KPC-producing *K. pneumoniae*.

A 2013 meta-analysis on *in vitro* synergy of polymyxins and carbapenems highlighted that most data for comparison involves non-*Enterobacteriaceae* such as *Pseudomonas aeruginosa* or *Acinetobacter baumannii*. In fact, the authors included only three studies[206,331,332] that evaluated polymyxin B in combination with a carbapenem (imipenem, doripenem, meropenem, or ertapenem) across a total of 34 unique isolates of *K. pneumoniae,* most of which were polymyxin-susceptible. Synergy rates for polymyxin B and a carbapenem were higher than synergy rates for colistin and a carbapenem (64% vs. 40%; P = 0.04), but substantial heterogeneity among these studies was present (I^2 = 51%).[212] Since the publication of the meta-analysis, only one other study has compared

polymyxin B in combination with a carbapenem *in vitro* against KPC-producing *Klebsiella pneumoniae*.[330]

Among the four studies evaluating the *in vitro* activity of polymyxin B in combination with a carbapenem against KPC-producing *K. pneumoniae*, two evaluated exclusively KPC-2-producing *K. pneumoniae* (18 isolates total),[206,330] one evaluated exclusively KPC-3-producing *K. pneumoniae* (4 isolates),[332] and the last evaluated both KPC-2- and KPC-3-producing *K. pneumoniae* (8 and 6 isolates, respectively). Our study evaluated KPC-3-producing *K. pneumoniae* (4 isolates). Although KPC-2 is considered the ancestral enzyme, KPC-3 has also been frequently observed in the United States. KPC-3 is very similar to KPC-2 in both structure and phenotypic resistance expression, differing only by a single nucleotide polymorphism and therefore also a single amino acid substitution of histidine for tyrosine (H272Y).[333] To date, there is no data to suggest distinguishing between KPC-2 or KPC-3 correlates with differences in phenotypic resistance or clinical outcome in meropenem and polymyxin B combinations, and so this difference among studies was accepted. Finally, definitions of synergy and bactericidality among studies were consistent except when noted.

Polymyxin B or a Carbapenem Alone

Comparing results of monotherapy was not possible among all studies because complete time-kill data was only provided by Lee *et al.*[332] Data for 0 hours and 24 hours was provided by Bratu *et al.*,[206] but the other studies only provided the difference from 0 hours to 24 hours.[330,331] Lee *et al.* studied the four KPC-3 isolates most similar to this study,

exhibiting polymyxin B MICs ranging from 0.125 - 0.25 µg/mL (this study: MICs 0.06 - 0.125 µg/mL) and doripenem (DOR) MICs ranging from 16 - 32 µg/mL (this study: MEM MICs 4 - 128 µg/mL). Polymyxin B at 2 x MIC displayed similar killing to the present study, but we observed regrowth sooner (4 hours) than did Lee *et al.* (8 hours), despite our use of higher concentrations relative to the MIC (Figure 4.11 vs. Figure 4.3, Figure 4.5, Figure 4.7, and Figure 4.9). Concerning carbapenem therapy, the bacteriostatic activity and growth observed with doripenem used alone (Figure 4.11) was similar to the present study, despite our use of meropenem instead.[332]

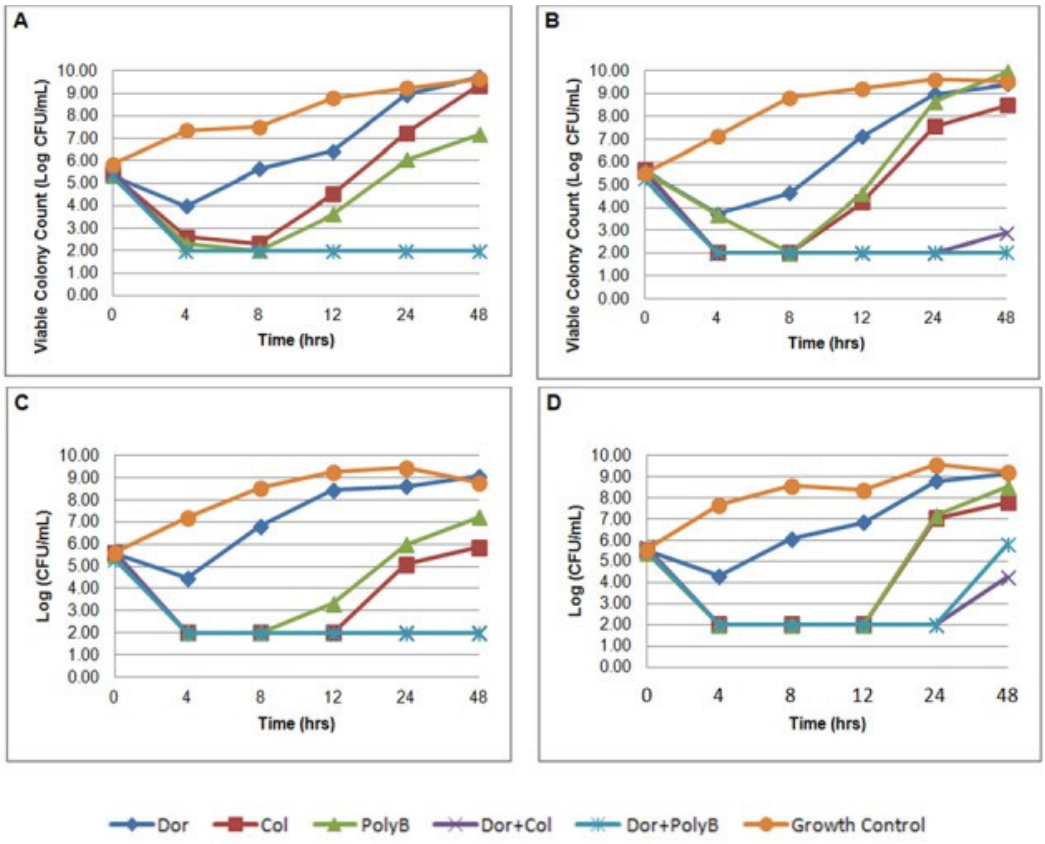

Figure 4.11: Time-kill Curves for Four KPC-3-producing *K. pneumoniae*. Reprinted[332]Abbreviations: Dor - Doripenem, Col - Colistin, PolyB - Polymyxin B

Comparing the data provided by Bratu *et al.*, polymyxin B alone at 0.5, 1, 2, and 4 µg/mL remained bactericidal at 24 hours for 2, 7, 12, and 13 isolates, respectively, out of 16 total. Interestingly, isolates from this study had at least 4-fold lower polymyxin B MICs than those studied by Bratu *et al.* (0.06 - 0.125 µg/mL vs. 0.5 - 16 µg/mL), but we, in contrast, observed regrowth in all polymyxin B concentrations tested alone before 24 hours. Comparing carbapenem therapy, imipenem (IPM) 4 µg/mL alone displayed growth in all 16 isolates. For the present study, the three isolates most similar to those evaluated by Bratu *et al.* (IPM MICs 8 - >32) were KP 22 (MEM MIC 16 µg/mL), KP 24 (MEM MIC 32 µg/mL), and KP 44 (MEM MIC 128 µg/mL). Like Bratu *et al.*, meropenem alone showed growth at 4 and 16 µg/mL.[206]

Polymyxin B and a Carbapenem in Combination

Lee *et al.* evaluated colistin or polymyxin B at 2 x MIC in combination with doripenem 6 µg/mL against four polymyxin-susceptible, KPC-3-producing *Klebsiella pneumoniae* (DOR MIC 16 - 32 µg/mL). Bactericidal, synergistic activity was observed throughout 24 hours for all isolates with some regrowth observed only at 48 hours for 1 of 4 isolates with polymyxin B and 2 of 4 with colistin.[332] Polymyxin B and meropenem showed similar activity against KP 22 and KP 24 (MEM MICs 16 and 32 µg/mL, respectively) in the present study at comparable concentrations (MEM 4 µg/mL and 16 µg/mL). One notable difference compared to our study was that meropenem 4 µg/mL in combination with polymyxin B at 0.25 or 1 µg/mL (2 and 8 x MIC, respectively) did not maintain bactericidal or synergistic activity by 24 hours against KP 24. However,

meropenem concentrations ≥1 x MIC in combination with polymyxin B did retain bactericidal activity and a synergistic interaction. The difference between meropenem and doripenem in combination with polymyxin B cannot be explained with good evidence, but clinically, others have observed equivalent efficacy of doripenem compared to other carbapenems when being used in lower doses but at extended infusions.[334]

Pankey *et al.* evaluated polymyxin B at 1/4, 1/2, and 1 x MIC in combination with meropenem 1 x MIC against 14 KPC-producing *Klebsiella pneumoniae* (MER MIC 16 - >32 µg/mL, PMB MIC ≤2 for 11 of 14 isolates). Synergy was observed for 9 of 14 isolates for all concentrations tested with meropenem and polymyxin B. Non-synergistic isolates showed indifferent/additive activity, but only 1 of 5 was resistant to polymyxin B (PMB MIC 32 µg/mL) before study. The authors did not comment on the killing activity (bactericidal vs. bacteriostatic) of meropenem and polymyxin B in combination against these isolates. Compared to the present study, we observed a loss of synergistic activity between meropenem and polymyxin B related to increasing meropenem MIC. A similar assessment is difficult to make in the study by Pankey *et al.* because detection of synergy did not depend on meropenem MIC, but a much smaller range of carbapenem resistance was evaluated (MEM MIC 16 - >32 µg/mL). Similarly, carbapenem MIC did not change the observation of synergy in the study by Lee *et al.*, but again, a smaller range (16 - 32 µg/mL) of carbapenem MIC was evaluated whereas we evaluated MEM MICs 4 - 128 µg/mL.[331]

Bratu *et al.* evaluated polymyxin B at 1 µg/mL and 1/2 x MIC in combination with imipenem 4 µg/mL against 16 KPC-2-producing *Klebsiella pneumoniae* that were mostly polymyxin-susceptible (14 of 16 isolates). Imipenem MICs were all >32 µg/mL except for

one isolate which had an imipenem MIC of 8 µg/mL. Data were unavailable for each isolate tested individually, but synergy was reported for 10 of 16 isolates with polymyxin B at 0.5 x MIC in combination with imipenem. Interestingly, antagonism was observed in 3 of 16 isolates. Although antagonism was not observed with polymyxin B and meropenem in the present study, the antagonism reported by Bratu *et al.* is consistent with our observed loss or reduction of synergy as the carbapenem MIC increases. It is important to note that a majority of isolates evaluated by Bratu *et al.* had imipenem MICs > 32 µg/mL while we observed loss of synergy at lower concentrations of meropenem (4 µg/mL) when meropenem MICs were at least 32 µg/mL. It is also interesting that synergy was still observed in a majority of highly carbapenem-resistant isolates (10 of 16 isolates) at polymyxin B concentrations close to 1 µg/mL in combination with imipenem 4 µg/mL when the present study required meropenem concentrations ≥ 16 µg/mL in combination with polymyxin B 1 µg/mL to maintain synergy.[206]

In the most recently published study, Barth *et al.* evaluated polymyxin B at 0.5, 1, and 2 µg/mL in combination with meropenem or imipenem at 4 µg/mL against two KPC-2-producing *Klebsiella pneumoniae*. Both isolates had a polymyxin B MIC of 2 µg/mL and a meropenem MIC of 32 µg/mL but had different imipenem MICs of 8 and 32 µg/mL. Synergy was observed in both strains at all combinations studied which is in contrast to this study where polymyxin B at 0.5 and 1 µg/mL in combination with meropenem 4 µg/mL was not synergistic for KP 24, despite having a lower polymyxin B MIC (MEM MIC 32 µg/mL, PMB MIC 0.125 µg/mL). Although not as directly comparable, but interesting, Barth *et al.* also evaluated two strains each of *Escherichia coli* and *Serratia marcescens* for which synergy was also found in all the same concentrations of polymyxin

B in combination with either meropenem or imipenem. This result was most surprising for *S. marcescens* which is intrinsically resistant to polymyxins. The *E. coli* strains had meropenem MICs of 64 µg/mL whereas the *S. marcescens* strains had meropenem MICs of 128 and 256 µg/mL. The polymyxin B MICs for *E. coli* were 2 µg/mL whereas the *S. marcescens* stains had polymyxin B MICs of 64 and >64 µg/mL. The author did characterize the killing of these antimicrobial agents against the strains, however their definition of bactericidality was different from most studies, including the present one. The authors assessed bactericidal activity based on a 10^3 CFU/mL difference between the colony count of the combination and the colony count of the most active agent alone, which, while appropriate for synergy, could actually mean that growth (as defined in this and most studies) occurred but to a lesser extent than the most active agent. Since only this difference was reported, it is not possible to compare the killing activity in the experiments by Barth *et al.* to the killing activity observed in this study.[330]

Overall, other studies evaluating polymyxin B in combination with a carbapenem by time-kill assay observed bactericidal activity and synergistic interaction maintained throughout 24 hours most of the time[206,330-332] which is in agreement with our findings. Antagonism was rarely observed, and it was only seen in a minority of isolates with high carbapenem MICs (IPM MICs > 32)[206] which is also similar to our findings because a lower extent of synergy was also observed in this study when carbapenem MICs were elevated (MEM MICs ≥ 32 µg/mL). A carbapenem alone exhibited similar activity in this study as compared to the Lee *et al.*[332] and Bratu *et al.*[206] Polymyxin B alone exhibited variable activity depending on the study. Our results were more similar to Lee *et al.* where growth was consistently observed, but at a slower rate.[332] In contrast, Bratu *et al.* observed

growth in only a fraction of the isolates, depending on the concentration of polymyxin B. This might best be explained by variable heteroresistant subpopulations among KPC-producing *K. pneumoniae* isolates,[140] however this has not been well characterized and was not discussed by Bratu *et al.*[206]

Staggering the Administration of Polymyxin B and Meropenem in Time-kill against Carbapenem-resistant *Enterobacteriaceae* Exhibiting a Wide Range of Meropenem MICs

Published: *Diagnostic Microbiology and Infectious Disease.* 2018. In press as a research note

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Kulengowski contribution: Experimental design, execution, data analysis, drafting, revisions and editing of final manuscript

Abstract

Little is known regarding the appropriate timing and sequencing of a carbapenem and polymyxin in combination against CRE. Meropenem and polymyxin B were administered simultaneously or one agent two hours prior to the other, *in vitro*. The carbapenem should be administered prior to the polymyxin when used in combination.

Keywords: Carbapenem-resistant *Enterobacteriaceae*, Polymyxin B, Meropenem, Time-kill, Staggered administration

Introduction

CRE are resistant to nearly all antimicrobials and are associated with staggering mortality (50%).[14] CRE are often treated with combination therapy, but optimal therapy has not been established. Polymyxin B (PMB) and meropenem (MEM) are commonly utilized against CRE, and the meropenem MIC is known to influence their interaction.[274] The aim of this study was to determine whether the sequence of administration of MEM or PMB affects the efficacy of this combination against CRE with varying MEM MICs.

Methods

CRE isolates expressing KPC-2 (isolates 10 and 17) or KPC-3 (isolates 24 and 44) with PMB MICs 0.06-0.125 mg/L and MEM MICs 8-128 mg/L were selected for analysis. Whole genome sequencing was previously performed on these isolates.[274,335] We conducted separate-day duplicate time-kill studies of clinically achievable concentrations by typical human dosing of PMB (0.25 and 1 mg/L) and MEM (4 and 16 mg/L) alone and in combination against two *Enterobacter cloacae* (MEM MICs: 8, and 32 mg/L) and two *Klebsiella pneumoniae* isolates (MEM MICs: 32 and 128 mg/L). MEM 64 mg/L was also tested alone and in combination with PMB 0.25 and 1 mg/L for the isolate with a MEM MIC of 128 mg/L. Sampling occurred at 0, 1, 2, 4, 8, 24, and 48 hours. For simultaneous administration, both antimicrobials were added at time 0. Otherwise, either PMB or MEM was added at time 0 and the remaining antimicrobial (MEM or PMB) was added at 2 hours. This 2-hour delay was selected based on maximal killing for polymyxin B monotherapy occurring around that time (Figure 4.12), and our working theory was that administering

polymyxin B first may improve the permeability of meropenem to its target site. Samples were diluted, plated using a spiral plater, which controlled for antimicrobial carryover, and enumerated by a laser colony counter.[277,336] Kill curves were constructed to characterize the activity of staggered vs. simultaneous administration. Standard definitions of bactericidal activity, bacteriostatic activity (colony count less than or equal to starting inoculum but not bactericidal), regrowth (any increase in colony count from previous time point), synergy, indifference, and antagonism were utilized.[336]

Results

For PMB monotherapy against all isolates, bactericidal activity was observed by 2 hours, but regrowth occurred by 8 hours (Figure 4.12). For MEM monotherapy against all isolates, bacteriostatic activity was observed with regrowth by 24 hours (Figure 4.12). Administering polymyxin B first exhibited less killing activity than simultaneous administration or administering meropenem first for all isolates, regardless of MEM MIC (Figure 4.12, Figure 4.13). Against the isolate with a MEM MIC of 8 mg/L, bactericidal activity was maintained throughout 48 hours only when meropenem was administered first (Figure 4.12, panel A). Furthermore, all combinations of meropenem administered first were synergistic. Synergy and bactericidal activity at 24 hours for simultaneous administration was only observed for the two higher meropenem combination concentrations (MEM 16 mg/L) (Figure 4.12, panel A). There was not a clear difference between administering meropenem first and simultaneous administration when the MEM MICs were 32 mg/L, but both exhibited more killing activity than administering polymyxin

B first (Figure 4.12, panels B and C; Figure 4.13). For the isolate with a MEM MIC of 128 mg/L, only when meropenem was administered first and at the highest meropenem concentration (MEM 64 mg/L) was bactericidal activity maintained throughout 48 hours. Regrowth was observed by 8 hours for the remaining combinations against this isolate except for the simultaneous MEM 64 / PMB 1 mg/L and the polymyxin first MEM 64 / PMB 1 mg/L curves where regrowth was observed by 24 hours (Figure 4.12, panel D).

Discussion

Although we are not the first to employ staggered administration techniques with combination antimicrobials, this study is the first to look at the sequencing of a carbapenem and a polymyxin against CRE. Lewis et al. demonstrated polyene-azole antagonism when fluconazole was administer prior to amphotericin B in a dynamic *in vitro* model;[337] Zelenitsky et al. demonstrated significantly improved (six-fold) activity with simultaneous or beta-lactam-first staggering of ceftazidime and either ciprofloxacin or tobramycin against *P. aeruginosa*.[338] Based on our data, a carbapenem should be given prior to a polymyxin antimicrobial when these two agents are used in combination. Polymyxins should not be administered first, and neither agent should be administered as monotherapy. However, some clinical data with *A. baumannii* have suggested no difference in clinical failure with colistin monotherapy compared to colistin combinations with meropenem, rifampin, or fosfomycin.[339,340] Data from another clinical trial of colistin with meropenem against extensively drug resistant gram-negative bacilli are anxiously awaited (NCT01597973, ClinicalTrials.gov).

The mechanism explaining why polymyxin B administered first results in less killing activity of the combination has yet to be determined. As previously mentioned, we anticipated improved combination antimicrobial activity based on the idea that polymyxins disrupt the outer membrane of bacteria, improving permeability of other compounds.[341] Instead, polymyxin B may be decreasing bacterial growth and metabolism without improving beta-lactam activity on cell wall synthesis.[338] Alternatively, polymyxin B may have increased beta-lactamase concentration in the growth media by improving the permeability of beta-lactamase out of viable bacterial cells or causing its release upon cell death. Increased extracellular beta-lactamase may result in decreased beta-lactam concentrations, but data suggest a 30% increase in permeability of meropenem in the presence of polymyxin B after accounting for increased extracellular beta-lactamase. However, that data involved polymyxin B administered 20 minutes prior to meropenem (X. Tao et al., presented at ASM Microbe 2018, Atlanta, GA, 8 June 2018).

Conclusions

Although there is not a clear benefit to administering a carbapenem first compared to simultaneously in isolates exhibiting carbapenem MICs of 32 mg/L, a benefit is observable at lower and higher carbapenem MICs (8 and 128 mg/L). Additional data are needed to confirm these findings and to determine optimal staggering time since only a 2-hour delay was evaluated in the present study. Additional data are also needed to determine the mechanism for the decreased antimicrobial activity observed when polymyxin B was administered first relative to simultaneously or when meropenem was administered first.

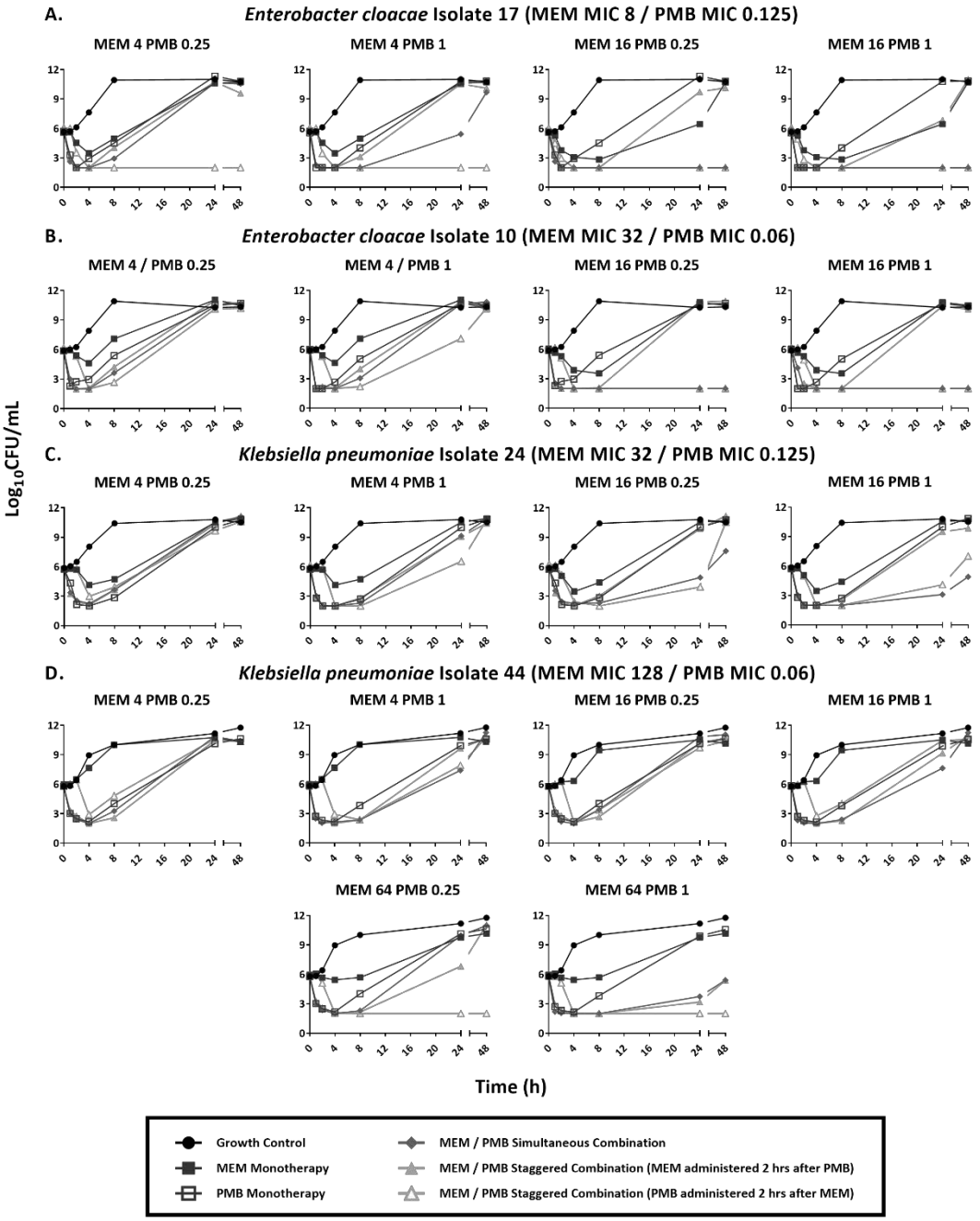

Figure 4.12: Time-kill Curves of Meropenem and Polymyxin B against Carbapenem-resistant *Enterobacteriaceae*. Curves represent geometric means of separate-day duplicate time-kill experiments conducted over 48 hours. Antibiotic concentrations in mg/L are subtitled above their corresponding graph.

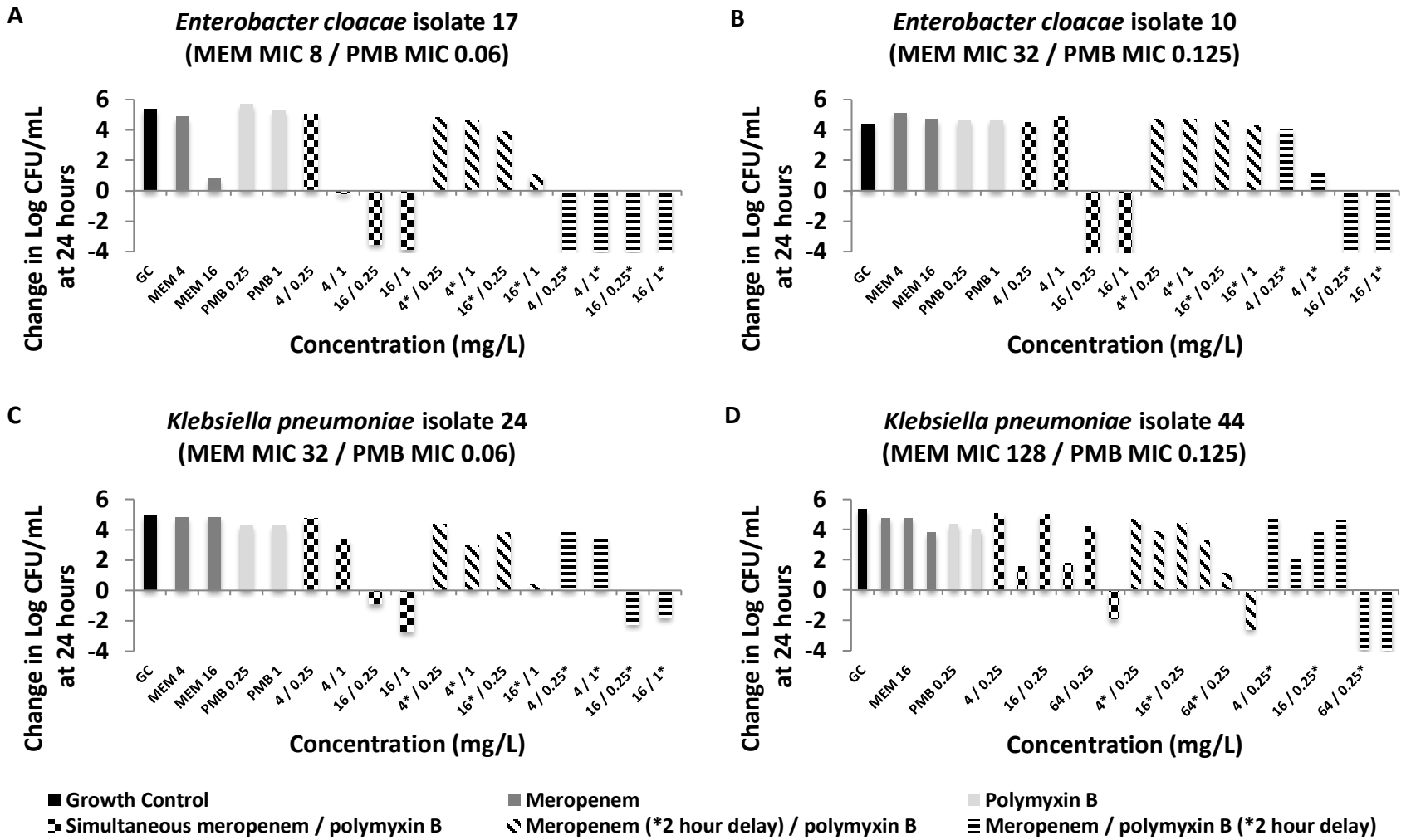

Figure 4.13: Change in Colony Count after 24 Hours of Meropenem and Polymyxin B against Carbapenem-resistant *Enterobacteriaceae*. Bars represent the change in geometric mean colony counts from time 0 to time 24 hours.
GC – Growth Control; MEM – Meropenem; PMB – Polymyxin B.

Killing Activity of Meropenem in Combination with Amikacin against VIM- or KPC-producing *Enterobacteriaceae* that Are Susceptible, Intermediate, or Resistant to Amikacin

Submitted: *Diagnostic Microbiology and Infectious Disease* as a research note

Authors: Brandon Kulengowski, Justin A. Clark, David S. Burgess

Kulengowski contribution: Experimental design, execution, data analysis, drafting, revisions and editing of final manuscript

Abstract

Amikacin is administered with a carbapenem to treat serious infections caused by carbapenem-resistant *Enterobacteriaceae*. The varying degrees of activity of the individual agents corresponds to differences in activity of the two in combination. Amikacin and meropenem are not bactericidal against amikacin-resistant CRE.

Keywords: carbapenem resistant *Enterobacteriaceae*; time kill; meropenem; polymyxin B; amikacin

Introduction

Carbapenem-resistant *Enterobacteriaceae* (CRE) exhibit resistance to most available antimicrobial agents, cause significant mortality, and there is no current treatment standard. Carbapenem-containing combination regimens have been the front-running choice clinically, along with recently approved contenders such as ceftazidime/avibactam and meropenem-vaborbactam which show activity against non-metallo-beta-lactamase-producing CRE.[14] The present study aimed to evaluate the killing activity of amikacin (AMK) in combination with meropenem (MEM) against KPC- or VIM-producing CRE exhibiting moderate resistance to MEM (MICs 16-32 mg/L) and variable activity ranging from susceptible to resistant with AMK (MICs 8-64 mg/L).

Methods

The selection of isolates and time-kill methodology has been previously described.[275] In brief, CRE isolates were identified as part of routine patient care and the carbapenemase gene was confirmed by the Verigene® system. MICs of AMK and MEM were determined by broth microdilution according to CLSI guidelines and four isolates were chosen for further study. A VIM-producing AMK susceptible isolate (MIC 8 mg/L; isolate 169), a KPC-producing AMK susceptible isolate (MIC 16 mg/L; isolate 32), a KPC-producing AMK intermediate isolate (MIC 32 mg/L; isolate 22), and a KPC-producing AMK resistant isolate (MIC 64 mg/L; isolate 37) were selected. The first three isolates exhibited MEM MICs of 16 mg/L, and the AMK resistant isolate had an MEM MIC of 32 mg/L. Time-kill assays were performed in at least duplicate using clinically achievable

concentrations with typical human dosing regimens. AMK alone (8 and 16 mg/L), MEM alone (4 and 16 mg/L), and all possible combinations were evaluated.[342,343] Standard definitions of bactericidal activity, bacteriostatic activity, regrowth, synergy, indifference, and antagonism were utilized.[275]

Results

AMK and MEM, when used alone, resulted in regrowth except for the highest MEM concentration (16 mg/L) against the VIM-producing isolate, even though the MEM MICs for most isolates were 16 mg/L (Figure 4.14). All combinations maintained bactericidal activity against the two amikacin-susceptible CRE (isolates 169 and 32), and synergy was demonstrated in 5 of 8 combinations (Figure 4.14 and Figure 4.15). Synergy was not determinable in the remaining 3 combinations because the most active single agent was within 2-log CFU/mL of the lower limit of quantification of the laser colony counter. Against the amikacin-intermediate isolate, only the highest MEM-AMK combination maintained synergy and bactericidal activity (Figure 4.14 and Figure 4.15). However, no combination maintained bactericidal activity against the amikacin resistant strain, and none were synergistic (Figure 4.14 and Figure 4.15). Antagonism was never observed with any isolate.

Discussion

These data suggest that amikacin and meropenem are only reliably synergistic and bactericidal when bacterial strains are susceptible to amikacin (MICs ≤16 mg/L) and are exhibiting meropenem MICs of at most 16 mg/L. Previous data with polymyxins in combination with a carbapenem have suggested that *in vitro* killing activity (bactericidal activity and synergy) is dependent on the carbapenem MIC.[274] We have not observed a similar relationship for amikacin in combination with meropenem against other amikacin susceptible isolates.[275] Similar to the present study, Le et al. demonstrated synergy and bactericidal activity maintained in 2 of 3 KPC-3-producing amikacin intermediate isolates, but not in the 1 amikacin resistant isolate. Unlike our study, Le et al. did not analyze amikacin susceptible isolates near the CLSI susceptibility breakpoint (16 mg/L). Interestingly, the rate of killing was faster in the present study with maximal killing around 2-4 hours except in the VIM-1-producing isolate where maximal killing was around 8 hours (Figure 4.14). In the study by Le et al., maximal killing occurred around 8-12 hours, but meropenem MICs were reportedly higher (≥32 mg/L).[217] We have previously observed slower killing rates as meropenem MIC increases with no change in rates of bactericidal activity or synergy in carbapenem-resistant *Enterobacter cloacae*.[275] Others have also reported *in vitro* synergy of amikacin and a carbapenem using an Etest® strip interaction assay against carbapenem-resistant *K. pneumoniae*. The improved activity of doripenem was found to be dependent on the susceptibility phenotypes of each drug alone since doripenem became significantly more active with the addition of amikacin only in amikacin-susceptible strains and not amikacin-resistant strains.[344] Another study has quantified the MIC-lowering effect of amikacin on doripenem and concluded that the

addition of amikacin to doripenem lowers doripenem MICs by 8-16 fold against KPC-producing *K. pneumoniae.*[345]

Conclusions

Amikacin in combination with meropenem demonstrates not only synergy, but important bactericidal activity against amikacin-susceptible CRE, including VIM-producing CRE. Additional data are needed to ascertain the mechanism of reduced rates of killing against VIM-producing CRE and KPC-producing CRE with elevated carbapenem MICs (i.e. >32 mg/L). Additional data are also warranted in other MBL-producing CRE.

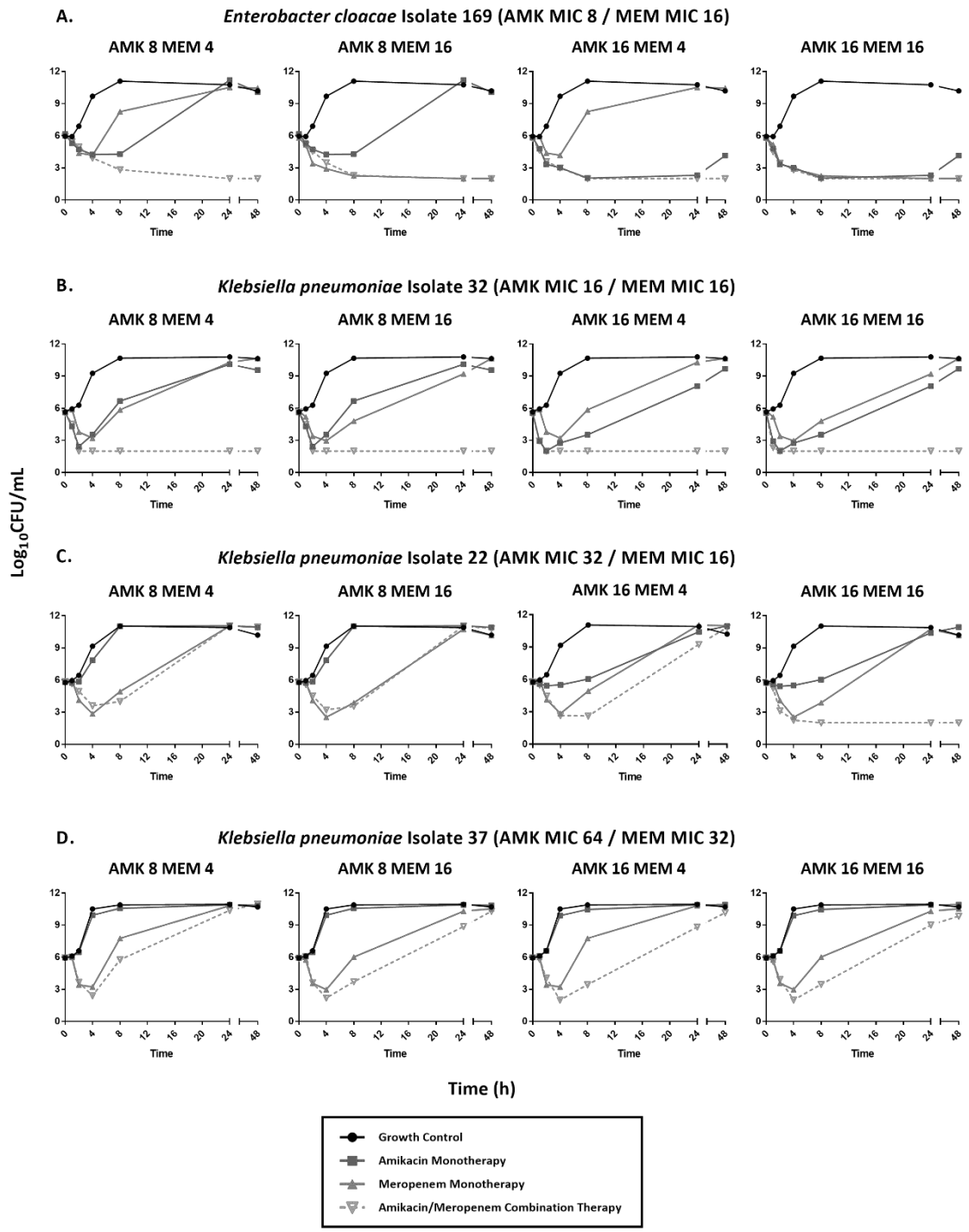

Figure 4.14: Time Kill Curves of Meropenem and Amikacin Alone and in Combination against Carbapenem-resistant *Enterobacteriaceae*. Filled circles represent growth controls. Filled squares represent amikacin alone. Filled triangles represent meropenem alone. Inverted hollow triangles represent amikacin and meropenem in combination. Data points are geometric means of replicate experiments (n = 2-3). The lower limit of quantification was 10^2 CFU/mL.

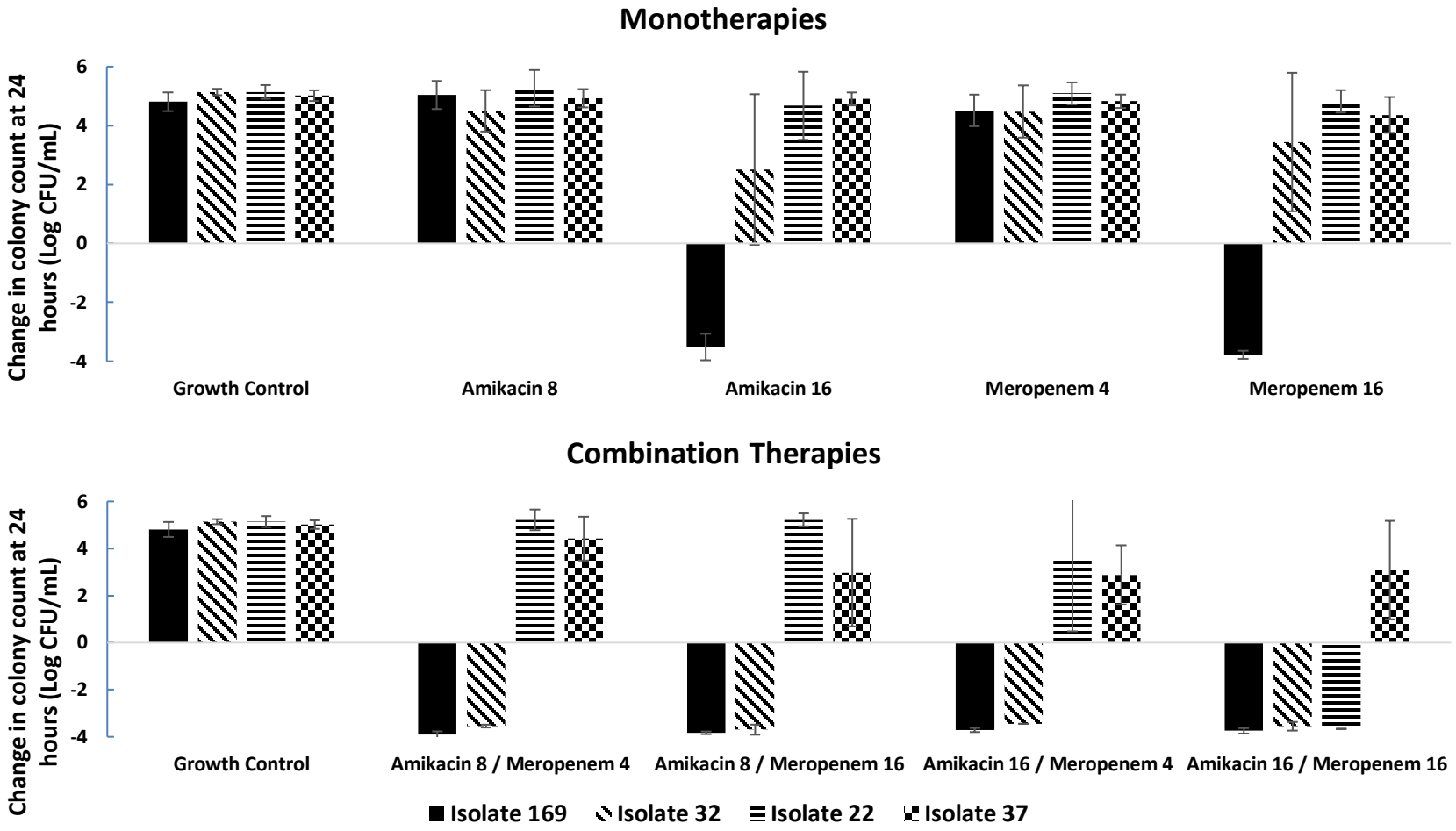

Figure 4.15: Twenty-four Hour Change in Colony Count for Meropenem and Amikacin Alone and in Combination against Carbapenem-resistant *Enterobacteriaceae*. Data are differences of geometric means at time points 0h and 24h with standard deviations as error bars of replicate experiments (n=2-3).

Effect of Increasing Meropenem MIC on the Killing Activity of Meropenem in Combination with Amikacin or Polymyxin B against MBL- and KPC-producing *Enterobacter cloacae*

Published: *Diagnostic Microbiology and Infectious Disease.* 2018. 92(3):262-266. Full manuscript

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Kulengowski contribution: Experimental design, execution, data analysis, drafting, revisions and editing of final manuscript

Special Note: In this manuscript, isolates were renamed according to meropenem MIC for reader reference. However, it should be known for the remainder of the dissertation that these isolates will be referred to by their original numbers. Specifically: EC 2 is isolate 17, EC 8 is isolate 19, EC 16 is isolate 40, and EC 32 is isolate 10.

Abstract

Carbapenem resistant *Enterobacteriaceae* (CRE) are a growing threat worldwide. Infections caused by these organisms have exhibited high rates of mortality (50%) for which there is no standard of care and a dearth of clinical trials. Most *in vitro* data on CRE focus on *Klebsiella pneumoniae*, but it is known that effective therapy may depend on species or even strain. To address this, meropenem, amikacin, and polymyxin B alone and in combination were evaluated by time kill against four carbapenem-producing *Enterobacter cloacae* clinical isolates representing a range of meropenem nonsusceptibility (2-32 mg/L) and resistance mechanisms (KPC 2 and/or VIM 1). As meropenem minimum inhibitory concentration (MIC) increased, bactericidal activity and synergy were maintained for 48 hours in isolates exposed to meropenem and amikacin, but synergy and bactericidal activity were not maintained in all isolates exposed to meropenem and polymyxin B.

Keywords: carbapenem resistant *Enterobacteriaceae* / time kill / meropenem / polymyxin B / amikacin

Introduction

Most antimicrobial agents have limited activity against carbapenem resistant *Enterobacteriaceae* (CRE).[14] Therefore, infections caused by these organisms typically require treatment with two or more antimicrobial agents.[203,346] Most commonly, a carbapenem in combination with a polymyxin (colistin or polymyxin B) is administered, but mortality remains relatively high.[14] Aminoglycosides are also a viable option for combination therapy, but have highly variable *in vitro* activity ranging from 2 to 80%, and very limited clinical experience.[14,347,348] However, we have observed amikacin (AMK) susceptibility as high as 95% at our tertiary academic medical center. Furthermore, carbapenem MICs of *K. pneumoniae* ≥16 mg/L have been associated with higher mortality in carbapenem and polymyxin combinations against *K. pneumoniae,* but not reported for carbapenem and polymyxin or amikacin combinations against *E. cloacae*.[200] The aminoglycoside MIC may also contribute to the activity of aminoglycoside and carbapenem combinations, but most clinical experience has been with gentamicin rather than amikacin.[349] Therefore, we investigated the *in vitro* killing of meropenem (MEM) alone and in combination with polymyxin B (PMB) or AMK against carbapenem-producing *E. cloacae* with varying MICs of MEM and varying resistance mechanisms (KPC 2 and VIM 1).

Material and Methods

Carbapenemase-producing *E. cloacae* (EC) clinical isolates were identified by modified Hodge test as part of routine clinical care at the University of Kentucky

HealthCare clinical microbiology laboratory. IRB approval was obtained and the requirement for informed consent was waived. MIC testing for MEM, PMB, and AMK were performed using broth microdilution according to CLSI guidelines.[262] Antimicrobial agents were obtained from Sigma Aldrich® (St. Louis, MO). CLSI does not have approved breakpoints or epidemiological cutoff values for PMB in *Enterobacteriaceae*, so the epidemiological cutoff value of 2 mg/L for colistin was used for PMB. However, all isolates had polymyxin B MICs ranging 0.06-0.125 mg/L. Four AMK susceptible strains with low PMB MICs (EC 2, EC 8, EC 16, and EC 32; Table 4.17) representing a wide range of MEM MICs were selected for time kill studies and for whole genome sequencing (WGS) using the MiSeq platform (Illumina) by the University of Kentucky Genomics Core Laboratory.

Time kill studies of MEM, AMK, and PMB alone and in combination (MEM with PMB and MEM with AMK) were performed over 48 hours using cation adjusted Mueller Hinton broth according to CLSI guidelines with a starting inocula of 10^6 CFU/ml for each isolate.[350] Clinically achievable concentrations with typical human dosing regimens were evaluated for MEM (4 and 16 mg/L),[342] AMK (8 and 16 mg/L),[343] and PMB (0.25 and 1 mg/L)[351] alone and in combination. The highest combinations (MEM 16 mg/L with PMB 1 mg/L and MEM 16 mg/L with AMK 16 mg/L) were not evaluated because of complete killing observed using combinations with lower concentrations. Separate day, replicate time kill assays were performed for each isolate, sampling at 0, 1, 2, 4, 8, 24, and 48 hours. Appropriately diluted aliquots were logarithmically plated onto Mueller Hinton agar using a spiral plater (Advanced Instruments, Norwood, MA), which controlled for antibiotic carryover.[277] A laser colony counter (Advanced Instruments, Norwood, MA) quantified

samples with a lower limit of quantification of 10^2 CFU/mL. Central zones of inhibition were not observed on agar plates for single or combination antimicrobials, so it was assumed that antibiotic carryover of the combinations were also controlled for by the spiral plater and laser colony counter.[277]

Bacteriostatic activity was defined as a <3 $\log_{10}$ CFU/mL decrease in colony count. Bactericidal activity was defined as a >3 $\log_{10}$ CFU/mL decrease in colony count. Regrowth was defined as an increase in colony count from a previous time point. Synergism was defined as a ≥ 2 $\log_{10}$ CFU/mL lower colony count at 24 hours compared to the most active agent alone. Indifference was defined as a change in colony count at 24 hours within 2 $\log_{10}$ CFU/mL compared to the most active agent alone. Antagonism was defined as ≥ 2 $\log_{10}$ CFU/mL higher colony count at 24 hours compared to the most active agent alone.[350]

Barcoded Nextera Libraries (Illumina, San Diego, CA) were generated according to manufacturer protocol. Following PCR, the amplified products were purified using AMPure XP beads (Beckman Coulter, Indianapolis, IN) where the sizes and concentrations of the amplification products were determined using the Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA). Library quality was assessed via qPCR using the KAPA library quantification kit (KAPA Biosystems, Wilmington, MA). Sequence data (250 bp, paired end reads) were acquired using the MiSeq platform (Illumina, San Diego, CA). Genome assemblies were generated with Newbler v2.9, in paired end mode and using default parameters. The presence and absence of known resistance mechanisms for each isolate were identified by BLAST against two databases, ResFinder and ARG ANNOT (Antibiotic Resistance Gene ANNOTation) following WGS.[284]

Results

Susceptibilities for all four strains are listed in Table 4.17. In summary, all isolates had low PMB MICs (MICs: 0.06 – 0.125 mg/L); all isolates were AMK susceptible (MICs: 1 – 16 mg/L); and all isolates were meropenem nonsusceptible (MICs: 2 – 32 mg/L). Each isolate belonged to a unique multi locus sequence type (MLST; Table 4.17) and numerous antimicrobial resistance genes were identified (Table 4.18). Concerning carbapenemase genes, we identified $bla_{KPC\ 2}$ alone in isolates EC 2 and EC 8, but $bla_{VIM\ 1}$ in EC 16. We detected both $bla_{KPC\ 2}$ and $bla_{VIM\ 1}$ in the most resistant isolate, EC 32. Other beta lactamase genes were present in these isolates as well (Table 4.18). No polymyxin resistance genes were identified by either ResFinder or ARG ANNOT (e.g. mcr 1). Numerous aminoglycoside modifying enzyme (AME) genes were detected in each isolate, and we report those capable of modifying amikacin based on site of activity and presence of appropriate amino or hydroxyl functional groups (Table 4.18).

Regrowth was observed by 24 hours for all monotherapy except for the highest meropenem concentrations tested against 2 of 4 isolates (Figure 4.16). Specifically, MEM alone at 4 and 16 mg/L was bacteriostatic, resulting in regrowth by 8 hours for all isolates except for MEM 16 mg/L with EC 2 and EC 16. For these two, bactericidal activity was observed by 8 hours and maintained throughout 48 hours. PMB alone at 0.25 and 1 mg/L was rapidly bactericidal for all isolates by 2 hours, but regrowth was observed by 8 hours in all instances. AMK 8 and 16 mg/L alone was bactericidal by 4 hours for the isolates with the lower AMK MICs (EC 2 and EC 32) but bacteriostatic for the isolates with the higher AMK MICs (EC 8 and EC 16). However, regrowth was observed by 24 hours for all isolates when AMK was used alone (Figure 4.16).

All combinations of MEM (4 or 16 mg/L) and AMK (8 or 16 mg/L) achieved bactericidal and synergistic activity against all isolates (Figure 4.17). However, synergy was indeterminate for MEM 16 mg/L combinations with EC 2 and EC 16 due to the excellent activity of MEM 16 mg/L alone. Higher meropenem MICs trended with slower killing activity, but bactericidal activity and synergy were maintained throughout 48 hours for all MEM and AMK combinations (Figure 4.16). Specifically, the isolate with the lowest meropenem MIC (EC 2) reached bactericidal activity by 2 hours whereas the isolates with the higher meropenem MICs (EC 16 and EC 32) reached bactericidal activity by 8 hours.

Bactericidal activity and synergy were observed for most combinations of MEM (4 or 16 mg/L) and PMB (0.25 or 1 mg/L) against each isolate, but not all for every isolate (Figure 4.17). All combinations of MEM (4 or 16 mg/L) and PMB (0.25 or 1 mg/L) achieved bactericidal activity, and synergy was observed for all MEM and PMB combinations against EC 2 and EC 16 (except indeterminate interaction with MEM 16 mg/L). However, synergy was observed only for the higher concentrations of the combinations (MEM 4 mg/L with PMB 1 mg/L and MEM 16 with PMB 0.25 mg/L) against EC 8, and the combination with the highest meropenem concentration (MEM 16 with PMB 0.25 mg/L) against the most MEM resistant isolate, EC 32 (Figure 4.17). Bactericidal activity among all isolates was only maintained for the higher MEM 16 with PMB 0.25 mg/L whereas regrowth was observed by 48 hours for combinations with lower concentrations for all isolates (Figure 4.16). Unlike in the MEM AMK combinations, the rate of killing was similar among isolates, and synergistic interactions for all combinations were not observed, specifically when the MEM MIC was ≥8 mg/L. Additionally,

bactericidal activity was only maintained for isolates when the MEM concentration was at least 16 mg/L or the PMB concentration was at least 1 mg/L.

Discussion

Carbapenemase genes were identified in each isolate studied, contributing to meropenem resistance. Other extended spectrum beta lactamase genes were also detected concurrently with the carbapenemase genes, particularly among the isolate with the highest meropenem resistance (EC 32; Table 4.18). Additionally, KPC-producing *E. cloacae* exhibited lower MEM MICs – 2 and 8 mg/L – than VIM-producing *E. cloacae* – 16 and 32 mg/L. The lack of detection of polymyxin B resistance genes supports MICs measured as 0.06 or 0.125 mg/L. Numerous genes that can modify amikacin were present in each isolate, but all isolates were susceptible. This study did not query gene expression levels for any resistance gene tested, which may have indicated silencing or upregulation of resistance mechanisms. Furthermore, the number of resistance genes did not correlate with increasing amikacin MICs given the isolate with the lowest amikacin MIC (EC 32, AMK MIC 1 mg/L) had all the amikacin resistance genes detected (Table 4.18). This may suggest differences in relative upregulation of these genes in other isolates.

Monotherapy with meropenem, polymyxin B or amikacin against CRE that are amikacin susceptible with low polymyxin MICs fails to maintain bactericidal activity throughout 48 hours, with regrowth occurring between 2-24 hours. Combination therapy with either meropenem and polymyxin B or meropenem and amikacin resulted in synergistic interactions and, importantly, sustained bactericidal activity. The AMK MIC

(compared to the MEM MIC) appeared to play a less significant role in the initial rate of killing for meropenem amikacin combinations given the isolate most susceptible to AMK (EC 32, AMK MIC 1 mg/L) had the slowest rate of killing and the isolate least susceptible to AMK (EC 8, AMK MIC 32 mg/L) had the second fastest rate of killing measured by 4h bactericidal activity (Figure 4.16). The synergistic interaction was not influenced by AMK MIC or MEM MIC for MEM in combination with AMK. Conversely, the synergistic activity of MEM and PMB was impacted by increased MEM MICs (Figure 4.17), whereas the rate of killing was not impacted by the MEM MICs (Figure 4.16). The PMB MICs were essentially the same among isolates so conclusions about PMB MIC dependence of the interaction could not be drawn. The observed regrowth in the combinations may indicate selection through antimicrobial pressure for resistant subpopulations, which has been observed in CRE populations by others.[140] Alternatively, the mechanism of resistance could be adaptive where environmental stimuli (e.g. polymyxin exposure) alters the outer membrane, conferring resistance.[137,140]

Additionally, a limitation of this study is that stability data for these antimicrobials in such an experiment are not well described. Meropenem is more likely impacted than amikacin or polymyxin B given its shorter stability time clinically, but the degree to which meropenem has degraded over 48 hours is unknown. Therefore, it is possible that the decreased antimicrobial concentrations could result in regrowth at later time points. It is worth mentioning that a similar limitation applies to broth dilution antimicrobial susceptibility studies which are the gold-standard for determining MICs, so the concentration exposures for the first 24 hours of the present experiment mimic that of broth dilution exposures. In only two cases (both meropenem-polymyxin B combinations) was

regrowth above the point of bactericidal activity observed at 48 hours where bactericidal killing had been maintained at 24 hours (Figure 4.16), and this does not impact rates of synergy discussed previously.

Bactericidal activity and synergy for polymyxins or aminoglycosides in combination with carbapenems against CRE have been described previously, but *K. pneumoniae* has typically been the representative isolate.[212,217] However, interactions between antimicrobial agents have exhibited a degree of dependence on bacterial strain.[14,195] Therefore, studies evaluating these antimicrobial combinations in CRE other than *K. pneumoniae* are important, and there are limited studies exploring amikacin or polymyxin B in combination with a carbapenem in *E. cloacae*. Cai et al. investigated polymyxin B combinations in four extensively drug resistant *E. cloacae* isolates harboring metallo beta lactamase enzymes NDM 1 or IMP 1.[352] More specifically, meropenem and polymyxin B were evaluated at 64 and 2 mg/L in combination by time kill, and bactericidal activity was only observed for 2 of 4 isolates at 24 hours. Regrowth was observed in all isolates by 24 hours. In contrast, we observed sustained bactericidal activity and synergy without regrowth throughout 48 hours when meropenem 16 was used in combination with polymyxin 0.25 mg/L for all four of our study isolates. However, the isolates in this study were KPC 2- and/or VIM 1-producing as opposed to NDM 1- or IMP 1- producing. In agreement with the present study, Barth et al. found polymyxin B in combination with meropenem for two KPC 2-producing *E. cloacae* to be bactericidal and synergistic at concentrations as low as 4 mg/L of meropenem and 0.5 mg/L of polymyxin, despite the meropenem MICs being higher than the present study.[330] Le et al. describes synergy maintained for 24 hours for four KPC 3-producing *K. pneumoniae* and bactericidal activity maintained for 24 hours for two KPC

3-producing *K. pneumoniae* for amikacin in combination with meropenem, which also supports the present findings.[217]

In contrast to the present *in vitro* results, a recently published open-label clinical trial for gram-negative bacteria suggests there is no difference in 14-day clinical failure for colistin monotherapy compared to colistin-meropenem combination therapy.[339] However, a majority (77%) of isolates were *Acinetobacter baumannii* and the trial was not powered to address monotherapy versus combination therapy with other bacterial isolates. Other trials involving *A. baumannii* have also suggested similar outcomes with colistin in combination with either rifampin or Fosfomycin.[340] However, important differences exist between *A. baumannii* and other gram-negative bacteria such as the virulence changes caused by shedding of lipopolysaccharide or bacterial density which may have implications on the antimicrobial therapy used.[340] We are still awaiting additional clinical trial data of colistin compared to colistin-meropenem against extensively drug resistant gram-negative bacilli (NCT01597973, ClinicalTrials.gov).

Novel agents with activity against KPC-producing *Enterobacteriaceae* have been approved, such as ceftazidime/avibactam and meropenem-vaborbactam, or are being developed (e.g. imipenem/relebactam) and may be more appropriate alternatives given data indicating superior cures rates.[353,354] However, beta-lactamase inhibitors like avibactam, vaborbactam, and relebactam do not inhibit metallo-beta-lactamase (MBL) enzymes, and so clinicians should be aware of their local susceptibility and genetic patterns. Other antimicrobial agents are under development, such as cefiderocol and aztreonam-avibactam, which do have activity against MBL-producing CRE. Also under development, plazomicin

and eravacycline are not beta-lactam antibiotics, but rather an aminoglycoside and a tetracycline, respectively, showing activity against CRE clinically.[353]

Conclusions

PMB or AMK in combination with MEM has bactericidal and synergistic activity against AMK susceptible E. cloacae with low polymyxin B MICs that produce KPC and/or VIM. Sustained bactericidal activity and synergy with MEM in combination with PMB is dependent on the MEM MIC. Bactericidal activity and synergy are unaffected by MEM or AMK MICs when MEM is used in combination with AMK. Additional *in vitro*, animal, and ideally human studies are warranted to further elucidate the impact that the carbapenem MIC has on the activity of a carbapenem in combination with a polymyxin or an aminoglycoside against carbapenem resistant *E. cloacae*, particularly against strains with carbapenem MICs ≥128 mg/L.

Table 4.17: Antimicrobial Activity and Carbapenemase of Selected Organisms

Isolate	MLST	Carbapenemase	MEM MIC (mg/L)	PMB MIC (mg/L)	AMK MIC (mg/L)
EC 2	**88***	**KPC-2**	**2**	**0.06**	**2**
EC 8	**80***	**KPC-2**	**8**	**0.125**	**16**
EC 16	**273***	**VIM-1**	**16**	**0.125**	**4**
EC 32	**484**	**KPC-2, VIM-1**	**32**	**0.06**	**1**

MLST – multilocus sequence type. MIC – minimum inhibitory concentration. MEM – meropenem. PMB – polymyxin B. AMK – amikacin.

*Best matched MLST reported because coverage for one of the housekeeping genes was too low to confirm a novel MLST

Table 4.18: Resistance Genes

	EC 2	*EC 8*	*EC 16*	*EC 32*
β-LACTAM RESISTANCE				
Ambler Class A				
CARB-2				✓
KPC-2	✓	✓		✓
SHV-69			✓	
SHV-73				✓
TEM-1A	✓	✓		
TEM-1B			✓	
TEM-124				✓
TEM-154				✓
Ambler Class B				
VIM-1			✓	✓
Ambler Class C				
ACT-6				✓
ACT-7	✓	✓	✓	✓
FOX-5				✓
Ambler Class D				
OXA-9	✓	✓		✓
AMIKACIN RESISTANCE				
Acetyltransferase				
aac(6')-Ib	✓	✓		✓
aac(6')Ib-cr	✓	✓		✓
aac(6')-IIc			✓	✓
aac(3)-IVa				✓
aac(3)-VIa		✓		✓
Phosphotransferase				
aph(3')-IIa				✓
aph(3')-Ia		✓		✓
Nucleotidyltransferase				
ant(2")-Ia			✓	✓

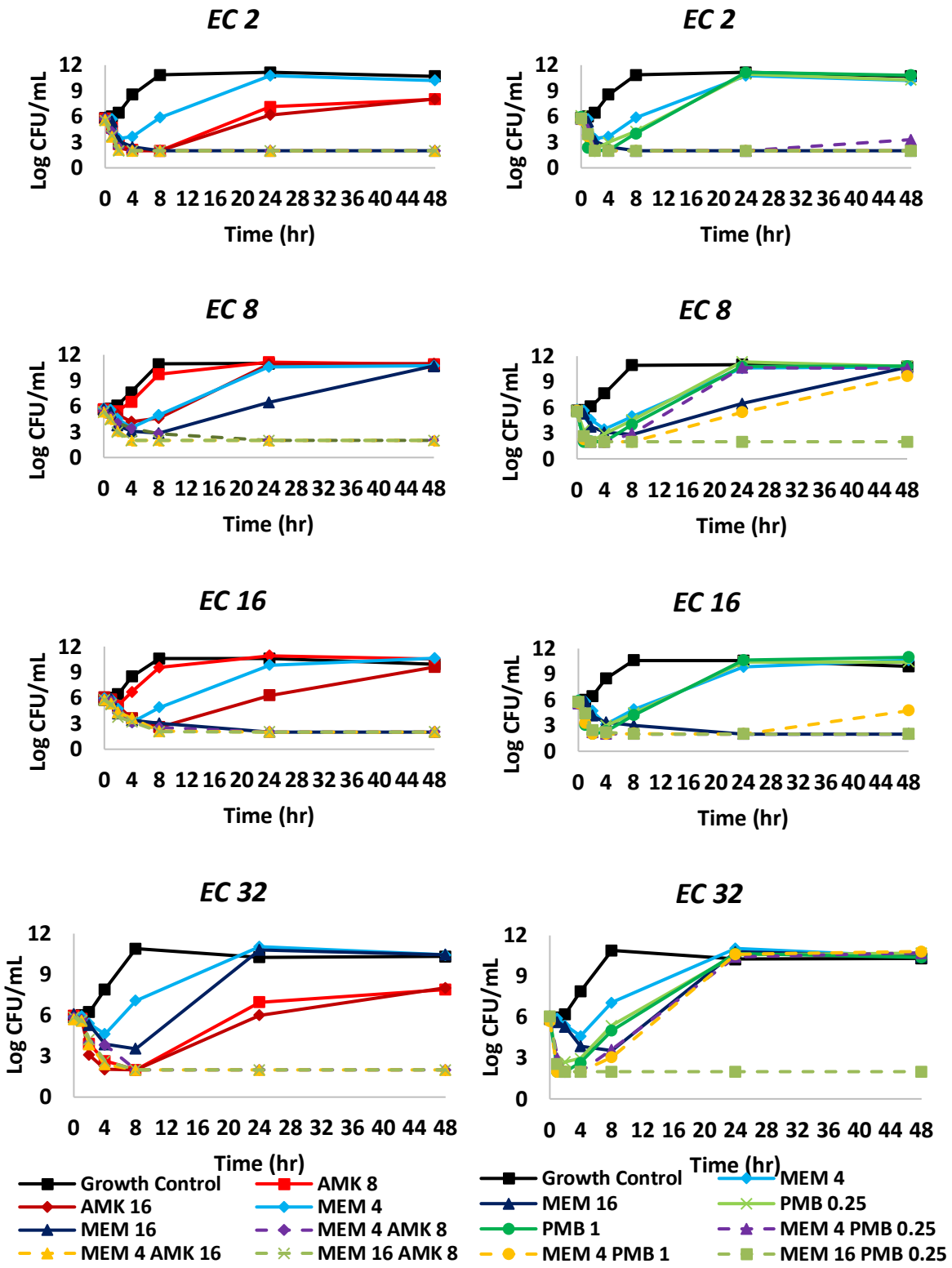

Figure 4.16: Time Kill Curves of Meropenem, Amikacin, and Polymyxin B Alone and in Combination against Carbapenemase-producing *Enterobacter cloacae* Strains. Data points are geometric means of replicate experiments (n = 2). The lower limit of quantification was 10^2 CFU/mL.

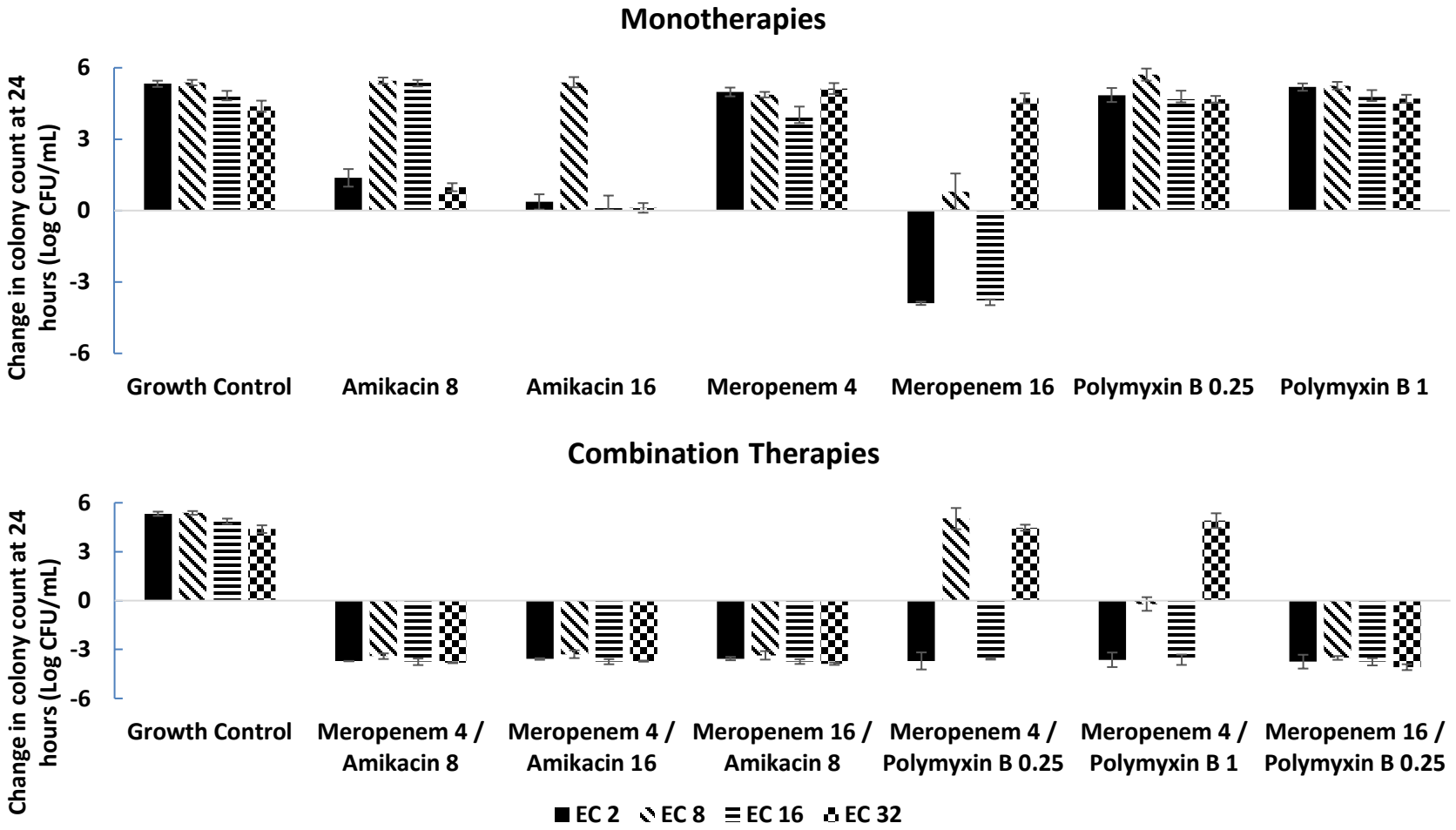

Figure 4.17: Twenty-four Hour Change in Colony Count for Meropenem, Polymyxin B, and Amikacin Alone and in Combination against Carbapenemase-producing *Enterobacter cloacae* Strains. Data are differences of geometric means at time points 0h and 24h with standard deviations of replicate experiments (n=2).

Resistance Development to Polymyxin B

Surviving or regrowing bacteria from time-kill studies often exhibit higher MICs to the antimicrobials to which they were exposed. This can be as a result of antimicrobial pressure which selects for resistant subpopulations or reveals adaptable or development of resistance.[140] MICs were reevaluated for polymyxin B in all regrowing colonies for two time-kill studies through broth microdilution susceptibility testing because others have reported rapid resistance development, attributed mostly to the selection of subpopulations.[137,140]

For surviving colonies, the MIC to polymyxin B increased at least 256-fold (from 0.06 - 0.125 µg/mL to 16 - >64 µg/mL) following exposure to polymyxin B alone in concentrations from 0.06 to 4 µg/mL (Table 4.19).

Table 4.19: MIC Testing of Isolates Following Exposure to Polymyxin B Alone

	PMB 0.06 µg/mL	**PMB 0.125 µg/mL**	**PMB 0.25 µg/mL**	**PMB 1 µg/mL**	**PMB 2 µg/mL**	**PMB 4 µg/mL**
KP 34	16	64	*	*	>64	>64
KP 22	>64	0.125	64	>64	>64	>64
KP 24	64	64	32	32	>64	>64
KP 44	64	16	32	64	>64	>64

*Concentration not tested, but growing colonies previously observed

Following exposure to meropenem alone, the polymyxin B MIC of surviving colonies remained in essential agreement (within one two-fold dilution) of the originally measured MIC of the corresponding isolate (0.06 µg/mL for KP 22 and KP 44 and 0.125 µg/mL for KP 34 and 24). This was expected given there was no antimicrobial pressure for the selection of subpopulations resistant to polymyxin B. However, cross-resistance or some other dependency relationship between meropenem and polymyxin B resistance may have explained a change (Table 4.20).

Table 4.20: MIC Testing of Isolates Following Exposure to Meropenem Alone

	MEM 4 µg/mL	**MEM 16 µg/mL**	**MEM 64 µg/mL**
KP 34	*	*	§
KP 22	0.06	0.06	§
KP 24	≤0.03	0.125	0.06
KP 44	0.06	0.06	0.06

*Concentration not tested, but growing colonies previously observed
§No growth of colonies during time-kill studies

Surviving colonies of meropenem and polymyxin B in combination were also evaluated for a change in polymyxin B MIC. Results were similar to exposure to polymyxin B alone (≥256-fold increase in MIC; Table 4.21).

Table 4.21: MIC Testing of Isolates Following Exposure to Combination

	MEM/PMB 4/0.25 µg/mL	**MEM/PMB 4/1 µg/mL**	**MEM/PMB 16/0.25 µg/mL**	**MEM/PMB 16/1 µg/mL**	**MEM/PMB 64/0.25 µg/mL**	**MEM/PMB 64/1 µg/mL**
KP 34	§	§	§	§	§	§
KP 22	§	§	§	§	§	§
KP 24	32	32	§	§	§	§
KP 44	16	16	16	64	16	16

[§]No growth of colonies during time-kill studies

In summary, the polymyxin B MICs of isolates exposed to polymyxin B alone or in combination with meropenem generally increased from 0.06 or 0.125 µg/mL at baseline to ≥16 µg/mL in all experiments where regrowth occurred. In contrast, the polymyxin B MICs for regrowing bacteria exposed only to meropenem remained at 0.06 or 0.125 µg/mL (Table 4.19, Table 4.20, and Table 4.21).

Subpopulation Analysis

To assess for the presence of subpopulations with increased MIC values relative to the overall population, growing bacteria were cultured onto agar plates infused with antimicrobials which would inhibit the growth of all colonies with MICs less than or equal to the antimicrobial concentration of the agar. Colonies were enumerated and then related to the overall population (see Subpopulation Analysis and Microfiltration). Table 4.22 summarizes the results of the growth control subpopulation time-kill study. The lower limit of quantification for microfiltration has been previously estimated to be 30 CFU/mL when 1000 µL samples were used.[282,283]

Table 4.22: Hetero-resistant Subpopulations of *Klebsiella pneumoniae* Isolates

	KP 34	KP 22	KP 24	KP 44
MEM MIC > 16	**7.2 CFU/mL**	**31.6 CFU/mL**	-	-
MEM MIC > 64	-	-	**6.7 CFU/mL**	**5.3 x 10^3 CFU/mL**
PMB MIC > 4	**<1 CFU/mL**	**2 CFU/mL**	**<1 CFU/mL**	**2.4 CFU/mL**

All reported values are proportionally corrected to a 10^6 CFU/mL overall population

For the isolates with meropenem MICs of 4 and 16 µg/mL, subpopulations with MICs > 16 µg/mL were 7 x 10^0 and 3 x 10^1 CFU/mL, respectively. For the isolates with meropenem MICs of 32 and 128 µg/mL, subpopulations with MICs > 64 µg/mL were 7 x 10^0 and 5 x 10^3 CFU/mL, respectively. Surprisingly, for all isolates, subpopulations with polymyxin B MICs > 4 µg/mL were almost non-detectable at < 3 CFU/mL.

Heteroresistance

KP 34, 22, 24, and 44 underwent preliminary characterization of the meropenem and polymyxin B MICs of any heteroresistant subpopulations. Table 4.22 displays the exact colony counts with respect to a 10^6 CFU/mL total population concentration. For a more complete subpopulation analysis, studies with higher inocula would provide higher sensitivity but would reduce the internal validity of comparing observations from the subpopulation study to our time-kill results, which was the primary purpose. The most interesting result was observing subpopulations with MICs at least 32 x the MIC of the total population (e.g. a strain with a population MIC of 0.125 µg/mL growing on an agar plate with 4 µg/mL polymyxin B). Better characterization of these heteroresistance isolates may elucidate the cause of regrowth observed throughout our experiments when polymyxin B was used alone or when combination therapy failed to prevent regrowth.

Other studies have also described polymyxin heteroresistance.[140] Among the four studies recently discussed, only Lee *et al.* reported post-exposure susceptibility testing on their regrowing isolates from time-kill studies. Similar to the present study, Lee *et al.* observed an increase in colistin or polymyxin B MIC from 0.125 - 0.25 µg/mL to 8 - 128 µg/mL whereas we observed changes from 0.06 - 0.125 µg/mL to 16 - >64 µg/mL. Lee *et al.* also observed no change in doripenem MICs following polymyxin exposure whereas we did not look at change in meropenem MICs. However, we observed no change in polymyxin MICs following meropenem exposure, suggesting insignificant (if any) cross-resistance between these antimicrobial classes. However, both studies observed polymyxin B and colistin MICs correlating very strongly together.[332]

Meletis *et al.* performed more thorough subpopulation studies on 16 carbapenemase-producing *K. pneumoniae* clinical isolates. In that study, subpopulations growing on agar plates impregnated with up to 8 μg/mL colistin had population MICs ranging from 1 - 4 μg/mL. Susceptibility testing on colonies growing on these agar plates ranged from 16 - 64 μg/mL, which was similar to both Lee *et al.* and our study, although we analyzed polymyxin B rather than colistin. The colony counts growing on agar plates containing colistin 8 μg/mL ranged from 3×10^0 to 4×10^3 CFU/mL whereas the colony counts we observed on agar plates containing 4 μg/mL polymyxin B was closer to 2×10^0 CFU/mL. Accounting for this difference may be the difference in the MIC of the populations since this study analyzed strains with much lower polymyxin B/colistin MICs (0.06 - 0.125 μg/mL) whereas Meletis *et al.* analyzed strains with colistin MICs $\geq$ 1 μg/mL. In other words, more similar colony counts may be observed in our isolates if we were to utilize agar plates impregnated with similar proportions of polymyxin B/colistin such as 0.5 - 1 μg/mL (approximately 4 - 8 x MIC).

Meletis *et al.* noticed that about 8 of 16 isolates did not exhibit heteroresistance which was demonstrated by a lack of growth on agar plates with colistin concentrations exceeding the colistin MIC of the population.[140] This observation may explain the differences observed between Lee *et al.*, our study, and Bratu *et al.* concerning regrowth with regimens containing either polymyxin alone or in combination. If heteroresistance impairs the synergistic interaction between polymyxins and carbapenems, then a lack of heteroresistance would explain the complete killing or indeterminate synergy observed by Bratu *et al.* since polymyxins alone seemed to be sufficient in preventing growth at concentrations above the MIC.[140] There may also be clinical relevance to these findings

such that regions where heteroresistance to polymyxins is low may demonstrate superior colistin/polymyxin B activity when compared to regions with abundant heteroresistance, confounding comparisons of polymyxin monotherapy and combination therapy.

Conclusions

Agar plates with lower polymyxin concentrations would better characterize the heteroresistance exhibited by KP 34, 22, 24, and 44, but we as well as others have observed wide variability in polymyxin MICs, even among the same strains.[140,332] The clinical role of polymyxin heteroresistance is not known at this time, but it is suspected to impair the synergistic interaction between carbapenems and polymyxins. Finally, not all carbapenem-producing *Klebsiella pneumoniae* have observable polymyxin heteroresistance.[140]

Ceftazidime/avibactam Disk Diffusion and MBL Etest®

Ceftazidime/avibactam is a recently approved antimicrobial for the treatment of CRE in the absence of MBL-production. In order to contribute to the growing knowledge of this novel antimicrobial agent, we evaluated the susceptibility of all CRE that we had collected to date (75 out of 164 isolates).

Metallo-β-lactamase (MBL)-production information from the University of Kentucky Clinical Microbiology Laboratory was available for four of the 75 isolates tested (isolates 26, 40, 41, and 42). Since metallo β-lactamases are not inhibited by avibactam, it was expected that these isolates would be resistant to ceftazidime/avibactam, but only three of four were resistant, verified by duplicate experiments.

Excluding the four known MBL-producers, six isolates met criteria for MBL testing. Three isolates were borderline susceptible to ceftazidime/avibactam with a zone of inhibition of 21 mm (isolates 21, 24, and 29), two isolates were resistant (isolates 53 and 134 with zones 17 mm and 15 mm, respectively) and one isolate was intermediate (22; zone 20 mm). In a duplicate experiment, only the isolate testing as intermediate changed in interpretation with the second test and the third test being susceptible (23 mm and 24 mm respectively; Table 4.23).

Table 4.23: Ceftazidime/avibactam Kirby Bauer Disk Diffusion Results

Organism	Isolate Number	Ceftazidime / Avibactam Zone of Inhibition (mm)	Interpretation (S/I/R)
Citrobacter amalonaticus	36	24	S
Citrobacter amalonaticus	91	25	S
Citrobacter freundii	27	29	S
Citrobacter freundii	50	22	S
Citrobacter freundii	54	30	S
Citrobacter freundii	101	27	S
Citrobacter freundii	127	27	S
Citrobacter freundii	145	31	S
Citrobacter freundii	147	30	S
Citrobacter youngae	136	26	S
Enterobacter aerogenes	97	30	S
Enterobacter cloacae	9	27	S
Enterobacter cloacae	10	28	S
Enterobacter cloacae	16	27	S
Enterobacter cloacae	17	27	S
Enterobacter cloacae	19	29	S
Enterobacter cloacae	20	27	S
Enterobacter cloacae	30	25	S
Enterobacter cloacae	39	27	S
Enterobacter cloacae*	**40**	**17, 16**	**R**
Enterobacter cloacae*	**41**	**18, 17**	**R**
Enterobacter cloacae	52	27	S
Enterobacter cloacae[§]	**53**	**17, 18**	**R**
Enterobacter cloacae	70	29	S
Enterobacter cloacae	96	26	S
Enterobacter cloacae	107	24	S
Enterobacter cloacae	121	25	S
Enterobacter cloacae	126	28	S
Enterobacter cloacae[§]	**134**	**15, 14**	**R**
Enterobacter cloacae	144	27	S
Escherichia coli	25	30	S
Escherichia coli	33	25	S
Escherichia coli	103	25	S
Enterobacter gergoviae	13	28	S
Enterobacter gergoviae	95	32	S
Enterobacter spp.	146	25	S
Klebsiella oxytoca	8	35	S
Klebsiella oxytoca	14	29	S
Klebsiella ozaenae	128	28	S
Klebsiella pneumoniae	7	32	S
Klebsiella pneumoniae[§]	21	21, 21	S

Table 4.23 (continued): Ceftazidime/avibactam Kirby Bauer Disk Diffusion Results

Organism	Isolate Number	Ceftazidime / Avibactam Zone of Inhibition (mm)	Interpretation (S/I/R)
***Klebsiella pneumoniae*§**	22	20, 23, 24	S
***Klebsiella pneumoniae*§**	24	21, 27	S
Klebsiella pneumoniae*	26	27, 26	S
Klebsiella pneumoniae	28	22	S
***Klebsiella pneumoniae*§**	29	21, 21	S
Klebsiella pneumoniae	31	31	S
Klebsiella pneumoniae	32	33	S
Klebsiella pneumoniae	34	28	S
Klebsiella pneumoniae	35	29	S
Klebsiella pneumoniae	37	26	S
Klebsiella pneumoniae*	**42**	**14, 14**	**R**
Klebsiella pneumoniae	43	25	S
Klebsiella pneumoniae	44	24	S
Klebsiella pneumoniae	45	24	S
Klebsiella pneumoniae	46	27	S
Klebsiella pneumoniae	47	27	S
Klebsiella pneumoniae	48	25	S
Klebsiella pneumoniae	49	27	S
Klebsiella pneumoniae	51	25	S
Klebsiella pneumoniae	55	23	S
Klebsiella pneumoniae	69	25	S
Klebsiella pneumoniae	77	30	S
Klebsiella pneumoniae	93	27	S
Klebsiella pneumoniae	98	23	S
Klebsiella pneumoniae	99	25	S
Klebsiella pneumoniae	105	27	S
Klebsiella pneumoniae	116	27	S
Klebsiella pneumoniae	119	25	S
Klebsiella pneumoniae	123	28	S
Klebsiella pneumoniae	129	23	S
Klebsiella pneumoniae	130	27	S
Klebsiella pneumoniae	142	26	S
Klebsiella pneumoniae	143	24	S
Klebsiella pneumoniae	152	25	S
TOTAL	**75**		**93% S**

*MBL identified by PCR at University of Kentucky Clinical Microbiology Laboratory
§MBL identified by Etest®

MBL-production was identified in all but one isolate tested (KP 24 was indeterminate twice; confirmed negative by the Verigene® system). MBL-production was identified in two borderline susceptible isolates (21 and 29), the initially intermediate isolate (KP 22), and both resistant isolates (53 and 134; Table 4.11). KP 24 was indeterminate upon initial testing because the resulting MIC ratio was ≥ 4 without a phantom zone or an ellipse deformation (Chapter 3: Methods "Etest® Procedure"). Isolate 21 was borderline negative after initial MBL testing but tested positive upon retest. All other isolates were interpreted as positive for both tests (Table 4.11).

Table 4.24: MBL MP/MPI Etest® Results

Isolate	MP/MPI MIC (µg/mL)	Interpretation	MP/MPI MIC (µg/mL)	Interpretation
21	**0.5 / 0.064**	**Negative**	**1.5 / 0.094**	**Positive**
22	**> 8 / ≤ 0.032**	**Positive**	**3 / 0.032**	**Positive**
24	**> 8 / > 2**	**Indeterminate**	**> 8 / > 2**	**Indeterminate**
29	**> 8 / ≤ 0.032**	**Positive**	**> 8 / ≤ 0.032**	**Positive**
53	**> 8 / ≤ 0.032**	**Positive**	**> 8 / ≤ 0.032**	**Positive**
134	**4 / ≤ 0.032**	**Positive**	**2 / ≤ 0.032**	**Positive**
Negative Control	**≤ 0.0125 / ≤ 0.032**	**Negative**	**≤ 0.0125 / ≤ 0.032**	**Negative**
Positive Control	**1.5 / ≤ 0.032**	**Positive**	**1.5 / ≤ 0.032**	**Positive**

Positive Control - Isolate 42 (Confirmed MBL by PCR)
Negative Control - *Klebsiella pneumoniae* ATCC® 700603

Overall, five of the 75 CRE isolates were resistant to ceftazidime/avibactam (93% susceptibility across all CRE tested), and all five were associated with MBL-production. All four of the MBL-producing *E. cloacae* isolates (40, 41, 53, and 134) were resistant to ceftazidime/avibactam whereas one isolate (42) of the four MBL-producing *K. pneumoniae* isolates (21, 22, 29, and 42) were resistant to ceftazidime/avibactam. Divided by phenotypic (determined by Etest®) compared to genotypic MBL-production (determined by PCR or by the Verigene® system from the University of Kentucky Clinical Microbiology Laboratory), two of five phenotypic MBL-producing isolates were resistant to ceftazidime/avibactam whereas three of four genotypic MBL-producing isolates were resistant to ceftazidime/avibactam.

Discussion

Other large studies have evaluated the activity of ceftazidime/avibactam against CRE. In 2015, Castanheira *et al.* reported 98% susceptibility (CZA MIC ≤ 8/4 μg/mL) among 153 CRE isolates collected from 71 U.S. medical centers as part of the International Network for Optimal Resistance Monitoring (INFORM) program (P = 0.07346 when compared to this study). The MIC_{50} was 0.5 μg/mL and the MIC_{90} was 2 μg/mL. Non-susceptibility was observed in two *Klebsiella pneumoniae* strains isolated from a Colorado medical center and one *Proteus mirabilis* strain of an unnamed source. All three CRE had ceftazidime/avibactam MICs > 32 μg/mL. Similar to our non-susceptible isolates, the *K. pneumoniae* isolates were found to harbor NDM-1 but the *P. mirabilis* isolate tested negative for CTX-M subgroups 1, 2, 8, 9, and 25; TEM wild type and ESBL; SHV wild type and ESBL; AmpC; KPC; and NDM-1 but positive only for TEM-212, a narrow-spectrum β-lactamase inhibitor (e.g. tazobactam, clavulanic acid, sulbactam) resistant β-lactamase.[355] The authors did not discuss the *P. mirabilis* strain further, but TEM-212 may also be resistant to the β-lactamase inhibitor, avibactam, and high ceftazidime MICs (≥32 μg/mL) have been observed in two *Providencia stuartii* isolates.[356] However, alternative explanations may exist in non-β-lactamase mediated mechanisms of resistance that still warrant exploration.

By 2016, de Jonge *et al.* evaluated ceftazidime/avibactam against 961 meropenem-non-susceptible *Enterobacteriaceae* collected from Europe, Asia/Pacific, Latin America, and the Middle East/Africa as part of the INFORM program. Of these, susceptibility to ceftazidime/avibactam was reported in 83.5%. Upon excluding MBL-producing isolates, 97.7% susceptibility was observed among 816 isolates, which is not significantly different

from our study upon also excluding MBL-producing isolates (P=0.2113). A most interesting result, however, was the decreased susceptibility observed among 207 carbapenemase-negative meropenem-non-susceptible *Enterobacteriaceae* compared to 609 carbapenemase-positive, MBL-negative, meropenem-non-susceptible *Enterobacteriaceae* (94.7% vs. 98.7%; P=0.0009). Among those 207, AmpC, ESBL or both genes were identified in only 195 isolates. Among the 12 remaining isolates, 8 (67%) were susceptible to ceftazidime/avibactam. Although a small subgroup, this suggests that non-enzyme mediate resistant may play a role in ceftazidime/avibactam non-susceptibility. In total, the authors identified only 19 of 961 isolates for which ceftazidime/avibactam non-susceptibility couldn't be explained by the presence of MBLs.[357] Target site modifications[358] and other MBLs not yet identified by PCR were among the most suspected whereas upregulation of efflux pumps were considered less likely after direct testing.[359] Finally, among 145 MBL-producing isolates, susceptibility to ceftazidime/avibactam was 3.4% whereas in the present study, 4 of 9 MBL-producing isolates were susceptible (44%; P<0.0001). The implications of this are not understood, but regional differences in resistance patterns combined with non-β-lactamase mediated resistance provide one hypothesis. Further studies are warranted to better understand this observation.

In the U.S., the first case report of ceftazidime/avibactam resistance in a KPC-3-producing *Klebsiella pneumoniae* clinical isolate was published in October 2015. The exact resistance mechanism in this isolate is not suspected to be related to KPC-3-production since the amino acid sequence encoded by $bla_{\text{KPC-3}}$ in this isolate was unaltered. This adds to the growing evidence that there may be non-β-lactamase resistance mechanisms to ceftazidime/avibactam. Others have reported β-lactamase mediated ceftazidime/avibactam

resistance in KPC-3 producing CRE only in the KPC-3 Ω loop.[258,360,361] The clinical implications of these reports are that susceptibility testing of ceftazidime/avibactam may still be warranted, even in the setting of MBL-negative carbapenem resistance.[362] Another question that remains to be answered is could use of ceftazidime/avibactam in combination prevent the emergence of resistance?

Modified Disk Diffusion Procedure for the Detection of MBL and KPC Enzymes

All CRE that had been obtained at the time of experiment (n=122) underwent duplicate experiments to ascertain their carbapenemase phenotype (Table 4.25; see Modified Disk Diffusion Procedure for the Detection of MBL and KPC Enzymes). Additionally, some information from the University of Kentucky Clinical Microbiology Laboratory was available and is also displayed in Table 4.25.

Table 4.25: Carbapenem-resistant *Enterobacteriaceae* Carbapenemase Enzyme Phenotype (n=122)

Organism	Isolate Number	PCR/Verigene® MBL Type	Phenotypic Disk Diffusion
C. amalonaticus	36		KPC
C. amalonaticus	91		KPC
C. freundii	27		KPC
C. freundii	50		KPC
C. freundii	54		KPC
C. freundii	101		None
C. freundii	127		KPC
C. freundii	135	VIM	MBL
C. freundii	145	KPC	Both
C. freundii	324		None
C. youngae	136		KPC
C. youngae	435	KPC	MBL
E. aerogenes	97		KPC
E. aerogenes	179	VIM	MBL
E. aerogenes	187		KPC
E. aerogenes	438		KPC
E. cloacae	10		KPC
E. cloacae	17		KPC
E. cloacae	30		KPC
E. cloacae	39		KPC
E. cloacae	40	VIM	MBL

Table 4.25 (continued): Carbapenem-resistant *Enterobacteriaceae* Carbapenemase Enzyme Phenotype (n=122)

Organism	Isolate Number	PCR/Verigene® MBL Type	Phenotypic Disk Diffusion
E. cloacae	41	VIM	Both
E. cloacae	52		KPC
E. cloacae	53	VIM	MBL
E. cloacae	70		None
E. cloacae	96		None
E. cloacae	107		KPC
E. cloacae	121		KPC
E. cloacae	126		KPC
E. cloacae	134	VIM	MBL
E. cloacae	144		KPC
E. cloacae	167	VIM	MBL
E. cloacae	168	KPC*	Non-determinate
E. cloacae	169	VIM	MBL
E. cloacae	171	KPC*	KPC
E. cloacae	175	VIM	MBL
E. cloacae	189	VIM	MBL
E. cloacae	200		Both
E. cloacae	203	VIM	MBL
E. cloacae	209		KPC
E. cloacae	266	VIM	MBL
E. cloacae	335		None
E. cloacae	339		KPC
E. cloacae	369		None
E. cloacae	416	VIM	MBL
E. cloacae	476	VIM	Both
E. cloacae	515		KPC
E. coli	33		None
E. coli	103		KPC
E. coli	172	VIM	MBL
E. coli	176		KPC
E. coli	309		None
E. coli	390		None
E. gergoviae	95		None
E. hormaechei	186	VIM	MBL
E. hormaechei	398	VIM	MBL
Enterobacter sp.	146		KPC
Enterobacter sp.	210		KPC
K. oxytoca	166		KPC
K. oxytoca	177	KPC	Both

Table 4.25 (continued): Carbapenem-resistant *Enterobacteriaceae* Carbapenemase Enzyme Phenotype (n=122)

Organism	Isolate Number	PCR/Verigene® MBL Type	Phenotypic Disk Diffusion
K. oxytoca	330		KPC
K. ozaenae	128		KPC
K. ozaenae	407		KPC
K. pneumoniae	21		KPC
K. pneumoniae	22		KPC
K. pneumoniae	24		KPC
K. pneumoniae	28		KPC
K. pneumoniae	29		KPC
K. pneumoniae	31		KPC
K. pneumoniae	32		KPC
K. pneumoniae	34		KPC
K. pneumoniae	35		KPC
K. pneumoniae	37		KPC
K. pneumoniae	42	VIM	MBL
K. pneumoniae	43		KPC
K. pneumoniae	44		KPC
K. pneumoniae	45		KPC
K. pneumoniae	46		KPC
K. pneumoniae	47		KPC
K. pneumoniae	48		KPC
K. pneumoniae	49		KPC
K. pneumoniae	51		KPC
K. pneumoniae	55		KPC
K. pneumoniae	69		None
K. pneumoniae	77		None
K. pneumoniae	93		KPC
K. pneumoniae	98		None
K. pneumoniae	99		KPC
K. pneumoniae	105		KPC
K. pneumoniae	116		None
K. pneumoniae	119		KPC
K. pneumoniae	123		KPC
K. pneumoniae	129		KPC
K. pneumoniae	130		KPC
K. pneumoniae	142		KPC
K. pneumoniae	143		None
K. pneumoniae	152		None
K. pneumoniae	165		KPC
K. pneumoniae	170	VIM	MBL

Table 4.25 (continued): Carbapenem-resistant *Enterobacteriaceae* Carbapenemase Enzyme Phenotype (n=122)

Organism	Isolate Number	PCR/Verigene® MBL Type	Phenotypic Disk Diffusion
K. pneumoniae	173	VIM	MBL
K. pneumoniae	174	VIM	MBL
K. pneumoniae	230		KPC
K. pneumoniae	243		KPC
K. pneumoniae	256		KPC
K. pneumoniae	269		None
K. pneumoniae	284		None
K. pneumoniae	349		None
K. pneumoniae	352		None
K. pneumoniae	372		None
K. pneumoniae	385		None
K. pneumoniae	391		KPC
K. pneumoniae	411	VIM	MBL
K. pneumoniae	418		None
K. pneumoniae	423		KPC
K. pneumoniae	445		KPC
K. pneumoniae	446		KPC
K. pneumoniae	449		KPC
K. pneumoniae	452		KPC
K. pneumoniae	466		KPC
K. pneumoniae	482		KPC
K. pneumoniae	492		None
S. marcescens	514		KPC

*VIM by PCR performed by Olga Lomovskaya (The Medicines Co., Parsippany, NJ)
Isolates highlighted in color indicate stratification by patient
Isolates without highlighting indicate unique patient source

KPC production composed the major mechanism of resistance to carbapenem antimicrobials among CRE (58%; Figure 4.18), followed by MBL production (17%; Figure 4.18). Other mechanisms of carbapenem resistance (20%; Figure 4.18) composed a significant proportion of CRE, but additional information was not available by chart review. VIM was the only MBL type identified in these isolates by the Verigene® system (Table 4.25; see Modified Disk Diffusion Procedure for the Detection of MBL and KPC Enzymes).

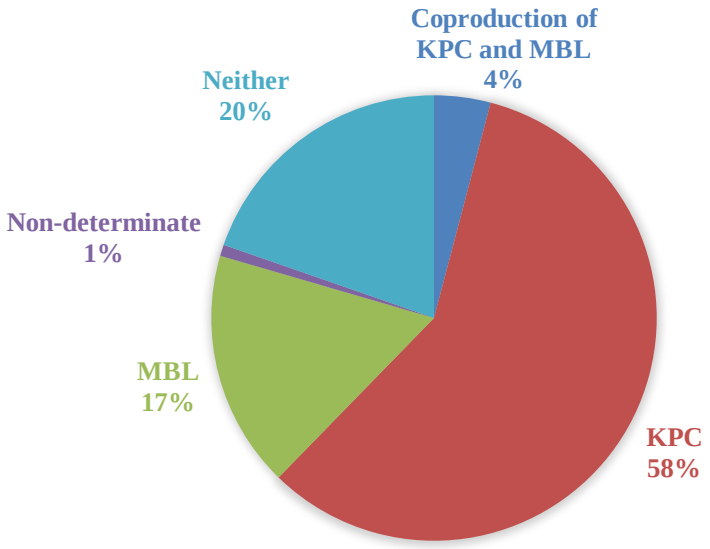

Figure 4.18: Carbapenemase Phenotype of All Carbapenem-resistant *Enterobacteriaceae* (n=122).

Pharmacodynamic Modeling

Pharmacodynamic Model of Isolates in Meropenem Monotherapy Experiments

According to the protocol discussed in Pharmacodynamic Mathematical Modeling, equation (1) and equation (11) were utilized for a total of 6 parameters, 52 time-kill experiments and 93 simultaneously solved differential equations. Individually modeled isolates are presented in Appendix H.

Table 4.26: Composite Model Initial Parameter Estimates for All Isolates against Meropenem Monotherapy

Parameter	g_S	g_R	Nmax	k_S	EC_{50S}	k'_R
Value	2.94	1.97	9.82E+10	5.74	0.28	0.23
Lower Bound	1.62	0.93	3.80E+09	3.56	0.00	0.00
Upper Bound	4.26	3.01	2.00E+11	7.93	0.83	0.49
Type	User-defined	User-defined	User-defined	User-defined	User-defined	User-defined
Source of estimate	Average of all isolate g_S	Average of all isolate g_R	Average of all isolate Nmax	Average of all isolate k_S	Average of all isolate EC_{50S}	Average of all isolate k'_R
Source of bounds	95% CI of mean	95% CI of mean	95% CI of mean	95% CI of mean	95% CI of mean	95% CI of mean

CI – Confidence interval; Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L); k' ($L/mg*h^{-1}$)

Table 4.27: Composite Model Final Parameter Estimates for All Isolates against Meropenem Monotherapy

Parameter	Estimate	SE	CV %	Lower univariate CI	Upper univariate CI
g_S	2.87	0.28	10	2.33	3.41
g_R	1.44	0.06	4	1.32	1.57
Nmax	6.87E+10	1.83E+10	27	3.25E+10	1.05E+11
k_S	5.55	0.34	6	4.88	6.23
EC_{50S}	0.14	0.03	20	0.09	0.20
k'_R	0.35	0.02	6	0.31	0.39

SE – Standard Error; CV – Coefficient of Variation; CI – 95% confidence interval; Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L); k' (L/mg*h^{-1})

The theoretical stationary concentration of the more resistant subpopulation was approximately 30-fold higher than the more susceptible majority population. The doubling time of the more resistant subpopulation was approximately twice that of the more susceptible majority population (Table 4.28).

Table 4.28: Composite Model Final Secondary Parameter Estimates for All Isolates against Meropenem Monotherapy

Parameter	Estimate	SE	CV %
SC_S (mg/L)	0.15*MIC	0.02	12
SC_R (mg/L)	4.11*MIC	0.12	3
d_S (min)	14.48	1.39	10
d_R (min)	28.80	1.28	4

SE – Standard Error; CV – Coefficient of Variation; SC – Stationary Concentration; d – doubling time

More support in the form of the Akaike score is provided to individually modeled isolates than parameters unifying isolates exhibiting a wide range of meropenem resistance. When the isolate with predicted parameters least like the others was excluding, improved support for the composite model was noted, but not to same degree of individually modeled isolates (Table 4.29).

Table 4.29: Akaike Scores of Competing Meropenem Monotherapy Models

	Individually Modeled	**Composite Model MIC Normalized***	**Composite Model MIC Normalized**
AIC	1200.8	1339.8	1401.7
Relative Likelihood	<0.0001		
Relative Likelihood		<0.0001	

Literature relative likelihood value 0.0067 corresponds to differences in AIC of 10.[290]
*Isolate 34 parameters were least like the other 3 isolates and was modeled individually

When MICs were fit as parameters, only one isolate showed reasonable agreement between the predicted and observed MIC. The other isolates exhibited MICs about 2-fold more or less than their corresponding predicted MIC which contributes to greater support for individually modeled isolates over a composite model when MICs are utilized to unify parameters (Table 4.30).

Table 4.30: Model Predicted MICs

Parameter	**Observed**	**Estimate**	**SE**	**CV %**	**Lower univariate CI**	**Upper univariate CI**
34 MIC	4	2.0	0.34	17	1.34	2.66
22 MIC	16	8.1	1.40	17	5.30	10.8
24 MIC	32	31.2	5.06	16	21.2	41.1
44 MIC	128	255.9	39.4	15	178	333

SE – Standard Error; CV – Coefficient of Variation; CI – 95% confidence interval; Units: MIC (mg/L)

Plots of observed and predicted data appeared congruent and simulated data fit the observed data well (Figure 4.19, Figure 4.20, Figure 4.21, and Figure 4.22). Gross systematic bias of the individually fit models was not observed (Figure 4.23). The linear assessment of bias was appropriate based on the randomly distributed residual plot (Figure 4.24).

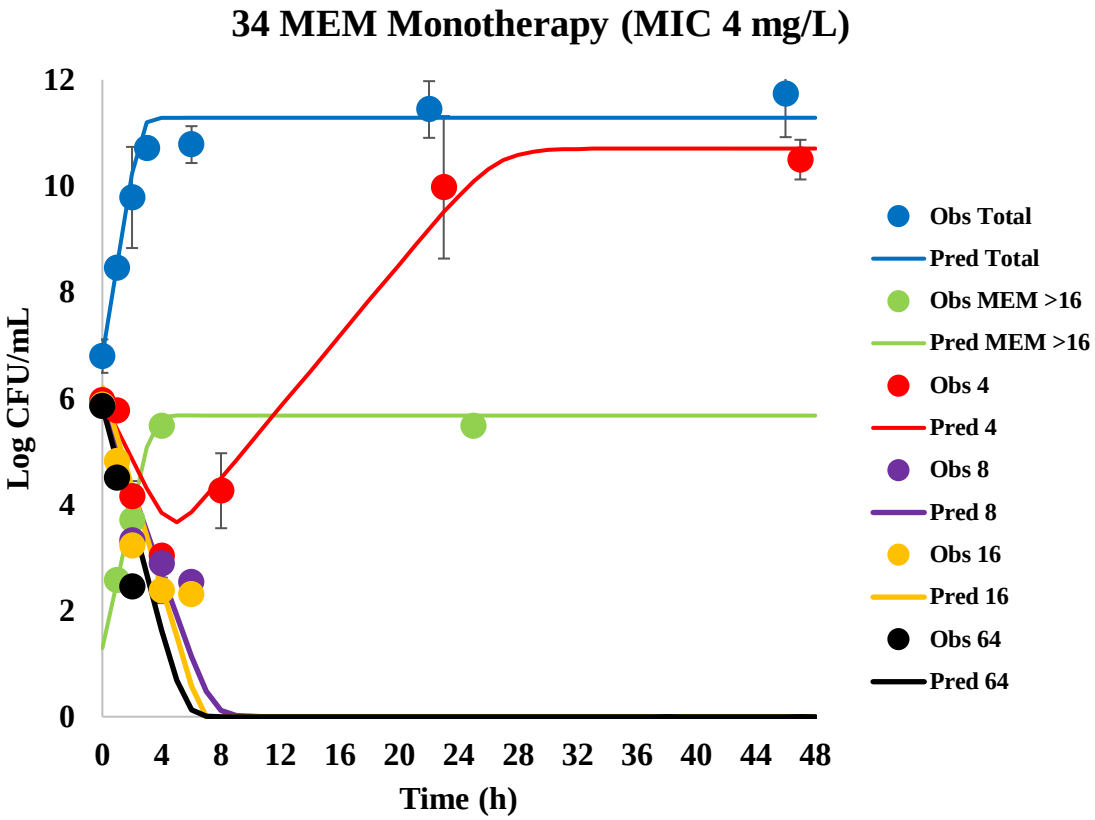

Figure 4.19: Predicted and Observed Time-kill Data for Meropenem against Isolate 34. Observed data represent geometric means with standard deviations of at least 2 time-kill experiments except for green data which was only performed once. Note that some error bars are smaller than the symbol. Blue data represent the sum of both populations. Green data represent the subpopulation with a meropenem MIC > 16 mg/L. Other data indicate the total colony count with the given concentration of meropenem present in the time-kill flask.

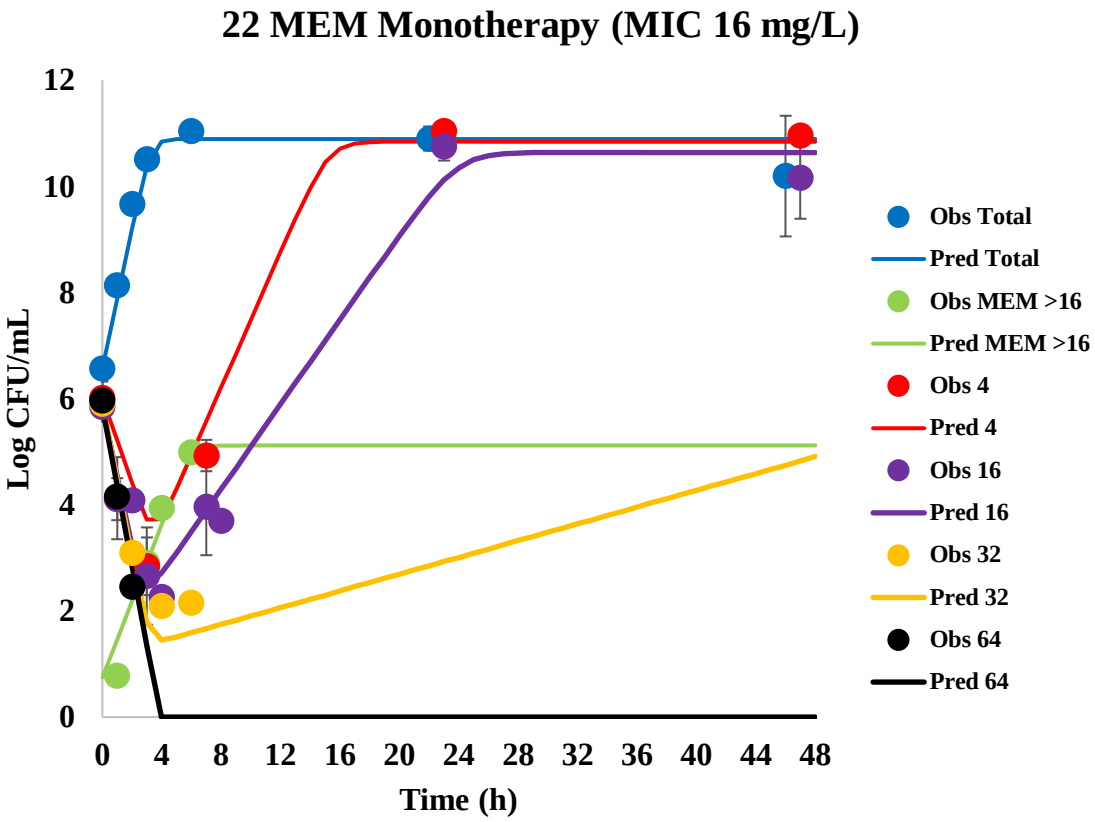

Figure 4.20: Predicted and Observed Time-kill Data for Meropenem against Isolate 22. Observed data represent geometric means with standard deviations of at least 2 time-kill experiments except for green data which was only performed once. Note that some error bars are smaller than the symbol. Blue data represent the sum of both populations. Green data represent the subpopulation with a meropenem MIC > 16 mg/L. Other data indicate the total colony count with the given concentration of meropenem present in the time-kill flask.

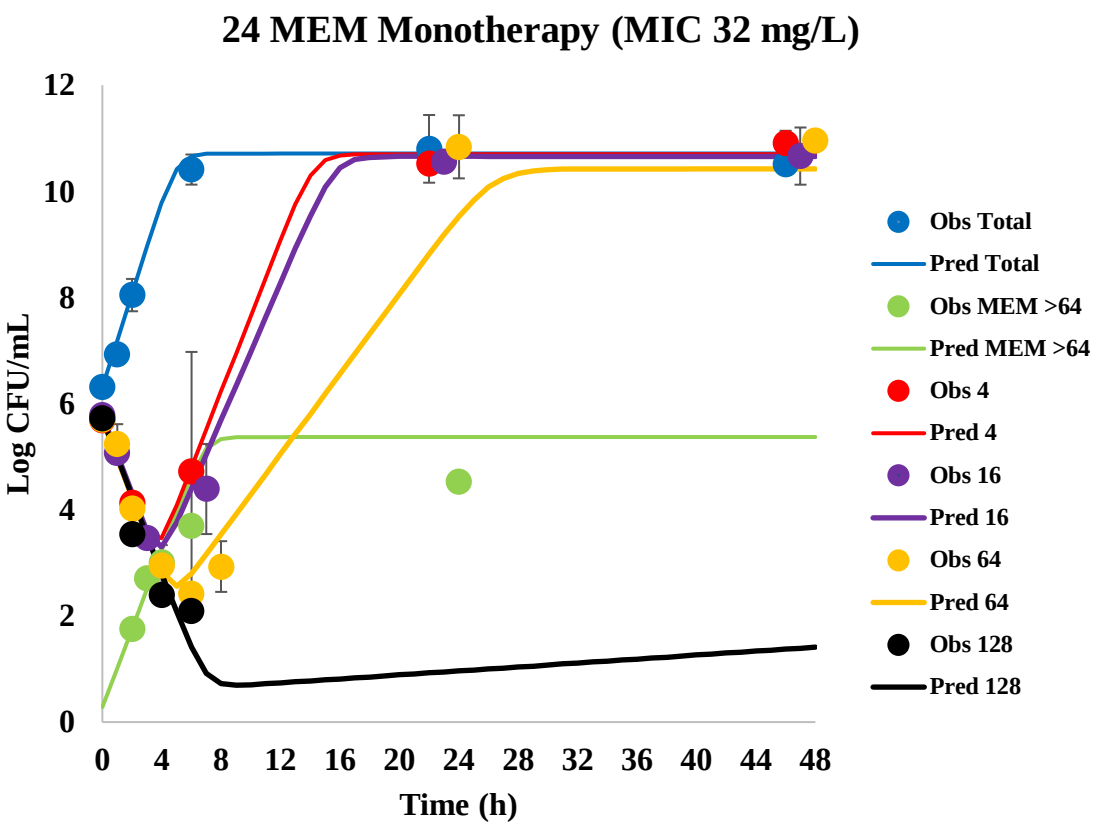

Figure 4.21: Predicted and Observed Time-kill Data for Meropenem against Isolate 24. Observed data represent geometric means with standard deviations of at least 2 time-kill experiments except for green data which was only performed once. Note that some error bars are smaller than the symbol. Blue data represent the sum of both populations. Green data represent the subpopulation with a meropenem MIC > 64 mg/L. Other data indicate the total colony count with the given concentration of meropenem present in the time-kill flask.

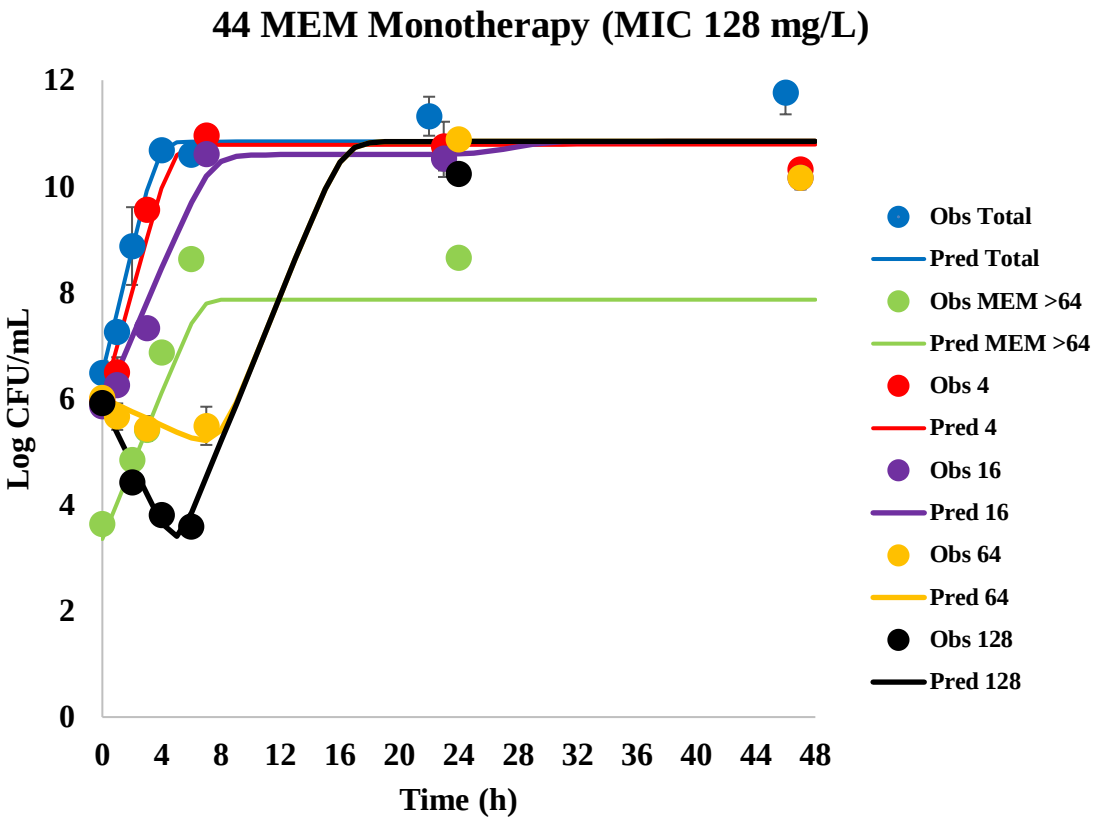

Figure 4.22: Predicted and Observed Time-kill Data for Meropenem against Isolate 44. Observed data represent geometric means with standard deviations of at least 2 time-kill experiments except for green data which was only performed once. Note that some error bars are smaller than the symbol. Blue data represent the sum of both populations. Green data represent the subpopulation with a meropenem MIC > 64 mg/L. Other data indicate the total colony count with the given concentration of meropenem present in the time-kill flask.

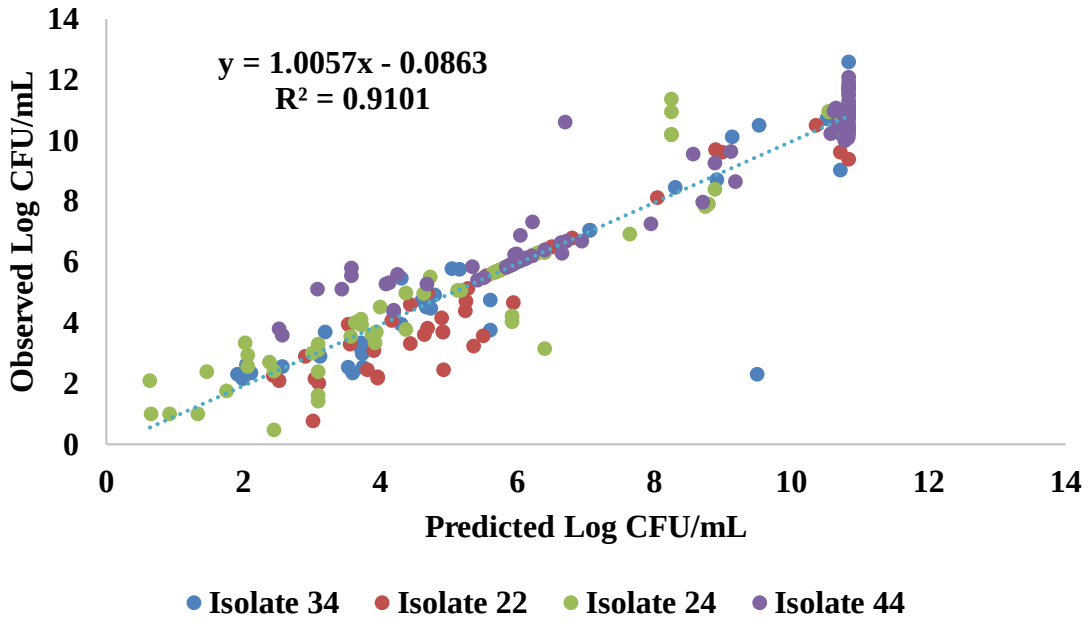

Figure 4.23: Individual Model Observed vs. Predicted Data for Meropenem Monotherapy. 95% confidence intervals: slope (0.97-1.04); intercept (-0.38-0.20).

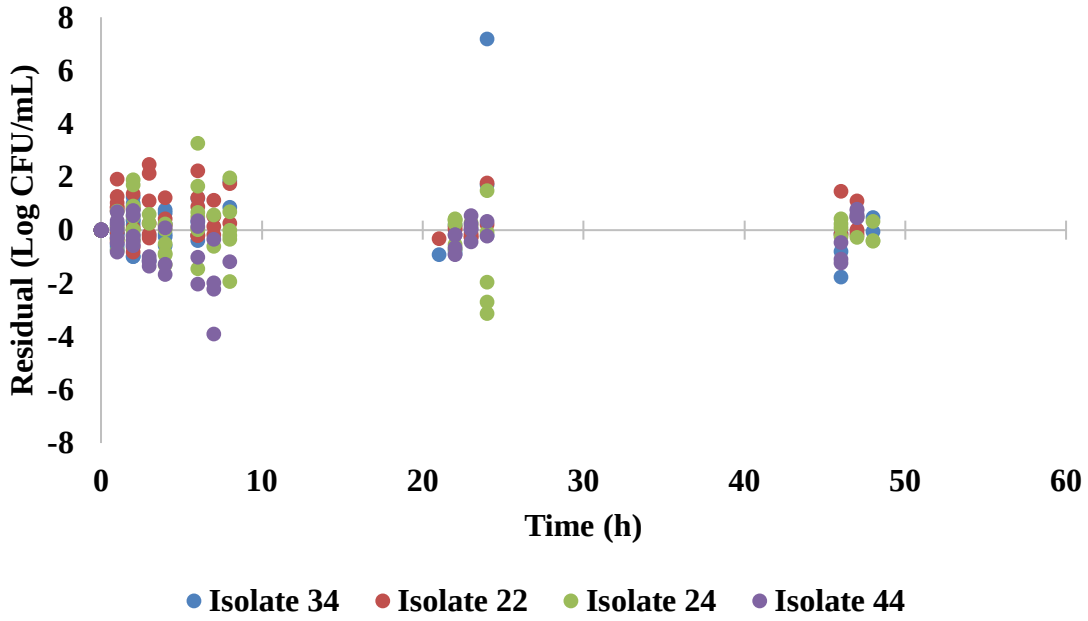

Figure 4.24: Individual Model Residual Data for Meropenem Monotherapy.

Pharmacodynamic Model of Isolates in Polymyxin B Monotherapy Experiments

According to the protocol discussed in Pharmacodynamic Mathematical Modeling, equation (1) and equation (2) were utilized for a total of 6 parameters, 78 time-kill experiments and 141 simultaneously solved differential equations. Individually modeled isolates are presented in Appendix H.

Table 4.31: Composite Model Initial Parameter Estimates for All Isolates against Polymyxin B Monotherapy

Parameter	g_S	g_R	Nmax	k_S	EC_{50S}	k'_R
Value	3.06	1.06	7.45E+10	9.03	0.06	0.07
Lower Bound	1.65	0.75	3.88E+10	6.39	0.00	0.02
Upper Bound	4.48	1.37	1.10E+11	11.67	0.23	0.12
Type	User-defined	User-defined	User-defined	User-defined	User-defined	User-defined
Source of estimate	Average of all isolate g_S	Average of all isolate g_R	Average of all isolate Nmax	Average of all isolate k_S	Average of all isolate EC_{50S}	Average of all isolate k'_R
Source of bounds	95% CI of mean	95% CI of mean	95% CI of mean	95% CI of mean	95% CI of mean	95% CI of mean

CI – Confidence interval; Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L); k' (L/mg*h^{-1})

Table 4.32: Composite Model Final Parameter Estimates for All Isolates against Polymyxin B Monotherapy

Parameter	Estimate	SE	CV %	Lower univariate CI	Upper univariate CI
g_S	2.85	0.27	9	2.32	3.38
g_R	1.06	0.03	3	1.00	1.12
Nmax	6.23E+10	1.29E+10	21	3.69E+10	8.77E+10
k_S	10.15	0.74	7	8.69	11.60
EC_{50S}	0.06	0.01	24	0.03	0.09
k'_R	0.09	0.01	12	0.07	0.11

SE – Standard Error; CV – Coefficient of Variation; CI – 95% confidence interval; Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L); k' (L/mg*h^{-1})

The theoretical stationary concentration of the more resistant subpopulation was approximately 400-fold higher than the more susceptible majority population. The doubling time of the more resistant subpopulation was approximately twice that of the more susceptible majority population (Table 4.33).

Table 4.33: Composite Model Final Secondary Parameter Estimates for All Isolates against Polymyxin B Monotherapy

Parameter	Estimate	SE	CV %
SC_S (mg/L)	0.025	0.003	13
SC_R (mg/L)	11.0	0.98	9
d_S (min)	15.8	1.62	10
d_R (min)	36.6	1.15	3

SE – Standard Error; CV – Coefficient of Variation; SC – Stationary Concentration; d – doubling time

More support in the form of the Akaike score is provided to the composite model than individually modeled isolates. Furthermore, the most support is provided to the model with parameters that were MIC-independent (Table 4.34).

Table 4.34: Akaike Scores of Competing Polymyxin B Monotherapy Models

	Individually Modeled	Composite Model	Composite Model MIC Normalized
AIC	1968.1	1441.0	1448.0
Relative Likelihood	<0.0001		
Relative Likelihood		0.03	

Literature relative likelihood value 0.0067 corresponds to differences in AIC of 10.[290]

Plots of observed and predicted data appeared congruent and simulated data fit the observed data well (Figure 4.25, Figure 4.26, Figure 4.27, and Figure 4.28). Gross systematic bias of the individually fit models was not observed (Figure 4.29). The linear assessment of bias was appropriate based on the randomly distributed residual plot (Figure 4.30).

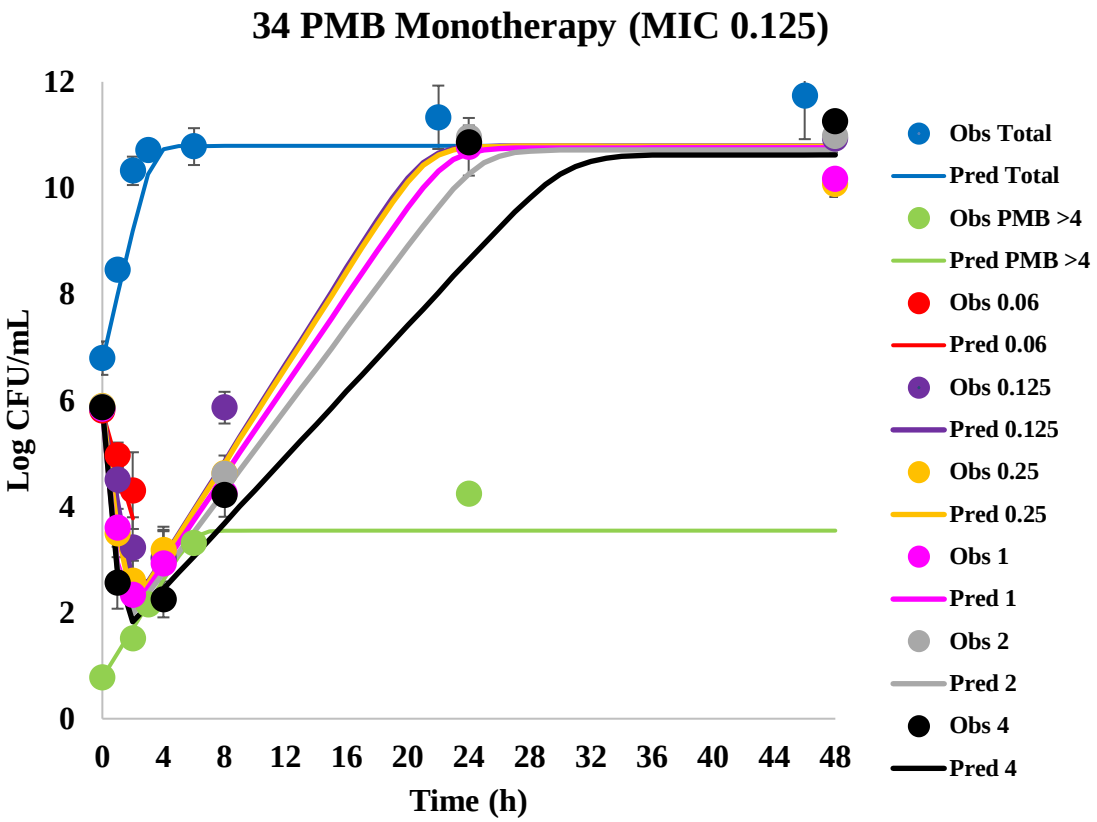

Figure 4.25: Composite Model Predicted and Observed Time-kill Data for Polymyxin B against Isolate 34. Observed data represent geometric means with standard deviations of at least 2 time-kill experiments except for green data which was only performed once. Note that some error bars are smaller than the symbol. Blue data represent the sum of both populations. Green data represent the subpopulation with a polymyxin B MIC > 4 mg/L. Other data indicate the total colony count with the given concentration of polymyxin B present in the time-kill flask.

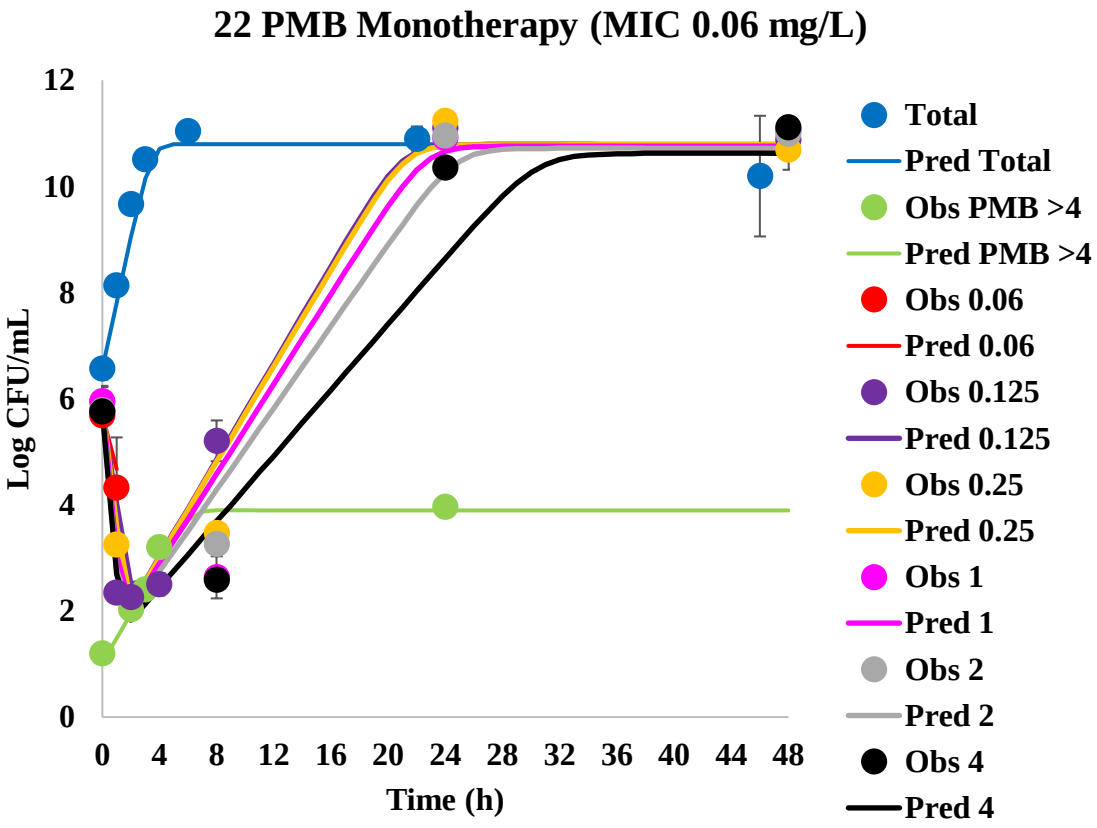

Figure 4.26: Composite Model Predicted and Observed Time-kill Data for Polymyxin B against Isolate 22. Observed data represent geometric means with standard deviations of at least 2 time-kill experiments except for green data which was only performed once. Note that some error bars are smaller than the symbol. Blue data represent the sum of both populations. Green data represent the subpopulation with a polymyxin B MIC > 4 mg/L. Other data indicate the total colony count with the given concentration of polymyxin B present in the time-kill flask.

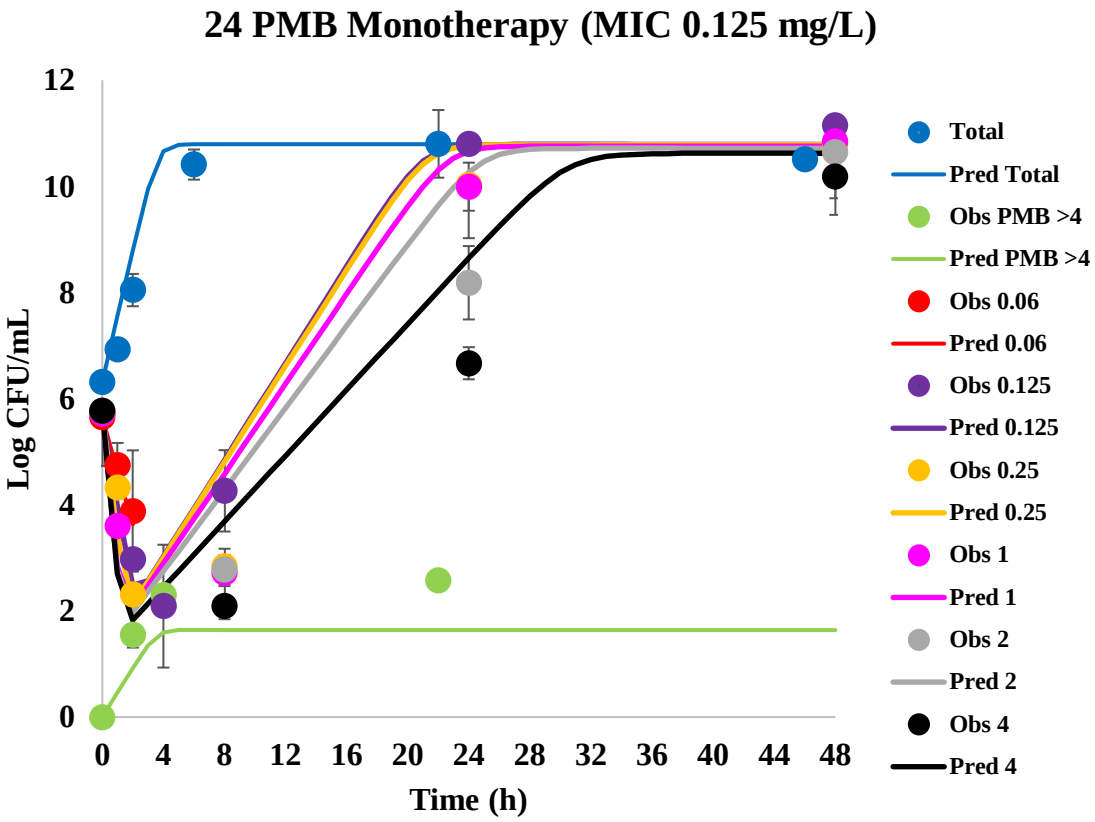

Figure 4.27: Composite Model Predicted and Observed Time-kill Data for Polymyxin B against Isolate 24. Observed data represent geometric means with standard deviations of at least 2 time-kill experiments except for green data which was only performed once. Note that some error bars are smaller than the symbol. Blue data represent the sum of both populations. Green data represent the subpopulation with a polymyxin B MIC > 4 mg/L. Other data indicate the total colony count with the given concentration of polymyxin B present in the time-kill flask.

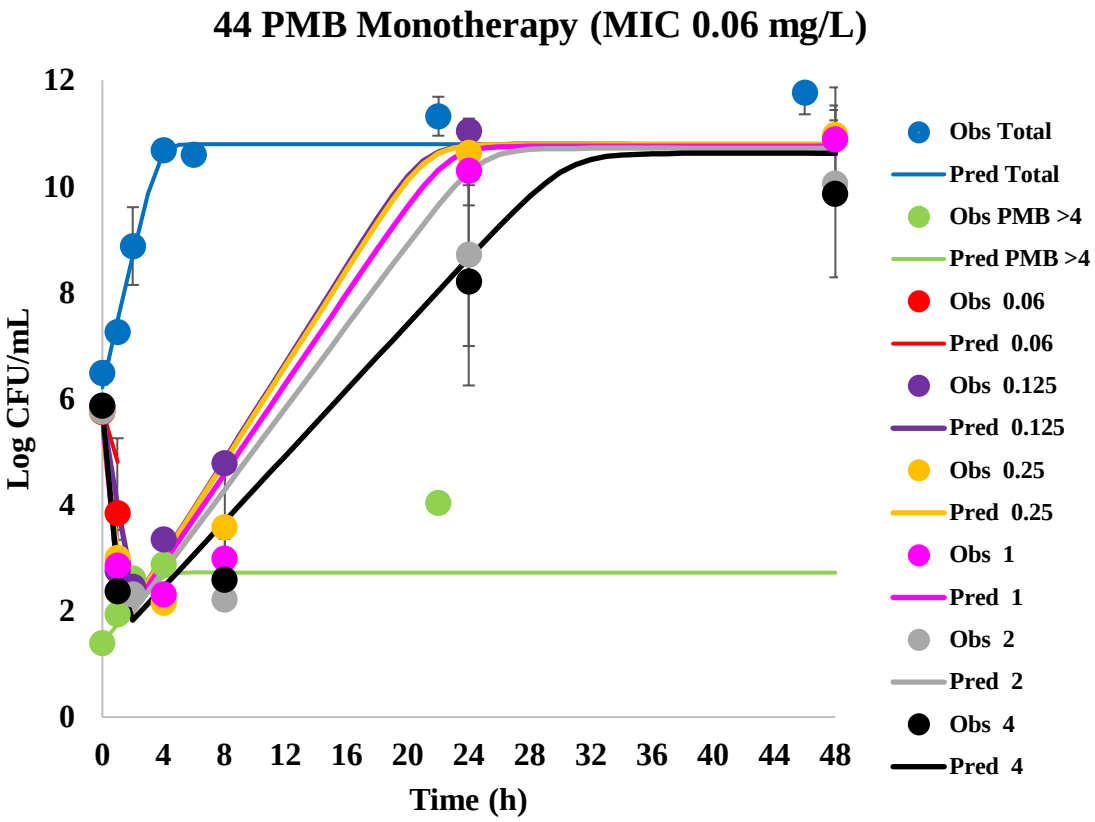

Figure 4.28: Composite Model Predicted and Observed Time-kill Data for Polymyxin B against Isolate 44. Observed data represent geometric means with standard deviations of at least 2 time-kill experiments except for green data which was only performed once. Note that some error bars are smaller than the symbol. Blue data represent the sum of both populations. Green data represent the subpopulation with a polymyxin B MIC > 4 mg/L. Other data indicate the total colony count with the given concentration of polymyxin B present in the time-kill flask.

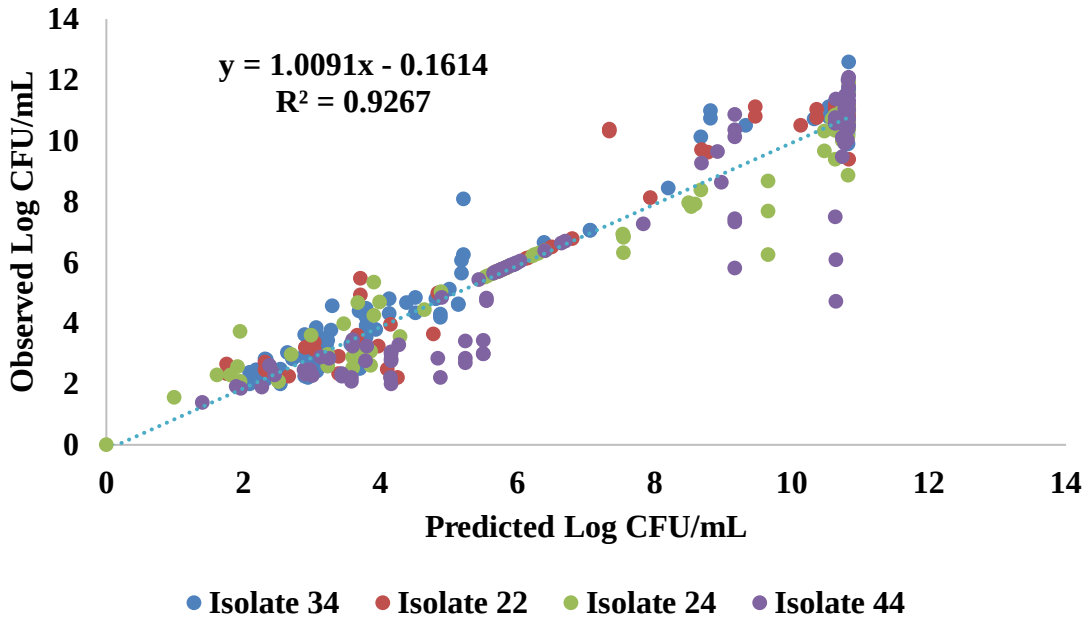

Figure 4.29: Composite Model Observed vs. Predicted Data for Polymyxin B Monotherapy. 95% confidence intervals: slope (0.98-1.04); intercept (-0.38-0.06).

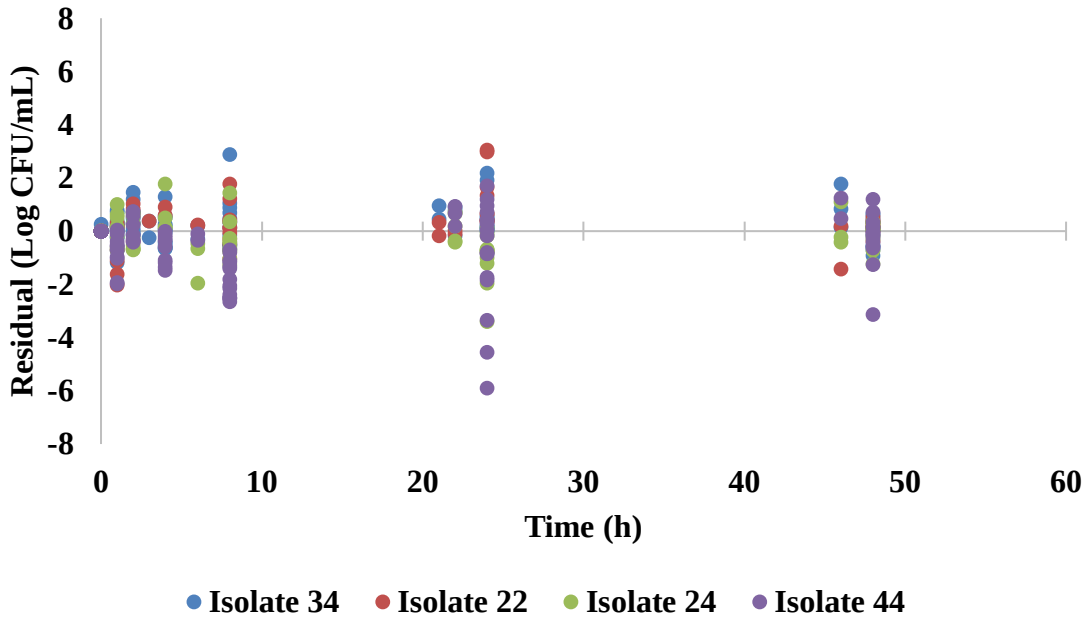

Figure 4.30: Composite Model Residual Data for Polymyxin B Monotherapy.

Discussion

In summary, a deterministic two-population model was assumed for each antimicrobial where a population of more susceptible bacteria exhibits unique growth and killing rate constants compared to a more resistant subpopulation. Additional subpopulations could be assumed in a deterministic model, but these more complex models were not evaluated after achieving good overall fit of a two-population model and lacking sufficient evidence to support an additional third population of organisms emerging. In reality, subpopulations likely exist as a continuum exhibiting variable growth rates and responses to antimicrobials.[363] A probabilistic model where the variance of the antimicrobial killing rate constants are fit as a parameters[364] or inclusion of a probabilistic mutation function to account for the step-wise mutations that may occur during the course of therapy may account for the variance observed in the time to regrowth of some of the experiments, particularly those of polymyxin B against isolate 44 (Figure H.19). A limitation of the current two-population model is that the average killing rate and time to regrowth are predicted by the model which may suggest lower concentrations than what are actually needed to prevent regrowth due to the variance in antimicrobial killing rate.[364] In other words, despite the deterministic nature of the present model, concentrations close to the theoretical "stationary concentration" may not always prevent the emergence of resistance in practice. However, in this large data set, admittedly involving few isolates (n=4), observed regrowth or killing was always predicted accurately by the deterministic two-population model for meropenem or polymyxin B.

In further summary, the rate of change of colony forming units over time was determined by a difference of the linear bacterial growth rate and the sigmoidal

antimicrobial kill rate, except for the resistant subpopulation where a linear killing rate was observed. Concentrations around and exceeding the EC_{50} of the resistant subpopulation are required to more accurately ascertain this parameter but given that these concentrations are well above clinically achievable concentrations in patients, assumption of a linear killing rate on the resistant subpopulation yielded more precise parameters (CVs < 50%) and an Akaike score lending more support for the simpler linear killing model for the more resistant subpopulation.

The polymyxin B MICs of isolates 34, 22, 24, and 44 were within essential agreement (within one two-fold dilution) as defined by CLSI.[268] In this context, the increase in Akaike score when parameter estimates were normalized by the MIC (Table 4.34) suggests that this normalization is not supported and instead these isolates likely have much closer MICs to one another than a 2-fold difference as determined by broth microdilution (see Broth Microdilution Susceptibility Testing). This result importantly highlights the well-described significant limitation of antimicrobial susceptibility testing methods. Specifically, MICs are associated with significant variance and they are reproducible only within one two-fold dilution.[268] This limitation significantly hampers efforts to standardize and compare isolates exhibiting different resistance phenotypes to the tested antimicrobial agents, as exemplified in the meropenem results where isolates were best modeled individually rather than as an overall composite (Table 4.29).

However, differences in the antimicrobial killing rate based on the organism MIC weren't the only factors contributing to an ill-fitting composite model. Growth rate constants among the more susceptible majority populations of bacteria ranged 2.0-4.0 hr^{-1} and growth rate constants of the more resistant subpopulations ranged 1.6-2.9 hr^{-1} for

meropenem and 0.8-1.2 hr^{-1} for polymyxin B (Appendix H). In general, the higher the antimicrobial MIC (or presumed higher MIC for the more resistant subpopulation), the lower the growth rate constants were for both populations. This may indicate a fitness cost as resistance mechanisms accrue, described in greater detail by previous literature.[288,289] Therefore, the ill-fitting meropenem composite model also suggests that if CRE with polymyxin B MICs much higher than 0.125 mg/L were evaluated, then a similar pattern may emerge. Essentially, differences in isolate fitness combined with the poor precision of MIC information may indicate isolate-specific models are better supported than a composite model in the setting of different isolate MICs. Improvements on MIC measurement precision and a better understanding of the factors underlying the fitness cost associated with additional resistance mechanisms is therefore warranted before a unifying model can be obtained.

The overall observed vs. predicted regression of the best fitting meropenem and polymyxin B models were good. In brief, at least 90% of the linear variation of the log-transformed colony count is accounted for by the models (Figure H.20 and Figure 4.29). Upon cursory visual inspection, some data points (particularly around 10^5-10^7 CFU/mL) fall extremely close to the line of unity whereas others are more widely distributed around the line within the same region (10^5 – 10^7 CFU/mL). This is explained by the greater variability associated with the regrowing heteroresistant subpopulation when compared to the more susceptible majority population. Essentially, the initial concentration and killing of the more susceptible population are very tightly modeled and characterized but the regrowth of the subpopulation is not as well described. Additional sampling of the heteroresistant subpopulation during this phase of regrowth would increase the number of

data points in this region. Further, the 95% confidence intervals of the slopes and intercepts of the observed vs. predicted plots contain 1 and 0, respectively, indicating that the models are not differentially biased at lower versus higher colony counts and also not systematically biased at a higher or lower colony counts. Furthermore, this analysis utilized the approach suggested by Piñeiro et al. to ensure appropriate determination of bias whereas others have traditionally plotted predicted vs. observed and arrived at erroneous conclusions because underestimation of the slope and overestimation of the y-intercept increases as r^2 values decrease.[365]

In general, the proposed models of polymyxin B and meropenem against KPC-producing *K. pneumoniae* suggest that, under ideal growth conditions for bacteria (i.e. nutrient rich environment, optimal temperature, sufficient oxygenation, etc.), meropenem concentrations up to 4x the measured meropenem MIC are required for bacteriostatic activity against the resistant subpopulation, but concentrations as low as 0.15x MIC may be required for bacteriostatic activity against the less resistant population (Table 4.28). This is supported by clinical data suggesting that regimens containing carbapenems using aggressive dosing strategies (e.g. meropenem 2g every 8h over a 3h infusion) are more effective in treating patients infected with CRE exhibiting MICs ≤8 mg/L, given that such regimens are predictive of carbapenem concentrations > MIC for at least 40% of the dosing interval.[340] Regarding polymyxin B, concentrations as low as 0.025 mg/L are bacteriostatic against the majority population whereas concentrations as high as 11 mg/L are needed to prevent the resistant subpopulation from regrowing (Table 4.33). However, it is necessary to mention that the present model did not evaluate concentrations as high as 11 mg/L. This suggests that lower doses of polymyxin B should be utilized to kill the more susceptible

population and also minimize the risk of renal injury while another antimicrobial agent could be used to kill the more resistant subpopulation, perhaps alleviating the very aggressive doses of polymyxins currently utilized clinically.[124] For both isolates, the growth rate of the more resistant subpopulation is approximately half that of the susceptible population, but the clinical implications of this observation are unknown. Again, this fits the present understanding of a fitness cost in exchange for higher degrees of resistance expression in isolates.[288,289]

To the best of my knowledge, this is the first study to model meropenem and polymyxin B against KPC-producing *Klebsiella pneumoniae* exhibiting a wide range of meropenem MICs. However, others have modeled meropenem against isolates like *Pseudomonas aeruginosa* using a variety of modeling techniques, some of which were employed in the present study.[286,363,364] Limitations of the present work include that insufficient data are available to validate the model. Although the model fits the training data very well, it remains to be seen how predictive this model will be of the activities of meropenem and polymyxin B of similar isolates. Furthermore, the definition of similar isolates may be nuanced by the isolate MIC, the genotype/phenotypic expression of resistance mechanisms, and perhaps even by the genus or species of the organism. Therefore, additional work is needed in this area to determine how similar in response other carbapenem-resistant organisms are. For example, clinically, patients infected with *Acinetobacter baumannii* seemingly respond to polymyxin monotherapy whereas patients infected with other carbapenem-resistant organisms may exhibit more frequent clinical failures, similar to what the present model might suggest in its prediction of rapid and consistent regrowth despite utilization of concentrations of up to 64x the MIC.[251]

Chapter Five:

Conclusions and Future Work

Part of the research contained within this chapter has been published as Kulengowski, B. *In vitro* activity of polymyxin B and meropenem alone and in combination against carbapenem-resistant *Enterobacteriaceae*. 2016. *Theses and Dissertations—Pharmacy*. 57.

Carbapenem-resistant *Enterobacteriaceae* (CRE) are a growing national and international threat.[39,94,95,120-123] Specifically, a "Call to Action" has been issued by various organizations (e.g. CDC, PACCARB, IDSA) hoping to raise awareness among the medical community as well as highlight the dwindling development of novel antimicrobial agents with activity against these hard-to-treat infections.[12] Furthermore, the United Nations released a report estimating that by 2050, antimicrobial resistant infections will kill more people annually than cancer.[366]

Compounding this issue is the wide variability in types of carbapenem resistance among CRE (e.g. KPC, MBL, OXA-48, and ESBL or AmpC with porin mutations) and the large differences observed among nations or even among hospitals within the same country, state, or province.[14] There are few randomized, controlled clinical trials evaluating optimal therapy for CRE treatment, and treatment strategies may vary depending on the type of resistance. It has also been suggested that strain-to-strain differences or bacteria genotype may be more important to optimal therapeutic decision-making than MIC or pharmacodynamic indices alone.[229]

In order to contribute to the growing knowledge of CRE management, this study focused on KPC-producing carbapenem-resistant *Enterobacteriaceae*, the most common CRE in the U.S., approximately 99% of CRE cases in some regions.[14] Some studies presented in this dissertation included VIM-producing CRE which emerged as a significant phenotype at UK HealthCare. In fact, the quantity of VIM-producing isolates within Kentucky is greater than all other states combined.[79] Specifically, we evaluated the use of amikacin, meropenem, and polymyxin B alone and in combination because, among the four "typically susceptible" antimicrobial agents, fosfomycin is only approved in the U.S. for uncomplicated urinary tract infections;[178] tigecycline is unable to reach effective serum concentrations for treatment of CRE bacteremia,[168,367] where mortality is estimated to be around 50%;[11] and colistin is associated with higher rates of nephrotoxicity, cumbersome therapeutic drug monitoring, and more difficult rational drug dosing when compared to polymyxin B.[130,131,145] All of these agents are associated with rapid resistance development when used as monotherapy,[14] and previous data have indicated that combination therapy, in particular combinations including a carbapenem, have a mortality benefit over monotherapy.[10,68,185,199-203]

To the best of my knowledge, this study was among the first to evaluate the *in vitro* interaction of polymyxin B and amikacin in combination with meropenem across a wide range of carbapenem resistance, testing multiple concentrations, evaluating staggered and sequential administration, and for a longer duration (48 hours) than previous studies which have typically evaluated combinations involving colistin.[201,206,207,331,368,369] Meropenem, amikacin, and polymyxin B alone and in combination were evaluated against KPC-producing and VIM-producing clinical isolates, representing polymyxin-susceptible (PMB

MICs < 2 µg/mL) CRE of varying meropenem resistance (MEM MICs 4 – 128 µg/mL) and amikacin susceptibility (AMK MICs 8 – 64 mg/L). These isolates were evaluated using CLSI-standardized *in vitro* laboratory methodology, designed and approved to minimize variability between laboratories to facilitate more meaningful comparisons of results.[262] The activity of novel antimicrobial agents such as ceftazidime/avibactam and imipenem/relebactam were evaluated and the agreement of antimicrobial susceptibility testing methods across a wide variety of antimicrobial agents were also compared according to CLSI guidelines. Finally, pharmacodynamic mathematical modeling was performed on a subset of *Klebsiella pneumoniae* isolates to better understand the interplay of the selection for and emergence of resistance in the presence and absence of common antimicrobial agents against CRE.

The most significant finding of this study is not simply that "susceptible" and "resistant" designations insufficiently describe antimicrobial activity, but rather that both the antimicrobial MIC as well as the specific resistance mechanisms influence optimal choice of therapy. The polymyxin B and meropenem time-kill studies against polymyxin susceptible KPC- and/or VIM-producing *K. pneumoniae* and *E. cloacae* suggest bactericidal activity can be attained against isolates exhibiting meropenem MICs ≤16 mg/L—with clinically achievable serum concentrations in patients. Similarly, the amikacin and meropenem time-kill studies against CRE suggest bactericidal interactions occur when the amikacin MIC is ≤16 mg/L. A wider variation in meropenem MICs should subsequently be evaluated to verify if this relationship holds for isolates exhibiting meropenem MICs ≤8 mg/L or >32 mg/L. Also, pharmacokinetic factors such as drug distribution will need to be addressed when considering infection sites that are not well

vascularized or are known to be difficult to reach such as central nervous system infections. If aggressive doses of these antimicrobial agents are used, our data suggest isolates exhibiting meropenem MICs ≤32 mg/L may be treatable. However, accounting for the within-one-10-fold-dilution variability in MIC tests would suggest that aggressive dosing should be used for isolates exhibiting a meropenem MIC of 16 mg/L. Isolates exhibiting MICs greater than 16 mg/L warrant additional exploration. One possibility is to further assess the utilization of the novel carbapenemase inhibitor combinations (e.g. ceftazidime/avibactam, meropenem/vaborbactam, etc.) and their interaction with antimicrobials like polymyxin B and amikacin using high through-put *in vitro* methods such as those described in this dissertation. Further work could also be conducted using triple (or higher degree) combinations to manage these organisms, but even high throughput *in vitro* methodology will be very time consuming to parse out the interactions between each pair of agents, the interaction component of all three agents together, and the activity of the antimicrobials individually. However, what makes this latter approach most interesting is the analogy to HIV treatment in that triple combination therapy is the standard for the prevention of resistance and not simply treatment of the acute-phase of the infection. It should be pointed out, however, that double agent HIV treatment is becoming more widespread in practice and, particularly with the novel compounds, is just as effective at preventing resistance while minimizing patient pill burden and toxicity. These points should also be considered for future work with CRE in that as regimen complexity increases, patients will require additional IV lines which pose increased risk as well as possible compounding toxicity issues like renal failure.

The next major finding of this dissertation is that the sequence of drug administration matters for meropenem and polymyxin B combinations. In fact, administering the beta-lactam agent first exhibited as good and sometimes superior activity to simultaneous administration of the two agents whereas administering the polymyxin agent first exhibited very poor activity against CRE. To date, no other study has demonstrated that meropenem should be administered before polymyxin B to improve the combination activity against CRE. However, a lot of additional work is needed to better understand why the interaction is different and to take full advantage of the interaction of sequence, timing, antimicrobial choice, and dose. More specifically, the present dissertation only explored one administration time difference of 2 hours when the optimal administration time difference may be more or less. Furthermore, following the first conclusion about the antimicrobials MICs influence the interaction, these MICs may also influence the timing and sequence necessary to optimize killing activity. The present dissertation supports the notion that the sequence of administration is not dependent on the carbapenem MIC of the organism, but the polymyxin B MIC or the timing may play a role on whether the sequence of antimicrobials should be a carbapenem first versus a polymyxin agent first. *In vitro* high throughput methodology like that of this dissertation would be most useful for initially addressing these hypotheses, but so too would animal and eventually human studies since the immune system and pharmacokinetics may influence the sequence and timing as well. For example, the same timing in a benchtop experiment may not be optimal when one drug distributes to the target site considerably faster than the other. Carbapenem resistance phenotypes other than KPC and VIM should also be included in future studies to determine if the sequence/timing interaction with carbapenems and

polymyxins is independent of genotype, and perhaps more importantly, phenotype. Finally, other antimicrobial agents in combination should be evaluated, at least *in vitro*, based on the present findings because such interactions may also be impacted by sequence and timing.

In the setting of current high-dose polymyxin dosing strategies exhibiting average free concentrations of polymyxin B around 1 m/gL, these dissertation findings suggest that lower polymyxin B concentrations can sufficiently kill the susceptible majority populations of CRE, substantiated by the model findings, and that when utilized in combination, lower concentration targets can exhibit equivalent killing activity as demonstrated by the time-kill studies. This information should subsequently be tested in an *in vivo* animal model, but it suggests that clinicians may be unnecessarily utilizing high doses of antimicrobials in combination like polymyxin B since the monotherapy dosing strategies are typically implemented. Lower doses may reduce toxicity. However, higher doses may address other important clinical endpoints such as the prevention of the emergence of resistance either on therapy or at a later point in a patient's life. Naturally, exploring this notion opens myriad opportunities to optimize therapeutic combinations and better characterize the relationship of clinical outcomes to antimicrobial dosing strategies.

Data in this dissertation do not support particular clinical testing methodologies for certain antimicrobials, namely polymyxins. More specifically, the Etest® should not be utilized to evaluate the activity of polymyxin B or colistin in the clinic. This conclusion is now well supported by other labs, including major organizations like CLSI and EUCAST. However, this dissertation also brings to light discrepancies that warrant further investigation between the traditional gold-standard broth dilution techniques and the

clinically efficient automated system, BD Phoenix™. Therefore, numerous questions are raised that warrant additional investigation.

It was previously discussed that resistance mechanism acquisition by bacteria are often associated with a fitness cost. Therefore, when bacteria are not under antimicrobial selective pressure (like when they are stored or re-subcultured prior to experimentation), the majority population may lose the mechanism of resistance in favor of more rapid growth. However, the present findings suggest that, in general, CRE tested by broth microdilution exhibited more resistance than the same CRE tested by BD Phoenix™ years prior. Since the degree of difference between testing methods was highly variable (it was not simply a normal distribution around, for example, 8-fold higher MICs), then attributing the difference only to differences in testing period or the time frozen is inappropriate. Instead, the data suggest a more complex explanation that warrants additional investigation as well as validation by other laboratories before insinuating that one testing method inherently produces biased results. An example of this complexity is also presented in this dissertation concerning the degree of heteroresistance observed with polymyxin B against CRE. Other laboratories have also attributed irreproducible or confounding results to heteroresistance observed among antimicrobials. These findings suggest that a single MIC report for an isolate are not sufficient for determining outcome, but instead are a guideline. If true, testing methodology should subsequently be developed to characterize not only the majority MIC measurement but also an assessment of the degree of heteroresistance or the uncertainty surrounding each MIC measurement. Such data could then be utilized in stochastic model to predict probabilities of treatment success. In other words, two isolates

exhibiting the same MIC but one having much more heteroresistant variability may warrant more aggressive treatment strategies to afford the same degree of clinical success certainty.

In summary, data from retrospective human studies have been compelling regarding the advantage of combination therapy, especially those containing a carbapenem, for the treatment of CRE. However, questions such as which antimicrobials, at what doses, for how long, and are there other factors that might determine clinical outcome still remain. Significant limitations such as heterogeneity and correlative evidence begs for randomized controlled trials evaluating antimicrobial combinations head-to-head. However, cost, coordination, and design hurdles nearly render this undertaking infeasible, which leads to *in vitro*, animal, and retrospective studies to address these questions. So far, time-kill studies have identified numerous combinations of antimicrobials with high rates of synergy, among them are polymyxins and aminoglycosides in combination with a carbapenem.

Limitations in antimicrobial susceptibility testing, such as the precision of MIC measurement, have been a long-standing issue for microbiologists. However, continued work on identifying isolate characteristics predictive of outcome and indicative of optimal therapy should be a continual pursuit in this field. Furthermore, as point-of-care and rapid diagnostic devices become available to assess patients and microorganisms for optimal treatment, special attention should be given to microorganisms possessing challenging-to-treat resistance mechanisms given the often-nuanced treatment necessary to effectively manage such infections. Essentially, precision medicine applies to infectious diseases, and the concept will continue to play a larger role as more information is available at the patient bedside when patient care teams are making decisions.

Data with *in vitro* pharmacodynamic and animal models more closely emulating human pharmacokinetics and the immune system are also warranted. Using the time-kill and pharmacodynamic modeling data from this study, pharmacokinetic models can be linked using published patient data or the use of bioreactors that mimic dynamic rather than static drug levels. Murine models can be used to incorporate the effects of an immune system and other factors of a living, infected host. Previous data, although limited, can serve as a foundation for experimental design and for comparison once data is obtained.

Finally, novel antimicrobial agents have just come to the market and should be investigated, both alone and in combination so that optimal treatment strategies can be developed. In particular, MBL-producing CRE are a major problem outside the U.S. but may easily become the predominant mechanism of resistance if judicious utilization of the novel carbapenemase inhibitors does not occur. Specifically, avibactam, relebactam, and vaborbactam show promise with inhibiting serine-based carbapenemases whereas plazomicin and eravacycline may demonstrate excellent activity against both KPC-producing and MBL-producing CRE. However, historically, bacteria have always developed resistance to any antimicrobial introduced to humans.

APPENDICES

Appendix A:

Minimum Inhibitory Concentrations Determined by BD Phoenix™

Tables A.1-A.2 provide the results of *in vitro* susceptibility testing performed by the University of Kentucky Clinical Microbiology Laboratory using BD Phoenix™.

The following abbreviations are used in Table A.1: AMP - Ampicillin; AMS - Ampicillin/Sulbactam; AZT - Aztreonam; CFZ - Cefazolin; CPM - Cefepime; FOX - Cefoxitin; CAZ - Ceftazidime; CAX - Ceftriaxone; CRM - Cefuroxime; ETP - Ertapenem; MEM - Meropenem; PTC - Piperacillin/Tazobactam.

The following abbreviations are used in Table A.2: AMK - Amikacin; CIP - Ciprofloxacin; GEN - Gentamicin; LEV - Levofloxacin; NIT - Nitrofurantoin; TET - Tetracycline; TOB - Tobramycin; SXT - Sulfamethoxazole/Trimethoprim.

Table A.1: Minimum Inhibitory Concentration of β-lactam Antimicrobials for University of Kentucky Carbapenem-resistant *Enterobacteriaceae* Clinical Isolates by BD Phoenix™ (n=164)

Organism	Isolate	AMP	AMS	AZT	CFZ	CPM	FOX	CAZ	CAX	CRM	ERT	MEM	PTC
C. amalonaticus	36	>16	>16/8	>16	>16	<=1	>16	>16	>32	>16	4	4	>64/4
C. amalonaticus	91	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	>4	4	>64/4
C. freundii	6	>16	>16/8	>16	>16	8	>16	4	>32	>16	2	<=1	>64/4
C. freundii	27	>16	>16/8	>16	>16	8	>16	8	>32	>16	2	<=1	>64/4
C. freundii	50	>16	>16/8	>16	>16	>16	16	>16	>32	>16	4	<=1	>64/4
C. freundii	54	>16	>16/8	>16	>16	4	>16	8	>32	>16	4	<=1	>64/4
C. freundii	101	>16	>16/8	>16	>16	2	>16	>16	32	>16	2	<=1	>64/4
C. freundii	127	>16	>16/8	>16	>16	2	>16	>16	>32	>16	>4	2	>64/4
C. freundii	135	>16	>16/8	<=2	>16	>16	>16	>16	>32	>16	<=0.5	4	>64/4
C. freundii	145	>16	>16/8	>16	>16	2	>16	>16	>32	>16	2	<=1	>64/4
C. freundii	147	>16	>16/8	>16	>16	8	>16	>16	>32	>16	2	<=1	>64/4
C. freundii	324	>16	>16/8	>16	>16	>16	>16	4	>32		1	<=0.5	>64/4
C. freundii	562	>16	>16/8	<=2	>16	>16	>16	>16	>32		>1	4	>64/4
C. freundii	593	>16	>16/8	>16	>16	>16	>16	>16	R		>1	<=0.5	>64/4
C. freundii	604	>16	>16/8	>16	>16	>16	>16	>16			>1	<=0.5	>64/4
C. youngae	136	>16	>16/8	>16	>16	16	>16	8	>32	>16	>4	4	>64/4
C. youngae	435	>16	>16/8	>16	>16	>16	>16	>16	>32		>1	8	>64/4
E. aerogenes	97	>16	>16/8	>16	>16	2	>16	>16	>32	>16	1	<=1	>64/4
E. aerogenes	179	>16	>16/8	<=2	>16	<=1	>16	>16	>32	>16	4	>8	>64/4
E. aerogenes	187	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	2	<=1	>64/4
E. aerogenes	438	>16	>16/8	>16	>16	16	>16	>16	>32			8	>64/4
E. cloacae	1	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	>4	8	>64/4
E. cloacae	3	>16	>16/8	>16	>16	8	>16	>16	>32	>16	>4	<=1	>64/4
E. cloacae	4	>16	>16/8	>16	>16	4	>16	>16	>32	>16	>4	<=1	>64/4
E. cloacae	5	>16	>16/8	>16	>16	16	>16	8	>32	>16	>4	2	>64/4
E. cloacae	9	>16	>16/8	>16	>16	16	>16	>16	>32	>16	4	>8	>64/4

Abbreviations: AMP - Ampicillin; AMS - Ampicillin/Sulbactam; AZT - Aztreonam; CFZ - Cefazolin; CPM - Cefepime; FOX - Cefoxitin; CAZ - Ceftazidime; CAX - Ceftriaxone; CRM - Cefuroxime; ETP - Ertapenem; MEM - Meropenem; PTC - Piperacillin/Tazobactam

Table A.1: Minimum Inhibitory Concentration of β-lactam Antimicrobials for University of Kentucky Carbapenem-resistant *Enterobacteriaceae* Clinical Isolates by BD Phoenix™ (n=164)

Organism	Isolate	AMP	AMS	AZT	CFZ	CPM	FOX	CAZ	CAX	CRM	ERT	MEM	PTC
E. cloacae	10	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	>4	>8	>64/4
E. cloacae	12	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	>4	>8	>64/4
E. cloacae	15	>16	>16/8	>16	>16	4	>16	>16	>32	>16	2	<=1	>64/4
E. cloacae	16	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	>4	<=1	>64/4
E. cloacae	17	>16	>16/8	>16	>16	4	>16	>16	>32	>16	>4	<=1	>64/4
E. cloacae	19	>16	>16/8	>16	>16	8	>16	>16	>32	>16	2	<=1	>64/4
E. cloacae	20	>16	>16/8	>16	>16	8	>16	>16	>32	>16	>4	8	>64/4
E. cloacae	23	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	>4	2	>64/4
E. cloacae	30	>16	>16/8	>16	>16		>16	>16	>32	>16	>4	<=1	>64/4
E. cloacae	39	>16	>16/8	>16	>16	8	>16	16	>32	>16	>4	<=1	>64/4
E. cloacae	40	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	4	8	>64/4
E. cloacae	41	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	4	>8	>64/4
E. cloacae	52	>16	>16/8	>16	>16	16	>16	8	>32	>16	>4	>8	>64/4
E. cloacae	53	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	>4	8	>64/4
E. cloacae	70	>16	>16/8	>16	>16	2	>16	>16	>32	>16	2	<=1	8/4
E. cloacae	96	>16	>16/8	>16	>16	<=1	>16	>16	>32	>16	1	>8	64/4
E. cloacae	107	>16	>16/8	>16	>16	8	>16	8	>32	>16	>4	8	>64/4
E. cloacae	121	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	>4	8	>64/4
E. cloacae	126	>16	>16/8	>16	>16	>16	>16	4	>32	>16	>4	>8	>64/4
E. cloacae	134	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	2	8	>64/4
E. cloacae	144	>16	>16/8	>16	>16	2	>16	16	>32	>16	>4	4	>64/4
E. cloacae	167	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	2	4	>64/4
E. cloacae	168	>16	>16/8	>16	>16	8	>16	16	>32	>16	>4	>8	>64/4
E. cloacae	169	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	2	4	>64/4
E. cloacae	171	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	>4	>8	>64/4
E. cloacae	175	>16	>16/8	<=2	>16	>16	>16	>16	>32	>16	2	4	>64/4

Abbreviations: AMP - Ampicillin; AMS - Ampicillin/Sulbactam; AZT - Aztreonam; CFZ - Cefazolin; CPM - Cefepime; FOX - Cefoxitin; CAZ - Ceftazidime; CAX - Ceftriaxone; CRM - Cefuroxime; ETP - Ertapenem; MEM - Meropenem; PTC - Piperacillin/Tazobactam

Table A.1: Minimum Inhibitory Concentration of β-lactam Antimicrobials for University of Kentucky Carbapenem-resistant *Enterobacteriaceae* Clinical Isolates by BD Phoenix™ (n=164)

Organism	Isolate	AMP	AMS	AZT	CFZ	CPM	FOX	CAZ	CAX	CRM	ERT	MEM	PTC
E. cloacae	189	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	4	4	>64/4
E. cloacae	200	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	4	<=1	>64/4
E. cloacae	203	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	>4	>8	>64/4
E. cloacae	209	>16	>16/8	>16	>16	>16	>16	16	>32	>16	>4	>8	>64/4
E. cloacae	266	>16	>16/8	>16	>16	>16	>16	>16	>32		>1	4	>64/4
E. cloacae	335	>16	>16/8	>16	>16	>16	>16	16	>32		>1	<=0.5	32/4
E. cloacae	339	>16	>16/8	>16	>16	>16	>16	>16	>32		>1	>8	>64/4
E. cloacae	369	>16	>16/8	>16	>16	>16	>16	>16	>32		>1	4	>64/4
E. cloacae	416	>16	>16/8	<=2	>16	>16	>16	>16	>32		>1	4	>64/4
E. cloacae	476	>16	>16/8	<=2	>16	>16	>16	>16	>32		1	8	>64/4
E. cloacae	515	>16	>16/8	>16	>16	16	>16	>16	>32		>1	2	>64/4
E. cloacae	561	>16	>16/8	>16	>16	4	>16	8	>32		>1	<=0.5	>64/4
E. cloacae	599	>16	>16/8	>16	>16	8	>16	>16	>32			2	>64/4
E. cloacae	606	>16	>16/8	>16	>16	16	>16	>16	>32		>1	4	>64/4
E. cloacae	607	>16	>16/8	>16	>16	>16	>16	>16	>32		>1	8	>64/4
E. cloacae	608	>16	>16/8	>16	>16	>16	>16	>16	>32		>1	>8	>64/4
E. cloacae	611	>16	>16/8	16	>16	2	>16	16	>32		>1	1	>64/4
E. cloacae	613	>16	>16/8	>16	>16	>16	>16	>16	>32		>1	8	>64/4
E. cloacae	615	>16	>16/8	>16	>16	>16	>16	>16	>32		>1	8	>64/4
E. coli	25	>16	>16/8	4	>16	<=1	8	4	>32	>16	4	<=1	>64/4
E. coli	33	>16	>16/8	>16	>16	8	>16	>16	>32	>16	4	<=1	>64/4
E. coli	103	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	1	<=1	32/4
E. coli	172	>16	>16/8	<=2	>16	>16	>16	>16	>32	>16	2	8	>64/4
E. coli	176	>16	>16/8	>16	>16	16	16	>16	>32	>16	>4	<=1	>64/4
E. coli	309	>16	>16/8	>16	>16	>16	>16	>16	>32		>1	4	64/4
E. coli	390	>16	>16/8	>16	>16	>16	>16	>16	>32		1	<=0.5	8/4

Abbreviations: AMP - Ampicillin; AMS - Ampicillin/Sulbactam; AZT - Aztreonam; CFZ - Cefazolin; CPM - Cefepime; FOX - Cefoxitin; CAZ - Ceftazidime; CAX - Ceftriaxone; CRM - Cefuroxime; ETP - Ertapenem; MEM - Meropenem; PTC - Piperacillin/Tazobactam

Table A.1: Minimum Inhibitory Concentration of β-lactam Antimicrobials for University of Kentucky Carbapenem-resistant *Enterobacteriaceae* Clinical Isolates by BD Phoenix™ (n=164)

Organism	Isolate	AMP	AMS	AZT	CFZ	CPM	FOX	CAZ	CAX	CRM	ERT	MEM	PTC
E. coli	508	>16	>16/8	>16	>16	>16	16	>16	>32		1	<=0.5	>64/4
E. coli	609	>16	>16/8	>16	>16	>16	>16	>16	>32		>1	>8	>64/4
E. gergoviae	13	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	>4	>8	>64/4
E. gergoviae	95	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	>4	<=1	>64/4
E. hormaechei	186	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	>4	>8	>64/4
E. hormaechei	398	>16	>16/8	>16	>16	>16	>16	>16	>32		>1	8	>64/4
Enterobacter sp.	146	>16	>16/8	>16	>16	8	>16	16	>32	>16	>4	>8	>64/4
Enterobacter sp.	210	>16	>16/8	>16	>16	>16	>16	16	>32	>16	>4	>8	>64/4
K. oxytoca	8	>16	>16/8	>16	>16	<=1	8	4	16	>16	>4	<=1	>64/4
K. oxytoca	14	>16	>16/8	>16	>16	8	8	8	>32	>16	>4	<=1	>64/4
K. oxytoca	166	>16	>16/8	>16	>16	8	<=4	2	>32	>16	>4	8	64/4
K. oxytoca	177	>16	>16/8	>16	>16	<=1	8	8	>32	>16	4	2	>64/4
K. oxytoca	330	>16	>16/8	>16	>16	>16	>16	8	>32		>1	8	>64/4
K. ozaenae	128	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	>4	<=1	>64/4
K. ozaenae	407	>16	>16/8	>16	>16	4	16	>16	>32		>1	<=0.5	>64/4
K. pneumoniae	2	>16	>16/8	8	>16	2	<=4	4	32	>16	4	<=1	>64/4
K. pneumoniae	7	>16	>16/8	>16	>16	>16	16	8	>32	>16	>4	8	>64/4
K. pneumoniae	11	>16	>16/8	>16	>16	8	>16	4	>32	>16	>4	<=1	>64/4
K. pneumoniae	18	>16	>16/8	>16	>16	8	>16	16	>32	>16	>4	<=1	>64/4
K. pneumoniae	21	>16	>16/8	16	>16	2	>16	>16	32	>16	2	<=1	>64/4
K. pneumoniae	22	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	>4	<=1	>64/4
K. pneumoniae	24	>16	>16/8	>16	>16	16	>16	>16	>32	>16	>4	2	>64/4
K. pneumoniae	26	>16	>16/8	>16	>16	<=1	>16	>16	8	>16	>4	8	>16/4
K. pneumoniae	28	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	>4	>8	>64/4
K. pneumoniae	29	>16	>16/8	>16	>16	<=1	>16	>16	>32	>16	>4	>8	>64/4
K. pneumoniae	31	>16	>16/8	8	>16	4	<=4	4	>32	>16	>4	<=1	>64/4

Abbreviations: AMP - Ampicillin; AMS - Ampicillin/Sulbactam; AZT - Aztreonam; CFZ - Cefazolin; CPM - Cefepime; FOX - Cefoxitin; CAZ - Ceftazidime; CAX - Ceftriaxone; CRM - Cefuroxime; ETP - Ertapenem; MEM - Meropenem; PTC - Piperacillin/Tazobactam

Table A.1: Minimum Inhibitory Concentration of β-lactam Antimicrobials for University of Kentucky Carbapenem-resistant *Enterobacteriaceae* Clinical Isolates by BD Phoenix™ (n=164)

Organism	Isolate	AMP	AMS	AZT	CFZ	CPM	FOX	CAZ	CAX	CRM	ERT	MEM	PTC
K. pneumoniae	32	>16	>16/8	>16	>16	>16	>16	4	>32	>16	>4	<=1	>64/4
K. pneumoniae	34	>16	>16/8	<=2	>16	<=1	<=4	2	>32	>16	>4	<=1	>64/4
K. pneumoniae	35	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	>4	>8	>64/4
K. pneumoniae	37	>16	>16/8	16	>16		16	16	>32	>16	>4	>8	>64/4
K. pneumoniae	42	>16	>16/8	<=2	>16	>16	>16	>16	>32	>16	2	<=1	>64/4
K. pneumoniae	43	>16	>16/8	>16	>16	16	>16	16	>32	>16	>4	>8	>64/4
K. pneumoniae	44	>16	>16/8	>16	>16	<=1	>16	>16	>32	>16	>4	>8	>64/4
K. pneumoniae	45	>16	>16/8	>16	>16	<=1	>16	>16	>32	>16	>4	>8	>64/4
K. pneumoniae	46	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	>4	>8	>64/4
K. pneumoniae	47	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	>4	>8	>64/4
K. pneumoniae	48	>16	>16/8	>16	>16	>16	16	>16	>32	>16	>4	<=1	>64/4
K. pneumoniae	49	>16	>16/8	>16	>16	16	>16	16	>32	>16	>4	>8	>64/4
K. pneumoniae	51	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	>4	>8	>64/4
K. pneumoniae	55	>16	>16/8	>16	>16	8	8	>16	>32	>16	>4	>8	>64/4
K. pneumoniae	69	>16	>16/8	>16	>16	<=1	>16	>16	>32	>16	2	<=1	8/4
K. pneumoniae	77	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	>4	2	>64/4
K. pneumoniae	93	>16	>16/8	>16	>16	>16	>16	16	>32	>16	>4	>8	>64/4
K. pneumoniae	98	>16	>16/8	>16	>16	<=1	>16	>16	>32	>16	2	<=1	>64/4
K. pneumoniae	99	>16	>16/8	>16	>16	8	>16	8	>32	>16	>4	8	>64/4
K. pneumoniae	105	>16	>16/8	>16	>16	8	8	16	>32	>16	>4	>8	>64/4
K. pneumoniae	116	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	>4	8	>64/4
K. pneumoniae	119	>16	>16/8	>16	>16	<=1	>16	>16	>32	>16	>4	>8	>64/4
K. pneumoniae	123	>16	>16/8	>16	>16	<=1	>16	>16	>32	>16	>4	>8	>64/4
K. pneumoniae	129	>16	>16/8	>16	>16	8	8	>16	>32	>16	>4	>8	>64/4
K. pneumoniae	130	>16	>16/8	>16	>16	>16	8	>16	>32	>16	>4	8	>64/4
K. pneumoniae	142	>16	>16/8	>16	>16	>16	>16	>16	>32	>16	>4	>8	>64/4

Abbreviations: AMP - Ampicillin; AMS - Ampicillin/Sulbactam; AZT - Aztreonam; CFZ - Cefazolin; CPM - Cefepime; FOX - Cefoxitin; CAZ - Ceftazidime; CAX - Ceftriaxone; CRM - Cefuroxime; ETP - Ertapenem; MEM - Meropenem; PTC - Piperacillin/Tazobactam

Table A.1: Minimum Inhibitory Concentration of β-lactam Antimicrobials for University of Kentucky Carbapenem-resistant *Enterobacteriaceae* Clinical Isolates by BD Phoenix™ (n=164)

Organism	Isolate	AMP	AMS	AZT	CFZ	CPM	FOX	CAZ	CAX	CRM	ERT	MEM	PTC
K. pneumoniae	143	>16	>16/8	>16	>16	<=1	>16	>16	>32	>16	4	2	>64/4
K. pneumoniae	152	>16	>16/8	<=2	>16	16	>16	16	<=2	>16	4	<=1	>64/4
K. pneumoniae	165	>16	>16/8	>16	>16	8	>16	16	>32	>16	>4	8	>64/4
K. pneumoniae	170	>16	>16/8	<=2	>16	>16	>16	>16	>32	>16	4	>8	>64/4
K. pneumoniae	173	>16	>16/8	<=2	>16	>16	>16	>16	>32	>16	2	8	>64/4
K. pneumoniae	174	>16	>16/8	<=2	>16	>16	>16	>16	>32	>16	2	>8	>64/4
K. pneumoniae	230	>16	>16/8	>16	>16	>16	>16	>16	>32		>1	>8	>64/4
K. pneumoniae	243	>16	>16/8	>16	>16	>16	>16	>16	>32		>1	<=0.5	>64/4
K. pneumoniae	256	>16	>16/8	>16	>16	>16	>16	16	>32		>1	>8	>64/4
K. pneumoniae	269	>16	>16/8		>16	<=1	>16	>16	>32		>1	<=0.5	>64/4
K. pneumoniae	284	>16	>16/8		>16	4	>16	>16	>32		>1	8	>64/4
K. pneumoniae	349	>16	>16/8	<=2	>16	<=1	>16	>16	>32		>1	2	>64/4
K. pneumoniae	352	>16	>16/8	>16	>16	>16	>16	16	>32		>1	<=0.5	>64/4
K. pneumoniae	372	>16	>16/8	>16	>16	<=1	>16	>16	>32		>1	4	>64/4
K. pneumoniae	385	>16	>16/8	>16	>16		>16	>16	>32		>1	1	>64/4
K. pneumoniae	391	>16	>16/8	>16	>16	>16	>16	>16	>32		>1	4	>64/4
K. pneumoniae	411	>16	>16/8	<=2	>16	>16	>16	>16	>32		>1	>8	>64/4
K. pneumoniae	418	>16	>16/8	>16	>16	<=1	>16	>16	>32		>1	1	>64/4
K. pneumoniae	423	>16	>16/8	>16	>16	>16	16	>16	>32		>1	4	>64/4
K. pneumoniae	445	>16	>16/8	>16	>16	>16	>16	>16	>32		>1	>8	>64/4
K. pneumoniae	446	>16	>16/8		>16	<=1	>16	>16	>32		>1	>8	>64/4
K. pneumoniae	449	>16	>16/8	>16	>16	16	>16	>16	>32		>1	8	>64/4
K. pneumoniae	452	>16	>16/8	>16	>16	<=1	>16	>16	>32		>1	2	>64/4
K. pneumoniae	466	>16	>16/8	>16	>16	>16	>16	>16	>32		>1	2	>64/4
K. pneumoniae	482	>16	>16/8	>16	>16	<=1	>16	>16	>32		>1	2	>64/4
K. pneumoniae	492	>16	>16/8	>16	>16	<=1	>16	>16	>32		>1	2	>64/4

Abbreviations: AMP - Ampicillin; AMS - Ampicillin/Sulbactam; AZT - Aztreonam; CFZ - Cefazolin; CPM - Cefepime; FOX - Cefoxitin; CAZ - Ceftazidime; CAX - Ceftriaxone; CRM - Cefuroxime; ETP - Ertapenem; MEM - Meropenem; PTC - Piperacillin/Tazobactam

Table A.1: Minimum Inhibitory Concentration of β-lactam Antimicrobials for University of Kentucky Carbapenem-resistant *Enterobacteriaceae* Clinical Isolates by BD Phoenix™ (n=164)

Organism	Isolate	AMP	AMS	AZT	CFZ	CPM	FOX	CAZ	CAX	CRM	ERT	MEM	PTC
K. pneumoniae	536	>16	>16/8	>16	>16	4	>16	>16	>32		>1	1	>64/4
K. pneumoniae	558	>16	>16/8	>16	>16	>16	>16	16	>32		>1	8	>64/4
K. pneumoniae	605	>16	>16/8	>16	>16	2	16	>16	>32		>1	8	>64/4
K. pneumoniae	610	>16	>16/8	>16	>16	8	8	>16	>32		>1	4	>64/4
K. pneumoniae	616	>16	>16/8	<=2	>16	>16	>16	>16	>32		>1	>8	>64/4
P. rettgeri	244	>16	8/4	<=2	>16	<=1	>16	2	2		>1	2	<=2/4
S. marcescens	514	>16	>16/8	>16	>16	>16	>16	>16	>32		>1	>8	>64/4
S. marcescens	560	>16	>16/8	>16	>16	>16	>16	>16	>32		>1	>8	>64/4

MIC values expressed in the table are in units of mg/L

Blank entries were not tested by University of Kentucky Microbiology Laboratory

Some organism and antimicrobial combinations were only tested by Etest® and reported as S, I, or R

Abbreviations: AMP - Ampicillin; AMS - Ampicillin/Sulbactam; AZT - Aztreonam; CFZ - Cefazolin; CPM - Cefepime; FOX - Cefoxitin; CAZ - Ceftazidime; CAX - Ceftriaxone; CRM - Cefuroxime; ETP - Ertapenem; MEM - Meropenem; PTC - Piperacillin/Tazobactam

Table A.2: Minimum Inhibitory Concentration of Non-β-lactam Antimicrobials for University of Kentucky Clinical Isolates by BD Phoenix™ (n=164)

Organism	Isolate	AMK	GEN	TOB	CIP	LEV	NIT	TET	SXT
C. amalonaticus	36	<=8	>8	>8	>2	>4	32	4	>2/38
C. amalonaticus	91	<=8	>8	>8	>2	>4	64	4	>2/38
C. freundii	6	<=8	<=2	<=2	>2	4	<=16	4	>2/38
C. freundii	27	>32	>8	>8	>2	>4	<=16	<=2	>2/38
C. freundii	50	<=8	>8	>8	>2	4	<=16	4	>2/38
C. freundii	54	<=8	>8	>8	>2	>4	<=16	4	<=0.5/9.5
C. freundii	101	<=8	8	4	>2	>4	<=16	>8	>2/38
C. freundii	127	<=8	>8	>8	>2	>4	<=16	>8	>2/38
C. freundii	135	<=8	<=2	8	<=0.5	<=1	<=16	<=2	>2/38
C. freundii	145	<=8	<=2	<=2	>2	>4	<=16	4	<=0.5/9.5
C. freundii	147	<=8	<=2	<=2	<=0.5	<=1	<=16	<=2	<=0.5/9.5
C. freundii	324	<=8	<=2	<=2	2	<=1	<=16	<=2	>2/38
C. freundii	562	<=8	<=2	8	<=0.5	<=1	<=16	<=2	>2/38
C. freundii	593	<=8	>8	8	>2	>4	<=16	>8	>2/38
C. freundii	604	<=8	>8	8	>2	>4	<=16	>8	>2/38
C. youngae	136	<=8	<=2	<=2	>2	>4	<=16	>8	>2/38
C. youngae	435	<=8	<=2	<=2	>2	>4	<=16	>8	<=0.5/9.5
E. aerogenes	97	<=8	<=2	<=2	<=0.5	<=1	<=16	<=2	<=0.5/9.5
E. aerogenes	179	<=8	8	>8	<=0.5	<=1	>64	<=2	>2/38
E. aerogenes	187	<=8	<=2	<=2	<=0.5	<=1	>64	>8	<=0.5/9.5
E. aerogenes	438	<=8	<=2	<=2	<=0.5	<=1	32	<=2	<=0.5/9.5
E. cloacae	1	<=8	>8	>8	>2	>4	>64	>8	>2/38
E. cloacae	3	<=8	>8	>8	1	<=1	64	<=2	>2/38
E. cloacae	4	<=8	>8	>8	1	<=1	64	<=2	>2/38
E. cloacae	5	<=8	>8	>8	<=0.5	<=1	64	<=2	>2/38
E. cloacae	9	<=8	>8	>8	>2	>4	64	8	<=0.5/9.5
E. cloacae	10	<=8	>8	>8	>2	>4	64	8	<=0.5/9.5

Abbreviations: AMK - Amikacin; GEN - Gentamicin; TOB - Tobramycin; CIP - Ciprofloxacin; LEV - Levofloxacin; NIT - Nitrofurantoin; TET - Tetracycline; SXT - Sulfamethoxazole/Trimethoprim

Table A.2: Minimum Inhibitory Concentration of Non-β-lactam Antimicrobials for University of Kentucky Clinical Isolates by BD Phoenix™ (n=164)

Organism	Isolate	AMK	GEN	TOB	CIP	LEV	NIT	TET	SXT
E. cloacae	12	<=8	>8	>8	>2	>4	64	8	<=0.5/9.5
E. cloacae	15	<=8	8	>8	1	<=1	32	<=2	<=0.5/9.5
E. cloacae	16	<=8	>8	>8	>2	>4	32	8	>2/38
E. cloacae	17	<=8	>8	>8	>2	>4	64	4	>2/38
E. cloacae	19	<=8	8	>8	1	<=1	64	<=2	<=0.5/9.5
E. cloacae	20	<=8	>8	>8	>2	>4	64	8	<=0.5/9.5
E. cloacae	23	<=8	>8	>8	>2	>4	64	8	<=0.5/9.5
E. cloacae	30	<=8	>8	>8	>2	>4	>64	>8	>2/38
E. cloacae	39	<=8	>8	>8	>2	2	32	<=2	<=0.5/9.5
E. cloacae	40	<=8	>8	>8	>2	>4	>64	4	>2/38
E. cloacae	41	16	>8	>8	1	<=1	64	>8	>2/38
E. cloacae	52	<=8	8	>8	>2	>4	32	8	<=0.5/9.5
E. cloacae	53	<=8	>8	>8	>2	>4	>64	>8	>2/38
E. cloacae	70	<=8	>8	8	>2	>4	>64	>8	>2/38
E. cloacae	96	<=8	<=2	<=2	<=0.5	<=1	<=16	<=2	<=0.5/9.5
E. cloacae	107	<=8	>8	>8	>2	>4	32	4	<=0.5/9.5
E. cloacae	121	<=8	>8	>8	1	<=1	>64	4	>2/38
E. cloacae	126	<=8	>8	>8	>2	>4	32	8	<=0.5/9.5
E. cloacae	134	<=8	>8	>8	1	<=1	>64	<=2	>2/38
E. cloacae	144	<=8	>8	>8	>2	2	<=16	<=2	<=0.5/9.5
E. cloacae	167	<=8	<=2	8	1		64	<=2	>2/38
E. cloacae	168	<=8	8	>8	>4	>4	64	8	<=0.5/9.5
E. cloacae	169	<=8	<=2	8	1	<=1	64	<=2	>2/38
E. cloacae	171	<=8	>8	>8	>2	>4	64	8	<=0.5/9.5
E. cloacae	175	<=8	>8	>8	1	<=1	32	<=2	>2/38
E. cloacae	189	<=8	<=2	8	<=0.5	<=1	64	<=2	>2/38
E. cloacae	200	<=8	>8	>8	>2	>4	64	8	>2/38

Abbreviations: AMK - Amikacin; GEN - Gentamicin; TOB - Tobramycin; CIP - Ciprofloxacin; LEV - Levofloxacin; NIT - Nitrofurantoin; TET - Tetracycline; SXT - Sulfamethoxazole/Trimethoprim

Table A.2: Minimum Inhibitory Concentration of Non-β-lactam Antimicrobials for University of Kentucky Clinical Isolates by BD Phoenix™ (n=164)

Organism	Isolate	AMK	GEN	TOB	CIP	LEV	NIT	TET	SXT
E. cloacae	203	<=8	>8	>8	1	<=1	64	<=2	>2/38
E. cloacae	209	<=8	>8	>8	>2	>4	>64	8	<=0.5/9.5
E. cloacae	266	<=8	>8	>8	<=0.5	<=1	64	<=2	>2/38
E. cloacae	335	<=8	<=2	<=2	>2	>4	>64	>8	>2/38
E. cloacae	339	<=8	>8	>8	>2	>4	>64	>8	>2/38
E. cloacae	369	<=8	<=2	<=2	>2	4	>64	>8	<=0.5/9.5
E. cloacae	416	<=8	>8	>8	<=0.5	<=1	64	<=2	>2/38
E. cloacae	476	<=8	<=2	>8	<=0.5	<=1	>64	<=2	>2/38
E. cloacae	515	<=8	4	4	>2	>4	64	8	>2/38
E. cloacae	561	<=8	>8	>8	>2	4	32	8	>2/38
E. cloacae	599	<=8	>8	>8	<=0.5	<=1		<=2	>2/38
E. cloacae	606	<=8	>8	>8	>2	>4		<=2	>2/38
E. cloacae	607	16	>8	>8	<=0.5	<=1	64	<=2	>2/38
E. cloacae	608	16	>8	>8	>2	4	>64	>8	>2/38
E. cloacae	611	<=8	>8	>8	<=0.5	<=1	64	<=2	>2/38
E. cloacae	613	<=8	>8	>8	>2	>4		>8	>2/38
E. cloacae	615	<=8	<=2	8	1	<=1		<=2	>2/38
E. coli	25	<=8	<=2	<=2	>2	>4	<=16	>8	>2/38
E. coli	33	<=8	>8	8	>2	>4	>64	<=2	<=0.5/9.5
E. coli	103	<=8	<=2	>8	>2	>4	64	>8	>2/38
E. coli	172	<=8	<=2	>8	1	<=1	<=16	<=2	>2/38
E. coli	176	<=8	<=2	<=2	>2	>4	<=16	<=2	>2/38
E. coli	309	<=8	<=2	<=2	>2	>4	32	>8	<=0.5/9.5
E. coli	390	<=8	>8	8	>2	>4	<=16	>8	>2/38
E. coli	508	<=8	>8	>8	>2	>4	<=16	>8	>2/38
E. coli	609	<=8	>8	8	>2	>4	<=16	>8	>2/38
E. gergoviae	13	<=8	>8	>8	>2	>4	64	8	<=0.5/9.5

Abbreviations: AMK - Amikacin; GEN - Gentamicin; TOB - Tobramycin; CIP - Ciprofloxacin; LEV - Levofloxacin; NIT - Nitrofurantoin; TET - Tetracycline; SXT - Sulfamethoxazole/Trimethoprim

Table A.2: Minimum Inhibitory Concentration of Non-β-lactam Antimicrobials for University of Kentucky Clinical Isolates by BD Phoenix™ (n=164)

Organism	Isolate	AMK	GEN	TOB	CIP	LEV	NIT	TET	SXT
E. gergoviae	95	<=8	<=2	<=2	<=0.5	<=1	64	>8	<=0.5/9.5
E. hormaechei	186	<=8	>8	>8	>2	4	>64	8	>2/38
E. hormaechei	398	<=8	>8	>8	<=0.5	<=1	64	<=2	>2/38
Enterobacter sp.	146	<=8	>8	>8	>2	>4	64	8	<=0.5/9.5
Enterobacter sp.	210	<=8	8	>8	>2	>4	<=16	4	<=0.5/9.5
K. oxytoca	8	<=8	<=2	<=2	<=0.5	<=1	<=16	<=2	1/19
K. oxytoca	14	<=8	>8	>8	2	2	<=16	4	>2/38
K. oxytoca	166	<=8	<=2	<=2	<=0.5	<=1	32	<=2	<=0.5/9.5
K. oxytoca	177	<=8	>8	>8	<=0.5	<=1	<=16	<=2	<=0.5/9.5
K. oxytoca	330	<=8	8	8	>2	4	<=16	8	>2/38
K. ozaenae	128	<=8	4	4	>2	>4	<=16	4	>2/38
K. ozaenae	407	<=8	>8	>8	2	2	<=16	4	<=0.5/9.5
K. pneumoniae	2	<=8	>8	>8	<=0.5	<=1	64	>8	>2/38
K. pneumoniae	7	<=8	<=2	<=2	<=0.5	<=1	64	<=2	>2/38
K. pneumoniae	11	<=8	<=2	<=2	<=0.5	<=1	>64	8	2/38
K. pneumoniae	18	<=8	<=2	<=2	<=0.5	<=1	>64	8	>2/38
K. pneumoniae	21	<=8	4	>8	1	<=1	<=16	<=2	>2/38
K. pneumoniae	22	32	>8	>8	>2	>4	>64	>8	>2/38
K. pneumoniae	24	<=8	8	>8	>2	>4	>64	4	<=0.5/9.5
K. pneumoniae	26	>32	>8	>8	>2	>4	>64	<=2	>2/38
K. pneumoniae	28	32	4	>8	>2	>4	>64	8	>2/38
K. pneumoniae	29	32	4	>8	>2	>4	>64	4	2/38
K. pneumoniae	31	<=8	>8	>8	<=0.5	<=1	>64	<=2	2/38
K. pneumoniae	32	<=8	<=2	<=2	>2	4	64	8	<=0.5/9.5
K. pneumoniae	34	<=8	<=2	<=2	<=0.5	<=1	64	<=2	<=0.5/9.5
K. pneumoniae	35	<=8	>8	>8	>2	>4	>64	8	<=0.5/9.5
K. pneumoniae	37	>32	>8	>8	<=0.5	<=1	32	<=2	<=0.5/9.5

Abbreviations: AMK - Amikacin; GEN - Gentamicin; TOB - Tobramycin; CIP - Ciprofloxacin; LEV - Levofloxacin; NIT - Nitrofurantoin; TET - Tetracycline; SXT - Sulfamethoxazole/Trimethoprim

Table A.2: Minimum Inhibitory Concentration of Non-β-lactam Antimicrobials for University of Kentucky Clinical Isolates by BD Phoenix™ (n=164)

Organism	Isolate	AMK	GEN	TOB	CIP	LEV	NIT	TET	SXT
K. pneumoniae	42	<=8	>8	>8	2	<=1	<=16	<=2	>2/38
K. pneumoniae	43	<=8	>8	>8	>2	>4	64	4	<=0.5/9.5
K. pneumoniae	44	<=8	>8	>8	>2	>4	>64	>8	1/19
K. pneumoniae	45	<=8	>8	>8	>2	>4	>64	4	2/38
K. pneumoniae	46	>32	>8	>8	>2	>4	>64	4	>2/38
K. pneumoniae	47	>32	>8	>8	>2	>4	>64	<=2	>2/38
K. pneumoniae	48	>32	>8	>8	>2	>4	>64	4	<=0.5/9.5
K. pneumoniae	49	<=8	>8	>8	>2	4	32	4	<=0.5/9.5
K. pneumoniae	51	<=8	8	8	>2	>4	64	4	<=0.5/9.5
K. pneumoniae	55	16	<=2	>8	>2	>4	64	>8	>2/38
K. pneumoniae	69	<=8	<=2	<=2	>2	>4	>64	>8	>2/38
K. pneumoniae	77	<=8	>8	8	>2	4	>64	<=2	<=0.5/9.5
K. pneumoniae	93	<=8	>8	>8	>2	>4	64	4	<=0.5/9.5
K. pneumoniae	98	<=8	4	>8	<=0.5	<=1	<=16	>8	<=0.5/9.5
K. pneumoniae	99	<=8	<=2	<=2	<=0.5	<=1	>64	>8	2/38
K. pneumoniae	105	<=8	<=2	<=2	<=0.5	<=1	64	<=2	1/19
K. pneumoniae	116	<=8	<=2	<=2	>2	>4	>64	>8	>2/38
K. pneumoniae	119	16	>8	>8	>2	>4	>64	4	>2/38
K. pneumoniae	123	<=8	>8	>8	>2	>4	>64	4	>2/38
K. pneumoniae	129	16	<=2	>8	>2	>4	32	<=2	>2/38
K. pneumoniae	130	<=8	>8	>8	1	<=1	>64	<=2	<=0.5/9.5
K. pneumoniae	142	<=8	<=2	<=2	<=0.5	<=1	64	<=2	<=0.5/9.5
K. pneumoniae	143	<=8	>8	8	>2	>4	>64	>8	>2/38
K. pneumoniae	152	<=8	<=2	<=2	>2	>4	>64	>8	>2/38
K. pneumoniae	165	<=8	>8	>8	>2	>4	64	8	<=0.5/9.5
K. pneumoniae	170	<=8	>8	>8	>2	4	32	>8	>2/38
K. pneumoniae	173	<=8	<=2	8	1	<=1	32	<=2	>2/38

Abbreviations: AMK - Amikacin; GEN - Gentamicin; TOB - Tobramycin; CIP - Ciprofloxacin; LEV - Levofloxacin; NIT - Nitrofurantoin; TET - Tetracycline; SXT - Sulfamethoxazole/Trimethoprim

Table A.2: Minimum Inhibitory Concentration of Non-β-lactam Antimicrobials for University of Kentucky Clinical Isolates by BD Phoenix™ (n=164)

Organism	Isolate	AMK	GEN	TOB	CIP	LEV	NIT	TET	SXT
K. pneumoniae	174	<=8	>8	>8	1	<=1	<=16	>8	>2/38
K. pneumoniae	230	<=8	4	>8	>2	>4	>64	8	>2/38
K. pneumoniae	243	<=8	<=2	<=2	>2	>4	>64	4	>2/38
K. pneumoniae	256	<=8	8	>8	>2	>4	64	8	<=0.5/9.5
K. pneumoniae	269	<=8	<=2	<=2	>2	>4	64	4	>2/38
K. pneumoniae	284	<=8	<=2	<=2	>2	>4	>64	>8	>2/38
K. pneumoniae	349	<=8	>8	8	>2	>4	>64	>8	>2/38
K. pneumoniae	352	<=8	>8	>8	>2	>4	>64	4	>2/38
K. pneumoniae	372	<=8	>8	>8	>2	>4	>64	>8	>2/38
K. pneumoniae	385	<=8	<=2	<=2	>2	>4	>64	4	>2/38
K. pneumoniae	391	<=8	<=2	<=2	>2	>4	>64	>8	>2/38
K. pneumoniae	411	<=8	8	8	>2	>4	32	>8	>2/38
K. pneumoniae	418	<=8	>8	8	>2	>4	>64	4	>2/38
K. pneumoniae	423	<=8	<=2	<=2	>2	4	>64	8	>2/38
K. pneumoniae	445	32	>8	>8	>2	>4	>64	8	>2/38
K. pneumoniae	446	<=8	<=2	<=2	>2	>4	>64	>8	>2/38
K. pneumoniae	449	16	>8	>8	>2	2	32	8	>2/38
K. pneumoniae	452	<=8	<=2	<=2	>2	>4	>64	4	>2/38
K. pneumoniae	466	<=8	<=2	<=2	>2	>4	<=16	>8	>2/38
K. pneumoniae	482	<=8	<=2	<=2	>2	>4	>64	8	>2/38
K. pneumoniae	492	<=8	>8	8	>2	>4	>64	>8	2/38
K. pneumoniae	536	<=8	>8	8	>2	>4	>64	8	>2/38
K. pneumoniae	558	<=8	<=2	<=2	<=0.5	<=1	<=16	8	<=0.5/9.5
K. pneumoniae	605	<=8	8	>8	2	2		4	<=0.5/9.5
K. pneumoniae	610	<=8	>8	>8	<=0.5	<=1		<=2	<=0.5/9.5
K. pneumoniae	616	<=8	<=2	8	<=0.5	<=1		<=2	<=0.5/9.5
P. rettgeri	244	<=8	>8	8	>2	>4	>64	>8	>2/38

Abbreviations: AMK - Amikacin; GEN - Gentamicin; TOB - Tobramycin; CIP - Ciprofloxacin; LEV - Levofloxacin; NIT - Nitrofurantoin; TET - Tetracycline; SXT - Sulfamethoxazole/Trimethoprim

Table A.2: Minimum Inhibitory Concentration of Non-β-lactam Antimicrobials for University of Kentucky Clinical Isolates by BD Phoenix™ (n=164)

Organism	Isolate	AMK	GEN	TOB	CIP	LEV	NIT	TET	SXT
S. marcescens	514	<=8	<=2	<=2	>2	>4	>64	>8	
S. marcescens	560	<=8	<=2	<=2	>2	>4	>64	>8	

MIC values expressed in the table are in units of mg/L
Blank entries were not tested by University of Kentucky Microbiology Laboratory
Some organism and antimicrobial combinations were only tested by Etest® and reported as S, I, or R

Abbreviations: AMK - Amikacin; GEN - Gentamicin; TOB - Tobramycin; CIP - Ciprofloxacin; LEV - Levofloxacin; NIT - Nitrofurantoin; TET - Tetracycline; SXT - Sulfamethoxazole/Trimethoprim

Appendix B:

Cumulative Antimicrobial Susceptibility Summary Report from BD Phoenix™

Tables B.1-B.3 provide the cumulative summary antimicrobial susceptibility results from the *in vitro* susceptibility testing performed by the University of Kentucky Clinical Microbiology Laboratory using BD Phoenix™.

Reports were only generated for species with testing data for ≥ 30 isolates.[292] Percentage intermediate or resistant were not included in the report.

The following abbreviations are used in Tables B.1-B.3: %S - percentage susceptible, MIC_{50} - Minimum Inhibitory Concentration (MIC) for 50% of isolates, MIC_{90} - Minimum Inhibitory Concentration (MIC) for 90% of isolates.

Table B.1: Cumulative Antimicrobial Susceptibility Summary Report for Carbapenem-resistant *Enterobacteriaceae* Clinical Isolates by the BD Phoenix™ System (n=164)

Antimicrobial	%S	MIC_{50}	MIC_{90}	MIC Range
Ampicillin	0%	>16	>16	>16 - >16
Ampicillin/Sulbactam	1%	>16/8	>16/8	8/4 - >16/8
Aztreonam	11%	>16	>16	<=2 - >16
Cefazolin	0%	>16	>16	>16 - >16
Cefepime	21%	>16	>16	<=1 - >16
Cefoxitin	8%	>16	>16	<=4 - >16
Ceftazidime	7%	>16	>16	2 - >16
Ceftriaxone	1%	>32	>32	2 - >32
Cefuroxime	0%	>16	>16	>16 - >16
Ertapenem	1%	>1	>4	<=0.5 - >4
Meropenem	34%	4	>8	<=0.5 - >8
Piperacillin/Tazobactam	2%	>64/4	>64/4	<=2/4 - >64/4
Amikacin	94%	<=8	16	<=8 - >32
Gentamicin	38%	>8	>8	<=2 - >8
Tobramycin	27%	>8	>8	<=2 - >8
Ciprofloxacin	32%	>2	>2	<0.5 - >4
Levofloxacin	36%	>4	>4	<=1 - >4
Nitrofurantoin	36%	64	>64	<=16 - >64
Tetracycline	52%	4	>8	<=2 - >8
Sulfamethoxazole/Trimethoprim	39%	>2/38	>2/38	<=0.5/9.5 - >2/38

MIC values expressed in the table are in units of mg/L

Table B.2: Cumulative Antimicrobial Susceptibility Summary Report for *Klebsiella pneumoniae* Clinical Isolates by the BD Phoenix™ System (n=68)

Antimicrobial	%S	MIC_{50}	MIC_{90}	MIC Range
Ampicillin	0%	>16	>16	>16 - >16
Ampicillin/Sulbactam	0%	>16/8	>16/8	8/4 - >16/8
Aztreonam	13%	>16	>16	<=2 - >16
Cefazolin	0%	>16	>16	>16 - >16
Cefepime	31%	16	>16	<=1 - >16
Cefoxitin	12%	>16	>16	<=4 - >16
Ceftazidime	7%	>16	>16	2 - >16
Ceftriaxone	1%	>32	>32	2 - >32
Cefuroxime	0%	>16	>16	>16 - >16
Ertapenem	0%	>1	>4	2 - >4
Meropenem	28%	<=1	>8	<=0.5 - >8
Piperacillin/Tazobactam	1%	>64/4	>64/4	8/4 - >64/4
Amikacin	87%	<=8	32	<=8 - >32
Gentamicin	46%	8	>8	<=2 - >8
Tobramycin	32%	>8	>8	<=2 - >8
Ciprofloxacin	26%	>2	>2	<0.5 - >4
Levofloxacin	31%	>4	>4	<=1 - >4
Nitrofurantoin	20%	>64	>64	<=16 - >64
Tetracycline	50%	4	>8	<=2 - >8
Sulfamethoxazole/Trimethoprim	41%	>2/38	>2/38	<=0.5/9.5 - >2/38

MIC values expressed in the table are in units of mg/L

Table B.3: Cumulative Antimicrobial Susceptibility Summary Report for *Enterobacter cloacae* Clinical Isolates by the BD Phoenix™ System (n=50)

Antimicrobial	%S	MIC_{50}	MIC_{90}	MIC Range
Ampicillin	0%	>16	>16	>16 - >16
Ampicillin/Sulbactam	0%	>16/8	>16/8	8/4 - >16/8
Aztreonam	6%	>16	>16	<=2 - >16
Cefazolin	0%	>16	>16	>16 - >16
Cefepime	8%	>16	>16	<=1 - >16
Cefoxitin	0%	>16	>16	<=4 - >16
Ceftazidime	2%	>16	>16	4 - >16
Ceftriaxone	0%	>32	>32	2 - >32
Cefuroxime	0%	>16	>16	>16 - >16
Ertapenem	0%	>1	>4	1 - >4
Meropenem	26%	8	>8	<=0.5 - >8
Piperacillin/Tazobactam	2%	>64/4	>64/4	8/4 - 64/4
Amikacin	100%	<=8	<=8	<=8 - 16
Gentamicin	18%	>8	>8	<=2 - >8
Tobramycin	8%	>8	>8	<=2 - >8
Ciprofloxacin	42%	>2	>2	<0.5 - >4
Levofloxacin	44%	4	>4	<=1 - >4
Nitrofurantoin	22%	64	>64	<=16 - >64
Tetracycline	52%	4	>8	<=2 - >8
Sulfamethoxazole/Trimethoprim	34%	>2/38	>2/38	<=0.5/9.5 - >2/38

MIC values expressed in the table are in units of mg/L

Appendix C:

Minimum Inhibitory Concentrations Determined by Broth Microdilution Susceptibility Testing, Disk Diffusion, and Etest®

Table C.1 provide the results of *in vitro* susceptibility testing performed by broth microdilution for all isolate and antimicrobial combinations except ceftazidime/avibactam for which Etest® or disk diffusion was utilized.

The following abbreviations are used in Table C.1: AZT – Aztreonam; CPM – Cefepime; CAZ – Ceftazidime; CZA – Ceftazidime/avibactam; PTZ – Piperacillin/tazobactam; ERT – Ertapenem; MEM – Meropenem; IMI – Imipenem; IMR – Imipenem/relebactam.

The following abbreviations are used in Table C.2: AMK - Amikacin; GEN - Gentamicin; TOB - Tobramycin; CST – Colistin; PMB – Polymyxin B; MIN – Minocycline; TIG – Tigecycline; LEV - Levofloxacin; NIT – Nitrofurantoin.

Table C.1: Minimum Inhibitory Concentration (MIC) Values for β-lactam Antimicrobials against Carbapenem-resistant *Enterobacteriaceae* Clinical Isolates (n=122)

Organism	Isolate	AZT	CPM	CAZ	CZA	PTZ	ERT	MEM	IMI	IMR
C. amalonaticus	36	512	32	>512	S	>512	64	16	4	1
C. amalonaticus	91	512	>256	512	S	>512	32	64	4	1
C. freundii	27	64	4	8	S	256	4	2	2	0.5
C. freundii	50	256	32	>512	S	>512	128	64	16	0.25
C. freundii	54	256	32	256	S	512	8	2	2	0.5
C. freundii	101	>512	16	>512	S	>512	8	2	4	1
C. freundii	127	256	16	256	S	512	64	4	32	1
C. freundii	135	256	128	>512	>256	>512	8	8	8	16
C. freundii	145	128	4	256	S	512	16	8	16	8
C. freundii	324	256	16	64	0.125	512	8	4	16	0.5
C. youngae	136	>512	128	512	S	>512	128	32	128	0.25
C. youngae	435	512	>256	512	0.38	>512	32	16	32	32
E. aerogenes	97	128	64	128	S	512	4	0.25	1	0.5
E. aerogenes	179	32	32	512	>256	512	2	4	16	16
E. aerogenes	187	512	64	>512	1.5	>512	64	32	16	0.5
E. aerogenes	438	512	128	512	0.25	>512	16	8	4	0.25
E. cloacae	10	512	256	128	S	>512	128	64	64	0.5
E. cloacae	17	>512	256	512	S	>512	64	4	8	0.25
E. cloacae	30	512	128	>512	S	>512	128	64	8	0.5
E. cloacae	39	512	64	>512	S	>512	64	32	4	1
E. cloacae	40	256	128	512	>256	>512	128	64	64	32
E. cloacae	41	128	32	512	192	512	4	8	16	16
E. cloacae	52	512	128	512	S	>512	128	64	4	0.25
E. cloacae	53	256	128	>512	>256	512	16	16	16	32
E. cloacae	70	256	128	512	S	256	2	0.25	0.25	0.125

Abbreviations: AZT – Aztreonam; CPM – Cefepime; CAZ – Ceftazidime; CZA – Ceftazidime/avibactam; PTZ – Piperacillin/tazobactam; ERT – Ertapenem; MEM – Meropenem; IMI – Imipenem; IMR – Imipenem/relebactam

Table C.1: Minimum Inhibitory Concentration (MIC) Values for β-lactam Antimicrobials against Carbapenem-resistant *Enterobacteriaceae* Clinical Isolates (n=122)

Organism	Isolate	AZT	CPM	CAZ	CZA	PTZ	ERT	MEM	IMI	IMR
E. cloacae	96	256	>256	512	S	512	8	2	4	0.5
E. cloacae	107	512	32	256	0.125	512	64	32	8	1
E. cloacae	121	>512	64	>512	S	>512	128	32	64	1
E. cloacae	126	512	64	512	0.5	512	128	64	128	0.5
E. cloacae	134	256	128	>512	>256	512	64	32	64	32
E. cloacae	144	512	256	512	S	>512	64	64	16	1
E. cloacae	167	128	64	>512	>256	512	8	16	64	>32
E. cloacae	168	256	64	512	>256	>512	32	16	32	1
E. cloacae	169	256	128	>512	>256	512	8	16	64	>32
E. cloacae	171	512	128	512	>256	>512	32	32	8	0.25
E. cloacae	175	32	32	512	>256	512	4	8	32	>32
E. cloacae	189	128	256	>512	>256	>512	8	16	32	>32
E. cloacae	200	>512	>256	>512	4	>512	8	1	8	8
E. cloacae	203	256	128	>512	>256	>512	4	4	32	32
E. cloacae	209	512	64	512	0.75	>512	64	32	8	0.125
E. cloacae	266	128	64	>512	>256	512	8	8	32	32
E. cloacae	335	128	128	512	1	256	4	0.25	1	0.5
E. cloacae	339	512	128	>512	1.5	>512	32	8	16	1
E. cloacae	369	256	32	>512	1	>512	8	0.5	1	1
E. cloacae	416	16	64	>512	>256	>512	128	32	64	>32
E. cloacae	476	512	>256	512		>512	128	32	16	32
E. cloacae	515	>512	32	>512		>512	16	4	16	0.5
E. coli	33	512	64	>512	S	>512	128	64	16	0.125
E. coli	103	512	>256	>512	S	>512	8	0.5	1	1
E. coli	172	64	32	256	96	>512	8	4	16	16

Abbreviations: AZT – Aztreonam; CPM – Cefepime; CAZ – Ceftazidime; CZA – Ceftazidime/avibactam; PTZ – Piperacillin/tazobactam; ERT – Ertapenem; MEM – Meropenem; IMI – Imipenem; IMR – Imipenem/relebactam

Table C.1: Minimum Inhibitory Concentration (MIC) Values for β-lactam Antimicrobials against Carbapenem-resistant *Enterobacteriaceae* Clinical Isolates (n=122)

Organism	Isolate	AZT	CPM	CAZ	CZA	PTZ	ERT	MEM	IMI	IMR
E. coli	176	512	32	512	0.5	>512	32	8	2	0.25
E. coli	309	128	>256	256	0.125	32	32	2	1	0.5
E. coli	390	256	>256	>512	0.19	512	4	0.5	1	0.25
E. gergoviae	95	128	>256	512	S	512	64	4	2	0.25
E. hormaechei	186	512	128	>512	>256	>512	16	8	32	32
E. hormaechei	398	256	256	>512	>256	>512	32	16	16	16
Enterobacter sp.	146	512	64	512	S	>512	64	32	64	1
Enterobacter sp.	210	512	>256	128	0.5	>512	128	32	128	1
K. oxytoca	166	64	4	32	0.094	256	16	4	64	0.5
K. oxytoca	177	32	64	512	0.5	256	8	4	32	4
K. oxytoca	330	256	128	128	0.75	>512	64	32	64	1
K. ozaenae	128	256	256	256	S	512	128	128	>128	0.25
K. ozaenae	407	128	16	512	0.5	512	32	16	16	0.5
K. pneumoniae	21	64	2	128	0.75	256	32	2	2	0.5
K. pneumoniae	22	512	>256	512	0.25	>512	64	16	4	0.25
K. pneumoniae	24	512	64	>512	1.5	>512	128	64	16	1
K. pneumoniae	28	>512	>256	>512	0.75	>512	>128	>128	>128	1
K. pneumoniae	29	512	>256	>512	1.5	>512	128	32	32	1
K. pneumoniae	31	32	1	256	S	>512	1	0.25	0.25	0.5
K. pneumoniae	32	512	128	512	S	>512	64	16	8	0.25
K. pneumoniae	34	256	128	16	S	>512	16	16	8	0.25
K. pneumoniae	35	>512	>256	>512	S	>512	128	128	64	0.5
K. pneumoniae	37	512	>256	512	S	>512	128	32	16	0.125
K. pneumoniae	42	64	64	>512	>256	>512	64	16	32	32
K. pneumoniae	43	>512	>256	>512	S	>512	128	64	8	0.5

Abbreviations: AZT – Aztreonam; CPM – Cefepime; CAZ – Ceftazidime; CZA – Ceftazidime/avibactam; PTZ – Piperacillin/tazobactam; ERT – Ertapenem; MEM – Meropenem; IMI – Imipenem; IMR – Imipenem/relebactam

Table C.1: Minimum Inhibitory Concentration (MIC) Values for β-lactam Antimicrobials against Carbapenem-resistant *Enterobacteriaceae* Clinical Isolates (n=122)

Organism	Isolate	AZT	CPM	CAZ	CZA	PTZ	ERT	MEM	IMI	IMR
K. pneumoniae	44	512	>256	512	S	>512	>128	128	64	0.5
K. pneumoniae	45	>512	>256	>512	1	>512	>128	128	64	0.25
K. pneumoniae	46	>512	>256	512	S	>512	128	64	16	1
K. pneumoniae	47	>512	>256	512	0.5	>512	128	64	128	1
K. pneumoniae	48	512	32	512	S	>512	16	8	2	0.0625
K. pneumoniae	49	512	>256	512	S	>512	64	64	32	0.25
K. pneumoniae	51	>512	>256	>512	S	>512	128	128	32	0.25
K. pneumoniae	55	256	256	512	S	512	64	64	4	0.125
K. pneumoniae	69	256	>256	256	S	256	4	0.25	1	0.5
K. pneumoniae	77	128	128	128	S	>512	16	0.25	0.25	0.25
K. pneumoniae	93	>512	>256	256	S	>512	64	128	16	1
K. pneumoniae	98	>512	32	>512	S	>512	8	1	0.25	0.25
K. pneumoniae	99	>512	>256	>512	S	>512	64	32	8	1
K. pneumoniae	105	>512	>256	>512	S	>512	128	32	32	0.25
K. pneumoniae	116	256	>256	256	S	512	8	4	1	0.5
K. pneumoniae	119	>512	>256	>512	0.25	>512	>128	128	>128	1
K. pneumoniae	123	>512	>256	>512	0.75	>512	>128	128	128	1
K. pneumoniae	129	256	256	512	S	>512	32	32	64	1
K. pneumoniae	130	256	256	256	S	>512	32	16	4	0.25
K. pneumoniae	142	>512	>256	256	S	>512	64	32	64	1
K. pneumoniae	143	256	>256	256	S	>512	8	1	1	0.5
K. pneumoniae	152	4	16	128	S	>512	2	<=0.125	0.5	0.25
K. pneumoniae	165	512	64	256	0.5	>512	64	16	32	1
K. pneumoniae	170	64	32	>512	>256	>512	8	8	32	32
K. pneumoniae	173	16	32	512	>256	>512	8	16	16	>32

Abbreviations: AZT – Aztreonam; CPM – Cefepime; CAZ – Ceftazidime; CZA – Ceftazidime/avibactam; PTZ – Piperacillin/tazobactam; ERT – Ertapenem; MEM – Meropenem; IMI – Imipenem; IMR – Imipenem/relebactam

Table C.1: Minimum Inhibitory Concentration (MIC) Values for β-lactam Antimicrobials against Carbapenem-resistant *Enterobacteriaceae* Clinical Isolates (n=122)

Organism	Isolate	AZT	CPM	CAZ	CZA	PTZ	ERT	MEM	IMI	IMR
K. pneumoniae	174	64	16	512	>256	512	2	8	32	32
K. pneumoniae	230	256	>256	512	0.75	>512	128	64	32	0.25
K. pneumoniae	243	256	>256	512	0.38	>512	4	0.5	0.5	0.5
K. pneumoniae	256	256	32	256	0.75	512	32	16	8	0.25
K. pneumoniae	269	512	>256	512	0.75	>512	8	1	0.5	1
K. pneumoniae	284	512	>256	512	2	>512	32	4	1	0.125
K. pneumoniae	349	256	>256	>512	0.5	>512	8	1	0.5	0.5
K. pneumoniae	352	128	>256	256	0.125	64	4	0.25	2	0.25
K. pneumoniae	372	256	>256	512	0.38	>512	8	1	1	0.5
K. pneumoniae	385	512	>256	>512	0.5	>512	4	0.5	0.5	0.25
K. pneumoniae	391	256	>256	512	0.75	>512	16	8	16	0.5
K. pneumoniae	411	32	256	>512	>256	>512	16	32	32	32
K. pneumoniae	418	256	>256	>512	1	>512	4	0.5	1	0.5
K. pneumoniae	423	256	>256	512	0.75	512	32	16	16	1
K. pneumoniae	445	>512	>256	>512	2	>512	128	128	32	0.5
K. pneumoniae	446	512	>256	512		>512	>128	>128	64	0.25
K. pneumoniae	449	512	128	>512		>512	>128	64	8	0.0625
K. pneumoniae	452	512	>256	>512		>512	>128	128	128	0.5
K. pneumoniae	466	512	>256	512		>512	128	32	4	0.25
K. pneumoniae	482	512	>256	>512		>512	128	64	4	0.125
K. pneumoniae	492	512	>256	512		>512	8	1	0.5	0.5
S. marcescens	514	512	128	512		512	>128	64	>128	1

MIC values expressed in the table are in units of mg/L. Blank entries were not tested by our laboratory
Same color highlighted isolates were identified from the same patient
Some organism and antimicrobial combinations were only tested by disk diffusion and reported as S, I, or R

Abbreviations: AZT – Aztreonam; CPM – Cefepime; CAZ – Ceftazidime; CZA – Ceftazidime/avibactam; PTZ – Piperacillin/tazobactam; ERT – Ertapenem; MEM – Meropenem; IMI – Imipenem; IMR – Imipenem/relebactam

Table C.2: Minimum Inhibitory Concentration (MIC) Values for Non-β-lactam Antimicrobials against 122 Carbapenem-resistant *Enterobacteriaceae* Clinical Isolates (n=122)

Organism	Isolate	AMK	GEN	TOB	CST	PMB	MIN	TIG	LEV	NIT
C. amalonaticus	36	16	32	128	2	2	16	2	32	256
C. amalonaticus	91	8	128	>128	0.25	0.125	>64	2	>32	256
C. freundii	27	2	0.25	0.5	<=0.06	<=0.06	8	1	32	64
C. freundii	50	2	4	32	32	16	8	1	32	256
C. freundii	54	2	32	16	<=0.06	0.125	64	2	16	8
C. freundii	101	1	8	8	0.125	<=0.06	64	2	32	128
C. freundii	127	16	32	32	0.125	<=0.06	16	1	32	128
C. freundii	135	8	64	8	0.125	<=0.06	64	1	0.5	256
C. freundii	145	1	4	8	<=0.06	0.125	32	1	16	256
C. freundii	324	4	64	16	<=0.06	<=0.06	0.25	0.06	>32	2
C. youngae	136	4	64	16	<=0.06	<=0.06	64	1	>32	64
C. youngae	435	4	16	8	<=0.06	<=0.06	64	1	32	256
E. aerogenes	97	4	32	16	<=0.06	<=0.06	4	1	16	64
E. aerogenes	179	8	8	8	<=0.06	<=0.06	16	0.5	32	64
E. aerogenes	187	4	16	64	<=0.06	<=0.06	8	1	32	64
E. aerogenes	438	1	<=0.125	0.25	0.25	0.125	16	1	0.06	32
E. cloacae	10	2	32	32	<=0.06	<=0.06	8	1	4	16
E. cloacae	17	4	64	32	0.125	0.125	16	4	16	64
E. cloacae	30	16	128	64	8	4	16	2	>32	256
E. cloacae	39	1	8	16	>64	>64	4	2	1	32
E. cloacae	40	32	128	>128	0.125	0.25	8	2	8	256
E. cloacae	41	32	128	>128	0.125	0.125	32	2	>32	128
E. cloacae	52	4	64	32	>64	>64	8	2	16	256
E. cloacae	53	16	128	64	<=0.06	0.125	>64	8	8	256
E. cloacae	70	16	64	32	0.125	<=0.06	64	2	16	256

Abbreviations: AMK - Amikacin; GEN - Gentamicin; TOB - Tobramycin; CST – Colistin; PMB – Polymyxin B; MIN – Minocycline; TIG – Tigecycline; LEV - Levofloxacin; NIT – Nitrofurantoin

Table C.2: Minimum Inhibitory Concentration (MIC) Values for Non-β-lactam Antimicrobials against 122 Carbapenem-resistant *Enterobacteriaceae* Clinical Isolates (n=122)

Organism	Isolate	AMK	GEN	TOB	CST	PMB	MIN	TIG	LEV	NIT
E. cloacae	96	4	64	32	32	32	64	2	32	256
E. cloacae	107	4	4	16	>64	64	64	2	16	16
E. cloacae	121	16	64	64	<=0.06	<=0.06	64	2	>32	256
E. cloacae	126	16	32	128	>64	>64	16	2	>32	256
E. cloacae	134	8	128	16	<=0.06	<=0.06	32	0.5	2	256
E. cloacae	144	4	32	32	0.125	0.125	64	0.5	>32	256
E. cloacae	167	8	0.5	8	0.125	<=0.06	4	0.5	0.5	64
E. cloacae	168	4	4	16	>64	64	32	2	16	64
E. cloacae	169	8	0.5	8	<=0.06	0.125	4	0.5	1	64
E. cloacae	171	2	32	64	0.125	0.125	32	4	8	64
E. cloacae	175	16	8	32	<=0.06	<=0.06	32	1	1	64
E. cloacae	189	8	8	8	<=0.06	0.125	32	1	1	128
E. cloacae	200	2	128	16	<=0.06	<=0.06	16	2	32	64
E. cloacae	203	16	16	32	<=0.06	<=0.06	4	1	2	64
E. cloacae	209	16	32	64	0.25	0.125	16	4	8	32
E. cloacae	266	8	128	16	0.125	0.125	2	2	2	64
E. cloacae	335	2	16	8	0.125	0.25	64	16	>32	256
E. cloacae	339	16	64	32	<=0.06	<=0.06	32	4	>32	64
E. cloacae	369	2	0.25	0.5	8	4	>64	16	16	128
E. cloacae	416	16	16	32	0.125	0.25	16	1	0.5	64
E. cloacae	476	8	1	32	>64	>64	16	2	0.5	128
E. cloacae	515	1	2	4	>64	>64	16	2	32	64
E. coli	33	16	64	16	8	4	8	2	>32	256
E. coli	103	16	2	32	<=0.06	<=0.06	>64	2	>32	256
E. coli	172	16	8	32	<=0.06	<=0.06	4	1	4	32

Abbreviations: AMK - Amikacin; GEN - Gentamicin; TOB - Tobramycin; CST – Colistin; PMB – Polymyxin B; MIN – Minocycline; TIG – Tigecycline; LEV - Levofloxacin; NIT – Nitrofurantoin

Table C.2: Minimum Inhibitory Concentration (MIC) Values for Non-β-lactam Antimicrobials against 122 Carbapenem-resistant *Enterobacteriaceae* Clinical Isolates (n=122)

Organism	Isolate	AMK	GEN	TOB	CST	PMB	MIN	TIG	LEV	NIT
E. coli	176	2	4	4	<=0.06	<=0.06	8	2	32	32
E. coli	309	4	0.5	1	<=0.06	<=0.06	4	0.5	32	32
E. coli	390	4	128	8	<=0.06	<=0.06	16	0.25	16	16
E. gergoviae	95	1	64	32	0.125	<=0.06	64	1	8	256
E. hormaechei	186	8	32	32	<=0.06	<=0.06	8	2	8	128
E. hormaechei	398	16	16	16	<=0.06	<=0.06	16	2	1	128
Enterobacter sp.	146	2	32	64	<=0.06	<=0.06	32	2	8	128
Enterobacter sp.	210	<=0.5	<=0.125	0.5	>64	64	8	2	4	32
K. oxytoca	166	2	16	32	0.125	0.25	8	1	4	32
K. oxytoca	177	4	8	8	<=0.06	<=0.06	8	2	32	32
K. oxytoca	330	2	4	2	<=0.06	<=0.06	4	1	16	32
K. ozaenae	128	8	32	64	>64	>64	8	1	>32	256
K. ozaenae	407	4	8	16	0.25	0.5	4	1	8	16
K. pneumoniae	21	16	128	>128	1	0.5	8	2	>32	16
K. pneumoniae	22	32	>128	>128	<=0.06	<=0.06	8	4	>32	256
K. pneumoniae	24	2	4	8	0.125	0.125	4	2	>32	256
K. pneumoniae	28	32	128	32	16	8	16	2	>32	512
K. pneumoniae	29	32	128	64	64	64	8	2	>32	256
K. pneumoniae	31	16	4	16	0.25	0.25	4	0.5	32	256
K. pneumoniae	32	16	128	32	0.5	0.125	8	0.5	>32	256
K. pneumoniae	34	1	>128	0.25	0.25	0.125	2	0.5	0.06	128
K. pneumoniae	35	32	128	32	4	4	16	2	>32	256
K. pneumoniae	37	64	64	>128	0.25	0.125	4	0.5	8	128
K. pneumoniae	42	512	>128	128	8	8	32	4	>32	128
K. pneumoniae	43	32	64	>128	0.125	<=0.06	16	2	16	128

Abbreviations: AMK - Amikacin; GEN - Gentamicin; TOB - Tobramycin; CST – Colistin; PMB – Polymyxin B; MIN – Minocycline; TIG – Tigecycline; LEV - Levofloxacin; NIT – Nitrofurantoin

Table C.2: Minimum Inhibitory Concentration (MIC) Values for Non-β-lactam Antimicrobials against 122 Carbapenem-resistant *Enterobacteriaceae* Clinical Isolates (n=122)

Organism	Isolate	AMK	GEN	TOB	CST	PMB	MIN	TIG	LEV	NIT
K. pneumoniae	44	8	32	32	0.125	<=0.06	4	2	>32	512
K. pneumoniae	45	4	32	64	>64	>64	8	4	>32	512
K. pneumoniae	46	64	128	>128	16	16	8	1	>32	256
K. pneumoniae	47	64	64	>128	64	64	8	1	>32	256
K. pneumoniae	48	32	4	32	0.125	0.125	8	1	>32	512
K. pneumoniae	49	32	32	128	<=0.06	0.125	8	1	>32	128
K. pneumoniae	51	1	8	16	64	32	16	2	8	512
K. pneumoniae	55	32	0.5	16	<=0.06	0.125	4	0.5	16	64
K. pneumoniae	69	1	0.25	0.5	0.25	0.125	64	2	32	256
K. pneumoniae	77	2	128	128	0.125	0.125	16	0.5	8	256
K. pneumoniae	93	8	32	64	32	8	32	2	16	256
K. pneumoniae	98	<=0.5	2	16	0.125	<=0.06	8	1	0.25	32
K. pneumoniae	99	2	2	8	0.125	<=0.06	32	2	8	128
K. pneumoniae	105	4	2	16	0.125	0.125	64	1	32	64
K. pneumoniae	116	2	0.5	0.5	0.125	<=0.06	>64	4	>32	256
K. pneumoniae	119	32	64	>128	64	32	4	1	>32	512
K. pneumoniae	123	2	32	128	>64	>64	8	4	>32	256
K. pneumoniae	129	32	2	32	<=0.06	<=0.06	4	1	>32	64
K. pneumoniae	130	4	32	16	0.125	0.125	8	2	32	128
K. pneumoniae	142	16	4	16	0.125	0.125	>64	1	>32	64
K. pneumoniae	143	2	64	8	<=0.06	0.125	64	1	32	256
K. pneumoniae	152	2	4	4	0.25	0.125	>64	2	16	256
K. pneumoniae	165	1	32	64	>64	>64	16	4	8	32
K. pneumoniae	170	4	16	16	<=0.06	<=0.06	16	2	4	64
K. pneumoniae	173	16	4	16	<=0.06	0.125	8	0.5	2	128

Abbreviations: AMK - Amikacin; GEN - Gentamicin; TOB - Tobramycin; CST – Colistin; PMB – Polymyxin B; MIN – Minocycline; TIG – Tigecycline; LEV - Levofloxacin; NIT – Nitrofurantoin

Table C.2: Minimum Inhibitory Concentration (MIC) Values for Non-β-lactam Antimicrobials against 122 Carbapenem-resistant *Enterobacteriaceae* Clinical Isolates (n=122)

Organism	Isolate	AMK	GEN	TOB	CST	PMB	MIN	TIG	LEV	NIT
K. pneumoniae	174	16	8	16	0.125	0.125	32	1	2	64
K. pneumoniae	230	4	16	32	>64	64	64	16	>32	256
K. pneumoniae	243	2	<=0.125	0.25	<=0.06	0.125	16	1	32	256
K. pneumoniae	256	2	16	8	>64	>64	32	2	>32	64
K. pneumoniae	269	1	8	16	8	8	8	1	>32	256
K. pneumoniae	284	1	0.25	0.25	32	16	>64	1	32	256
K. pneumoniae	349	1	64	4	16	16	>64	8	>32	256
K. pneumoniae	352	16	64	16	<=0.06	<=0.06	8	2	>32	256
K. pneumoniae	372	1	64	8	32	32	>64	4	>32	256
K. pneumoniae	385	1	0.25	0.25	2	2	8	2	>32	256
K. pneumoniae	391	1	<=0.125	<=0.125	32	16	16	2	8	128
K. pneumoniae	411	8	8	8	<=0.06	0.125	8	2	8	32
K. pneumoniae	418	1	64	8	2	0.25	4	2	>32	256
K. pneumoniae	423	2	0.25	0.25	0.25	0.125	8	1	4	128
K. pneumoniae	445	32	>128	128	0.25	0.125	32	1	>32	256
K. pneumoniae	446	<=0.5	<=0.125	<=0.125	0.125	0.125	32	8	>32	256
K. pneumoniae	449	16	8	32	0.125	<=0.06	16	2	2	128
K. pneumoniae	452	1	0.25	0.25	0.125	0.125	32	8	>32	256
K. pneumoniae	466	1	<=0.125	<=0.125	0.125	0.25	64	2	32	128
K. pneumoniae	482	4	16	16	64	>64	8	2	>32	256
K. pneumoniae	492	1	32	8	32	64	>64	2	>32	256
S. marcescens	514	2	0.25	1	>64	>64	16	8	16	128

MIC values expressed in the table are in units of mg/L. Blank entries were not tested by our laboratory. Inhibitors fixed at 4 mg/L
Same color highlighted isolates were identified from the same patient
Some organism and antimicrobial combinations were only tested by disk diffusion and reported as S, I, or R

Abbreviations: AMK - Amikacin; GEN - Gentamicin; TOB - Tobramycin; CST – Colistin; PMB – Polymyxin B; MIN – Minocycline; TIG – Tigecycline; LEV - Levofloxacin; NIT – Nitrofurantoin

Appendix D:

Cumulative Antimicrobial Susceptibility Summary Report from Broth Microdilution

Tables D.1-D.3 provide the cumulative summary antimicrobial susceptibility results from the *in vitro* susceptibility testing performed using broth microdilution. Etest® and disk diffusion were utilized for ceftazidime/avibactam

Reports were only generated for species with testing data for ≥ 30 isolates.[292] Percentage intermediate or resistant were not included in the report.

The following abbreviations are used in Tables D.1-D.3: %S - percentage susceptible, MIC_{50} - Minimum Inhibitory Concentration (MIC) for 50% of isolates, MIC_{90} - Minimum Inhibitory Concentration (MIC) for 90% of isolates.

Table D.1: Cumulative Antimicrobial Susceptibility Summary Report for Carbapenem-resistant *Enterobacteriaceae* Clinical Isolates (n=122)

Antimicrobial	%S	MIC_{50}	MIC_{90}	MIC Range
Aztreonam	1%	512	>512	4 - >512
Cefepime	2%	128	>256	1 - >256
Ceftazidime	0%	512	>512	8 - >512
Ceftazidime/avibactam	80%	<=8	>256	0.094 - >256
Piperacillin/tazobactam	0%	>512	>512	32 - >512
Ertapenem	0%	32	128	1 - >128
Meropenem	17%	16	64	<=0.125 - >128
Imipenem	18%	16	64	0.25 - >128
Imipenem/relebactam	79%	0.5	32	0.06 - >32
Amikacin	86%	4	32	<=0.5 - 512
Gentamicin	31%	16	128	<=0.125 - >128
Tobramycin	18%	16	128	<=0.125 - >128
Colistin	70%	0.125	>64	<=0.06 - >64
Polymyxin B	70%	0.125	64	<=0.06 - >64
Minocycline	16%	16	64	0.25 - >64
Tigecycline	84%	2	4	0.06 - 16
Levofloxacin	15%	32	>32	0.06 - >32
Nitrofurantoin	16%	128	256	2 – 512

MIC values expressed in the table are in units of mg/L
Inhibitors fixed at 4 mg/L
Unable to evaluate ceftazidime/avibactam MIC_{50} due to use of disk diffusion

Table D.2: Cumulative Antimicrobial Susceptibility Summary Report for *Klebsiella pneumoniae* Clinical Isolates (n=58)

Antimicrobial	%S	MIC_{50}	MIC_{90}	MIC Range
Aztreonam	2%	512	>512	4 - >512
Cefepime	2%	>256	>256	1 - >256
Ceftazidime	0%	512	>512	16 - >512
Ceftazidime/avibactam	90%	<=8	<=8	0.125 - >256
Piperacillin/tazobactam	0%	>512	>512	64 - >512
Ertapenem	0%	64	>128	1 - >128
Meropenem	24%	16	128	<=0.125 - >128
Imipenem	26%	8	64	0.25 - >128
Imipenem/relebactam	91%	0.5	1	0.06 - >32
Amikacin	74%	4	32	<=0.5 - 512
Gentamicin	36%	16	128	<=0.125 - >128
Tobramycin	22%	16	>128	<=0.125 - >128
Colistin	64%	0.25	64	<=0.06 - >64
Polymyxin B	64%	0.125	64	<=0.06 - >64
Minocycline	16%	16	>64	2 - >64
Tigecycline	81%	2	4	0.5 - 16
Levofloxacin	9%	>32	>32	0.06 - >32
Nitrofurantoin	7%	256	512	16 – 512

MIC values expressed in the table are in units of mg/L
Inhibitors fixed at 4 mg/L
Unable to evaluate ceftazidime/avibactam MIC_{50} and MIC_{90} due to use of disk diffusion

Table D.3: Cumulative Antimicrobial Susceptibility Summary Report for *Enterobacter cloacae* Clinical Isolates (n=31)

Antimicrobial	%S	MIC_{50}	MIC_{90}	MIC Range
Aztreonam	0%	256	>512	16 - >512
Cefepime	2%	128	256	32 - >256
Ceftazidime	0%	512	>512	128 - >512
Ceftazidime/avibactam	55%	4	>256	0.125 - >256
Piperacillin/tazobactam	0%	>512	>512	256 - >512
Ertapenem	0%	32	128	2 - 128
Meropenem	13%	16	64	0.25 - 64
Imipenem	10%	16	64	0.25 - 128
Imipenem/relebactam	58%	1	>32	0.125 - >32
Amikacin	94%	8	16	1 - 32
Gentamicin	23%	32	128	0.25 - 128
Tobramycin	6%	32	64	0.5 - >128
Colistin	68%	0.125	>64	<=0.06 - >64
Polymyxin B	68%	0.125	>64	<=0.06 - >64
Minocycline	16%	16	64	2 - >64
Tigecycline	77%	2	4	0.5 - 16
Levofloxacin	32%	16	>32	0.5 - >32
Nitrofurantoin	13%	64	256	16 – 256

MIC values expressed in the table are in units of mg/L
Inhibitors fixed at 4 mg/L
Unable to evaluate ceftazidime/avibactam MIC_{50} and MIC_{90} due to use of disk diffusion

Appendix E:

Time-kill Studies

Table E.1 contains the time-kill data for all carbapenem-resistant *Enterobacteriaceae* exposed to amikacin, meropenem, and polymyxin B alone and in combination. Isolates are listed alphabetically by flask code, which designates the isolate, concentrations of antimicrobials used, any unique container conditions, type of flask, and any antimicrobial containing agar.

Flask code is of the format:

<Isolate_Number><Antimicrobial_1><Concentration_1><Antimicrobial_2><Concentration_2><Flask_Material><Time-kill_Type> on <Agar_Composition>

Any antimicrobial administration delays are denoted with parentheses following the corresponding antimicrobial concentration and surround the time in hours from time=0h when the antimicrobial was added to the flask.

Flask material “G” denotes glass flasks, all other flasks were polypropylene A time-kill type of “F” denotes microfiltration method, all other time-kill experiments were unmodified. GC – Growth Control (no antimicrobial agents present); A – Amikacin; M – Meropenem; P or PB – Polymyxin B.

Examples: “266M4P1(2)GF on PB4” uses the entire coding scheme discussed previously and means meropenem 4 mg/L and polymyxin B 1 mg/L in combination were tested against isolate 266 in glass flasks by the microfiltration method and plated on Mueller-Hinton agar containing polymyxin B 4 mg/L. “266GC” simply means isolate 266 was grown in a polypropylene flask without antimicrobial agents by the traditional time-kill methodology discussed in Time-Kill Procedure.

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
10A16	9/23/2014	8.12E+05	7.03E+05	1.53E+03		1.00E+02		1.00E+02	1.58E+06	1.37E+09
10A16	9/29/2014	7.26E+05	5.86E+05	1.02E+03		1.23E+02		1.00E+02	6.26E+05	8.18E+06
10A8	9/23/2014	1.03E+06	5.66E+05	5.36E+03		5.93E+02		1.00E+02	1.33E+07	7.15E+08
10A8	9/29/2014	8.74E+05	5.40E+05	1.20E+04		3.47E+02		1.00E+02	6.23E+06	8.58E+06
10GC	9/17/2014	9.18E+05	1.35E+06	1.29E+06		2.25E+08		5.25E+10	3.01E+10	2.46E+10
10GC	10/21/2014	6.17E+05	7.58E+05	2.35E+06		2.89E+07		1.21E+11	1.10E+10	1.83E+10
10M(2)16P0.25	9/6/2017	1.78E+06	4.70E+02	8.18E+01	0.00E+00	0.00E+00		1.43E+02	8.71E+10	7.44E+10
10M16	9/17/2014	1.73E+06	4.89E+05	4.71E+05		1.41E+04		1.61E+04	4.55E+10	4.56E+10
10M16	10/21/2014	8.56E+05	4.37E+05	8.19E+04		3.94E+03		7.56E+02	8.83E+10	1.85E+10
10M16(2)P0.25	9/29/2017	1.08E+06	4.09E+01	2.04E+01		0.00E+00		0.00E+00	4.83E+10	9.93E+10
10M16(2)P1	9/6/2017	1.32E+06	2.45E+02	1.12E+03	4.09E+01	0.00E+00		0.00E+00	1.37E+10	1.70E+09
10M16(2)P1	9/29/2017	1.26E+06	2.04E+01	0.00E+00		0.00E+00		0.00E+00	4.43E+10	9.03E+10
10M16A8	9/29/2014	8.73E+05	5.92E+05	9.65E+03		2.25E+02		1.00E+02	1.00E+02	1.00E+02
10M16A8	12/9/2014	6.23E+05	3.19E+05	2.78E+04		8.79E+02		1.00E+02	1.00E+02	1.00E+02
10M16P0.25(2)	9/26/2017	1.82E+06	1.79E+06	1.44E+05	0.00E+00	0.00E+00		0.00E+00	0.00E+00	0.00E+00
10M16P0.25(2)	9/29/2017	9.18E+05	1.15E+06	1.36E+05		0.00E+00		0.00E+00	0.00E+00	0.00E+00
10M16P1	9/26/2017	2.25E+06	1.60E+06	0.00E+00	0.00E+00	4.09E+01		2.04E+01	0.00E+00	0.00E+00
10M16P1	2/13/2018	8.49E+05	2.04E+01	0.00E+00		0.00E+00		1.23E+02	2.51E+10	6.87E+09
10M16P1	2/26/2018	7.19E+05	2.04E+01	0.00E+00		0.00E+00		0.00E+00	0.00E+00	0.00E+00
10M16P1(2)	9/26/2017	1.99E+06	1.67E+06	6.40E+04	0.00E+00	4.09E+01		0.00E+00	0.00E+00	0.00E+00
10M16P1(2)	9/29/2017	1.06E+06	1.17E+06	1.04E+05		0.00E+00		0.00E+00	0.00E+00	0.00E+00
10M16PB0.25	9/23/2014	1.82E+06	1.35E+03	1.00E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
10M16PB0.25	10/28/2014	7.97E+05	1.00E+02	1.00E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
10M4	9/17/2014	1.06E+06	1.03E+06	4.23E+05		9.67E+04		1.55E+07	6.96E+10	3.44E+10
10M4	10/21/2014	6.48E+05	7.05E+05	1.49E+05		1.77E+04		9.67E+06	1.77E+11	2.22E+10
10M4(2)P0.25	9/6/2017	1.81E+06	1.64E+02	4.09E+01	4.09E+01	1.43E+02		2.79E+04	1.95E+11	1.60E+10

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
10M4(2)P0.25	9/29/2017	1.90E+06	2.45E+02	2.04E+01		2.04E+01		1.05E+04	4.92E+10	1.13E+11
10M4(2)P1	9/6/2017	1.25E+06	4.09E+01	2.66E+02	8.18E+01	1.23E+02		1.91E+04	6.17E+10	2.41E+09
10M4(2)P1	9/29/2017	1.33E+06	0.00E+00	0.00E+00		0.00E+00		5.70E+03	7.33E+10	6.48E+10
10M4A16	9/29/2014	4.75E+05	4.11E+05	2.53E+03		1.00E+02		1.00E+02	1.00E+02	1.00E+02
10M4A16	12/9/2014	5.53E+05	3.78E+05	2.05E+04		6.34E+02		1.00E+02	1.00E+02	1.00E+02
10M4A8	9/29/2014	6.87E+05	5.20E+05	3.50E+03		6.73E+03		1.00E+02	1.00E+02	1.00E+02
10M4A8	12/9/2014	6.04E+05	3.94E+05	2.87E+04		6.90E+03		1.00E+02	1.00E+02	1.00E+02
10M4P0.25(2)	9/26/2017	2.06E+06	1.76E+06	2.65E+05	0.00E+00	0.00E+00		0.00E+00	0.00E+00	0.00E+00
10M4P0.25(2)	9/29/2017	1.10E+06	1.40E+06	2.31E+05		0.00E+00		4.70E+02	6.36E+10	8.31E+10
10M4P0.25(2)	2/26/2018	1.13E+06	9.91E+05	1.98E+05		2.04E+01		4.50E+02	2.72E+09	3.02E+09
10M4P1(2)	9/26/2017	1.85E+06	1.81E+06	3.64E+05	0.00E+00	2.04E+01		0.00E+00	0.00E+00	0.00E+00
10M4P1(2)	9/29/2017	9.61E+05	1.20E+06	2.06E+05		0.00E+00		0.00E+00	3.78E+04	4.42E+10
10M4P1(2)	2/26/2018	7.62E+05	9.50E+05	1.95E+05		0.00E+00		2.45E+02	4.11E+09	5.62E+09
10M4PB0.25	9/23/2014	1.38E+06	5.31E+02	1.00E+02		1.00E+02		8.38E+02	2.81E+10	7.50E+10
10M4PB0.25	10/28/2014	1.10E+06	1.00E+02	1.02E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
10M4PB0.25	12/9/2014	5.94E+05	1.98E+03	1.00E+02		1.00E+02		2.17E+04	2.57E+10	3.77E+10
10M4PB1	9/23/2014	1.41E+06	1.00E+02	1.00E+02		1.00E+02		7.42E+03	3.15E+10	9.01E+10
10M4PB1	10/28/2014	1.91E+05	1.00E+02	1.00E+02		1.00E+02		1.84E+02	5.52E+10	4.93E+10
10PB0.25	9/17/2014	1.05E+06	1.43E+02	2.92E+03		8.67E+03		9.32E+05	3.88E+10	3.73E+10
10PB0.25	10/21/2014	5.60E+05	2.66E+02	1.00E+02		1.00E+02		6.47E+04	3.42E+10	6.82E+10
10PB1	9/17/2014	1.38E+06	1.00E+02	1.00E+02		2.13E+03		2.29E+05	4.45E+10	3.77E+10
10PB1	10/21/2014	6.39E+05	1.00E+02	1.00E+02		1.00E+02		4.87E+04	4.90E+10	1.67E+10
17A16	9/23/2014	2.98E+05	3.15E+05	2.68E+05		4.09E+04		1.27E+05	1.34E+11	9.83E+10
17A16	9/29/2014	3.76E+05	5.64E+04	4.29E+04		5.68E+03		1.27E+04	5.13E+10	9.35E+10
17A8	9/23/2014	6.44E+05	3.86E+05	6.90E+05		8.67E+06		8.79E+10	1.24E+11	1.07E+11
17A8	9/29/2014	3.68E+05	1.17E+05	1.14E+05		1.27E+06		3.41E+08	1.57E+11	6.01E+10

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
17GC	9/17/2014	5.38E+05	6.36E+05	1.68E+06		1.34E+08		5.50E+10	1.13E+11	6.69E+10
17GC	10/21/2014	3.33E+05	4.15E+05	1.06E+06		1.37E+07		1.35E+11	8.99E+10	4.77E+10
17M16	9/17/2014	8.08E+05	3.11E+05	7.19E+03		3.37E+03		1.51E+03	1.75E+11	3.25E+10
17M16	10/21/2014	3.50E+05	2.78E+05	1.79E+04		5.33E+03		2.29E+03	5.06E+05	9.71E+10
17M16	12/16/2014	5.10E+05	1.27E+05	5.93E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
17M16	1/6/2015	3.35E+05	8.14E+04	1.59E+03		1.02E+02		1.00E+02	1.57E+07	2.64E+10
17M16	3/13/2018	5.28E+05	3.68E+04	2.86E+02		1.43E+02		6.13E+01	0.00E+00	0.00E+00
17M16(2)P0.25	9/6/2017	7.43E+05	2.90E+03	8.18E+01	0.00E+00	0.00E+00		0.00E+00	0.00E+00	0.00E+00
17M16(2)P0.25	9/29/2017	8.08E+05	1.21E+03	8.18E+01		0.00E+00		6.13E+01	2.40E+10	3.51E+10
17M16(2)P0.25	2/26/2018	5.29E+05	6.17E+03	6.13E+01		0.00E+00		0.00E+00	1.23E+09	5.28E+09
17M16(2)P1	9/6/2017	8.12E+05	3.47E+02	4.09E+01	4.09E+01	0.00E+00		0.00E+00	0.00E+00	0.00E+00
17M16(2)P1	9/29/2017	8.00E+05	8.18E+01	0.00E+00		0.00E+00		0.00E+00	2.95E+10	7.50E+10
17M16(2)P1	2/26/2018	4.84E+05	2.04E+02	0.00E+00		2.04E+01		2.04E+01	1.66E+03	1.33E+11
17M16A8	9/29/2014	3.58E+05	1.02E+05	2.53E+04		4.62E+03		1.49E+03	3.68E+03	2.23E+04
17M16A8	12/9/2014	1.25E+05	1.32E+04	7.36E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
17M16A8	12/16/2014	4.15E+05	6.00E+04	1.17E+03		1.00E+02		1.00E+02	1.00E+02	1.00E+02
17M16P0.25(2)	9/26/2017	1.56E+06	1.60E+04	1.04E+03	0.00E+00	0.00E+00		0.00E+00	0.00E+00	0.00E+00
17M16P0.25(2)	9/29/2017	7.34E+05	6.60E+04	8.18E+02		2.04E+01		0.00E+00	0.00E+00	0.00E+00
17M16P1	9/26/2017	1.05E+06	0.00E+00	0.00E+00	0.00E+00	4.09E+01		0.00E+00	2.04E+01	0.00E+00
17M16P1	2/13/2018	5.44E+05	4.09E+01	0.00E+00		0.00E+00		0.00E+00	2.04E+01	0.00E+00
17M16P1(2)	9/26/2017	1.65E+06	1.46E+05	5.40E+02	0.00E+00	6.13E+01		0.00E+00	0.00E+00	0.00E+00
17M16P1(2)	9/29/2017	8.52E+05	5.00E+04	7.77E+02		2.04E+01		0.00E+00	0.00E+00	0.00E+00
17M16PB0.25	9/23/2014	4.29E+05	1.00E+02	1.00E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
17M16PB0.25	10/28/2014	2.51E+05	1.92E+03	1.00E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
17M4	9/17/2014	6.92E+05	5.02E+05	2.59E+04		1.10E+03		1.10E+05	3.58E+10	3.11E+10
17M4	10/21/2014	4.29E+05	3.64E+05	4.85E+04		7.79E+03		7.97E+04	4.55E+10	9.01E+10

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
17M4(2)P0.25	9/6/2017	1.07E+06	1.55E+03	6.13E+01	6.13E+01	2.04E+01		1.17E+04	6.21E+10	3.68E+08
17M4(2)P0.25	9/29/2017	7.32E+05	8.79E+02	8.18E+01		4.09E+01		1.17E+04	5.16E+10	4.26E+10
17M4(2)P1	9/6/2017	9.65E+05	3.88E+02	2.04E+01	4.09E+01	2.04E+01		6.74E+02	2.90E+10	1.86E+09
17M4(2)P1	9/29/2017	7.32E+05	4.09E+01	2.04E+01		2.04E+01		2.62E+03	4.27E+10	8.83E+10
17M4A16	9/29/2014	3.70E+05	4.90E+04	3.17E+04		5.13E+03		1.23E+03	1.00E+02	2.96E+03
17M4A16	12/9/2014	1.12E+05	6.32E+03	4.91E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
17M4A16	12/16/2014	3.35E+05	1.72E+05	3.41E+03		1.00E+02		1.00E+02	1.00E+02	1.00E+02
17M4A8	9/29/2014	3.82E+05	1.33E+05	4.88E+04		1.72E+04		3.54E+03	1.00E+02	1.00E+02
17M4A8	12/9/2014	1.72E+05	2.14E+04	2.60E+03		4.09E+02		1.00E+02	1.00E+02	1.00E+02
17M4P0.25(2)	9/26/2017	1.61E+06	1.32E+06	3.72E+03	0.00E+00	0.00E+00		0.00E+00	0.00E+00	0.00E+00
17M4P0.25(2)	9/29/2017	8.90E+05	7.52E+05	3.13E+03		0.00E+00		0.00E+00	0.00E+00	0.00E+00
17M4P1(2)	9/26/2017	1.67E+06	1.39E+06	2.44E+03	0.00E+00	0.00E+00		0.00E+00	0.00E+00	0.00E+00
17M4P1(2)	9/29/2017	7.40E+05	7.20E+05	3.45E+03		0.00E+00		0.00E+00	0.00E+00	0.00E+00
17M4PB0.25	9/23/2014	5.67E+05	1.00E+02	1.00E+02		1.00E+02		7.89E+03	1.95E+11	1.39E+11
17M4PB0.25	10/28/2014	2.92E+05	2.25E+02	1.00E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
17M4PB0.25	12/9/2014	3.80E+05	1.81E+04	5.93E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
17M4PB0.25	12/16/2014	3.27E+05	1.31E+03	1.00E+02		1.00E+02		1.00E+02	1.34E+05	1.58E+10
17M4PB0.25	1/6/2015	3.13E+05	1.74E+03	1.00E+02		1.00E+02		1.00E+02	1.04E+10	8.77E+09
17M4PB1	9/23/2014	5.78E+05	1.00E+02	1.00E+02		1.00E+02		1.00E+02	3.07E+03	5.47E+09
17M4PB1	10/28/2014	1.60E+05	1.00E+02	1.00E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
17M4PB1	12/9/2014	4.41E+05	1.14E+03	1.00E+02		1.00E+02		1.00E+02	5.26E+05	1.54E+10
17M4PB1	12/16/2014	4.17E+05	1.00E+02	1.00E+02		1.00E+02		1.00E+02	5.33E+05	3.80E+09
17M4PB1	1/6/2015	3.45E+05	1.00E+02	1.00E+02		1.00E+02		1.00E+02	7.15E+04	1.90E+09
17PB0.25	9/17/2014	5.33E+05	4.09E+03	1.00E+02		7.42E+03		5.20E+05	3.65E+11	4.88E+10
17PB0.25	10/21/2014	3.25E+05	9.40E+02	1.00E+02		1.00E+02		2.15E+03	1.26E+11	8.51E+10
17PB1	9/17/2014	4.46E+05	1.00E+02	1.09E+02		1.00E+02		3.70E+04	4.72E+10	6.08E+10

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
17PB1	10/21/2014	2.82E+05	1.02E+02	1.00E+02		1.00E+02		2.98E+03	8.35E+10	7.99E+10
19A16	9/23/2014	1.05E+06	4.11E+05	4.91E+02		1.00E+02		1.00E+02	2.86E+06	1.53E+09
19A16	9/29/2014	4.42E+05	1.43E+03	1.00E+02		1.00E+02		1.00E+02	8.69E+05	8.18E+06
19A8	9/23/2014	1.10E+06	2.06E+05	2.02E+03		1.84E+02		1.00E+02	2.41E+07	1.51E+09
19A8	9/29/2014	3.06E+05	1.23E+04	1.84E+02		1.00E+02		1.00E+02	7.85E+06	6.95E+06
19GC	9/17/2014	8.00E+05	1.22E+06	3.17E+06		6.00E+08		7.44E+10	1.13E+11	2.50E+10
19GC	10/21/2014	6.11E+05	8.36E+05	2.26E+06		2.27E+08		7.07E+10	1.94E+11	1.09E+11
19M16	9/17/2014	9.24E+05	5.22E+05	7.56E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
19M16	10/21/2014	6.75E+05	1.21E+05	2.53E+03		6.74E+02		1.00E+02	1.00E+02	1.00E+02
19M16A8	9/29/2014	2.85E+05	2.41E+03	1.00E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
19M16A8	12/9/2014	4.54E+05	1.19E+04	1.00E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
19M16PB0.25	9/23/2014	1.47E+06	6.39E+04	1.00E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
19M16PB0.25	10/28/2014	2.09E+05	1.29E+03	1.00E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
19M4	9/17/2014	1.51E+06	4.87E+05	7.15E+02		1.02E+02		1.00E+02	1.16E+06	2.66E+09
19M4	10/21/2014	7.01E+05	6.17E+05	4.72E+03		1.51E+03		1.02E+04	8.75E+10	1.71E+10
19M4	12/16/2014	5.29E+05	4.29E+05	1.47E+03		1.29E+04		5.48E+07	3.93E+10	1.61E+10
19M4A16	9/29/2014	4.27E+05	1.86E+03	1.00E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
19M4A16	12/9/2014	3.39E+05	9.05E+03	1.84E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
19M4A8	9/29/2014	5.26E+05	9.88E+03	1.23E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
19M4A8	12/9/2014	5.01E+05	2.66E+04	3.68E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
19M4PB0.25	9/23/2014	1.81E+06	9.15E+04	1.43E+02		1.00E+02		1.00E+02	1.00E+02	6.78E+05
19M4PB0.25	10/28/2014	1.00E+05	5.89E+03	1.00E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
19M4PB0.25	12/9/2014	6.95E+05	5.52E+04	1.00E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
19M4PB1	9/23/2014	1.19E+06	3.56E+03	1.00E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
19M4PB1	10/28/2014	1.53E+05	6.13E+03	1.00E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
19PB0.25	9/17/2014	2.25E+06	8.18E+02	1.00E+02		9.42E+03		3.80E+04	1.23E+11	1.47E+10

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
19PB0.25	10/21/2014	6.84E+05	3.99E+03	2.45E+02		1.00E+02		8.63E+03	6.42E+10	2.38E+10
19PB1	9/17/2014	1.27E+06	1.00E+02	1.00E+02		1.43E+02		1.63E+04	1.19E+11	2.96E+10
19PB1	10/21/2014	7.19E+05	5.52E+02	1.00E+02		1.00E+02		6.89E+03	1.82E+11	1.56E+11
22A16	5/29/2014	6.02E+05	4.31E+05	5.02E+05		1.23E+05		4.89E+05	1.18E+09	8.19E+10
22A16	6/18/2014	4.47E+05	8.45E+05	2.99E+05		1.06E+06		3.84E+06	1.16E+11	1.19E+11
22A16	5/26/2017	5.84E+05	1.45E+05	1.17E+05		2.31E+05		6.57E+05	1.18E+11	6.72E+10
22A16M16	6/18/2014	4.67E+05	3.82E+05	4.50E+02		6.13E+01		1.00E+00	1.00E+00	1.00E+00
22A16M16	5/26/2017	4.55E+05	7.97E+04	4.07E+03		3.07E+02		0.00E+00	0.00E+00	0.00E+00
22A16M4	6/18/2014	4.64E+05	8.69E+05	3.77E+04		4.09E+01		1.00E+00	1.31E+07	9.03E+10
22A16M4	5/26/2017	6.96E+05	1.08E+05	2.21E+04		2.06E+03		1.72E+03	2.31E+11	4.39E+10
22A16M64	6/18/2014	4.73E+05	1.43E+04	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
22A64	5/29/2014	6.35E+05	2.91E+04	2.49E+03		2.04E+02		1.00E+00	1.00E+00	1.00E+00
22A64	5/26/2017	5.84E+05	1.84E+04	3.88E+02		2.04E+01		0.00E+00	0.00E+00	0.00E+00
22A8	5/26/2017	5.13E+05	4.52E+05	3.70E+05		4.27E+07		1.07E+11	3.09E+11	6.44E+10
22A8	2/13/2018	7.61E+05	6.87E+05	1.33E+06		1.17E+08		1.01E+11	4.28E+10	9.88E+10
22A8M16	5/26/2017	6.10E+05	2.05E+05	2.96E+04		2.49E+03		1.14E+03	1.71E+11	6.51E+10
22A8M16	2/13/2018	7.41E+05	7.11E+05	3.59E+04		1.10E+03		1.14E+04	7.13E+10	5.98E+10
22A8M4	5/26/2017	6.47E+05	2.80E+05	8.99E+04		8.11E+03		6.36E+03	2.36E+11	1.19E+11
22A8M4	2/13/2018	7.75E+05	7.08E+05	9.40E+04		1.90E+03		1.55E+04	5.70E+10	6.72E+10
22GC	5/29/2014	6.02E+05	8.00E+05	3.14E+06		4.21E+09		1.11E+11	5.23E+10	9.93E+10
22GC	8/23/2014	6.21E+05	9.91E+05	2.51E+06		5.15E+09		1.06E+11	6.69E+10	2.45E+09
22GC	6/11/2015	5.07E+05		2.55E+06	6.23E+06	1.35E+08	3.21E+10		1.43E+11	
22GC on M16	6/11/2015	1.00E+00		6.00E+00	7.93E+02	8.75E+03	9.69E+04		9.69E+04	
22GC on P4	6/11/2015	1.60E+01		1.08E+02	2.54E+02	1.58E+03	3.14E+01		9.18E+03	
22M16	5/14/2014	1.64E+06	1.26E+06	2.43E+04		2.02E+03		4.09E+04	9.27E+10	4.13E+09
22M16	5/20/2014	4.73E+05	3.52E+05	6.70E+03		1.02E+02		2.08E+03	6.68E+10	5.12E+10

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
22M16	2/13/2017	7.82E+05		1.22E+04		1.84E+02	1.64E+02	5.00E+03	2.86E+10	
22M16 on M16	2/13/2017	0.00E+00		0.00E+00		2.04E+01	8.18E+01	4.94E+03	3.19E+10	
22M16F on M16	2/13/2017	0.00E+00		0.00E+00		6.00E+00	1.54E+02	TNTC		
22M16PB0.25	5/14/2014	1.66E+06	1.08E+03	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
22M16PB0.25	5/20/2014	5.28E+05	1.02E+02	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
22M16PB1	5/14/2014	5.97E+05	1.00E+00	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
22M16PB1	5/20/2014	5.86E+05	6.13E+01	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
22M32	2/13/2017	8.00E+05		1.23E+03		1.23E+02	1.43E+02	6.13E+01	0.00E+00	
22M32 on M16	2/13/2017	0.00E+00		0.00E+00		0.00E+00	0.00E+00	0.00E+00	0.00E+00	
22M32F on M16	2/13/2017	0.00E+00		1.00E+00		0.00E+00	0.00E+00	0.00E+00	0.00E+00	
22M4	5/14/2014	1.65E+06	1.70E+06	4.70E+04		1.68E+03		1.37E+05	1.19E+11	7.98E+10
22M4	5/20/2014	4.93E+05	6.11E+05	3.78E+03		2.86E+02		5.23E+04	1.03E+11	1.01E+11
22M4PB0.25	5/14/2014	1.27E+06	5.84E+03	2.04E+01		1.00E+00		1.00E+00	1.00E+00	1.00E+00
22M4PB0.25	5/20/2014	5.17E+05	2.04E+02	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
22M4PB1	5/14/2014	8.79E+05	2.45E+02	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
22M4PB1	5/20/2014	5.10E+05	2.04E+01	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
22M64	5/14/2014	9.34E+05	1.43E+04	2.86E+02		1.00E+00		1.00E+00	1.00E+00	1.00E+00
22M64	5/20/2014	5.28E+05	4.19E+03	8.18E+01		4.09E+01		2.04E+01	1.00E+00	1.00E+00
22M64PB0.25	5/14/2014	1.17E+06	1.23E+02	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
22M64PB0.25	5/20/2014	5.88E+05	8.18E+01	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
22M64PB1	5/14/2014	7.68E+05	1.00E+00	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
22M64PB1	5/20/2014	5.02E+05	1.00E+00	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
22PB0.06	6/10/2014	5.27E+05	9.81E+04	1.31E+05		9.39E+06		1.28E+11	7.98E+10	5.62E+10
22PB0.06	6/18/2014	4.56E+05	4.48E+03	1.00E+03		1.12E+03		8.08E+05	1.13E+11	1.12E+11

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
22PB0.125	6/18/2014	4.93E+05	3.07E+02	2.04E+01		2.25E+02		3.00E+05	1.29E+11	6.66E+10
22PB0.125	7/9/2014	6.91E+05	1.64E+02	1.84E+02		4.50E+02		8.58E+04	1.26E+11	8.75E+10
22PB0.25	5/14/2014	1.34E+06	1.78E+03	1.00E+00		1.00E+00		3.94E+03	2.21E+11	2.65E+10
22PB0.25	5/20/2014	5.01E+05	4.09E+01	2.04E+01		4.09E+01		2.23E+03	1.35E+11	9.15E+10
22PB1	5/14/2014	1.43E+06	1.00E+00	1.00E+00		1.00E+00		2.25E+02	5.95E+10	1.04E+11
22PB1	5/20/2014	5.53E+05	1.00E+00	2.04E+01		1.00E+00		8.18E+02	1.08E+11	1.23E+11
22PB16	2/13/2017	5.86E+05	0.00E+00	0.00E+00		0.00E+00		6.74E+02	4.95E+09	
22PB16 on PB4	2/13/2017	0.00E+00	0.00E+00	0.00E+00		0.00E+00		0.00E+00	0.00E+00	
22PB16F on PB4	2/13/2017	2.29E+02	0.00E+00	1.00E+00		2.00E+00		5.10E+02		
22PB2	5/29/2014	6.68E+05	1.00E+00	2.04E+01		1.00E+00		2.41E+03	6.37E+10	1.21E+11
22PB2	6/10/2014	5.64E+05	1.00E+00	1.00E+00		1.00E+00		1.37E+03	1.29E+11	8.03E+10
22PB32	2/13/2017	6.06E+05	0.00E+00	0.00E+00		0.00E+00		0.00E+00	0.00E+00	
22PB32 on PB4	2/13/2017	0.00E+00	0.00E+00	0.00E+00		0.00E+00		0.00E+00	0.00E+00	
22PB32F on PB4	2/13/2017	1.90E+01	1.00E+00	0.00E+00		1.00E+00		0.00E+00	2.00E+00	
22PB4	5/29/2014	6.60E+05	1.00E+00	1.00E+00		1.00E+00		2.86E+02	2.11E+10	1.13E+11
22PB4	6/10/2014	5.00E+05	1.00E+00	1.00E+00		1.00E+00		5.31E+02	2.38E+10	1.52E+11
24A16	5/29/2014	7.00E+05	1.95E+05	1.00E+00		3.68E+02		4.29E+02	2.56E+06	9.69E+10
24A16	6/18/2014	3.94E+05	6.91E+04	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
24A16	5/26/2017	1.31E+06	6.57E+05	3.88E+02		0.00E+00		2.04E+01	3.78E+05	2.27E+09
24A16M16	6/18/2014	3.19E+05	5.35E+04	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
24A16M4	6/18/2014	3.95E+05	4.46E+04	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
24A16M64	6/18/2014	3.92E+05	2.62E+03	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
24A64	5/29/2014	7.14E+05	5.31E+04	1.00E+00		1.00E+00		6.13E+01	1.00E+00	1.00E+00
24A8	5/26/2017	1.50E+06	6.62E+05	1.64E+03		0.00E+00		6.13E+01	9.39E+06	2.82E+09

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
24A8M16	5/26/2017	1.63E+06	3.86E+05	7.36E+02		1.02E+02		6.13E+01	0.00E+00	0.00E+00
24A8M4	5/26/2017	1.53E+06	4.71E+05	1.59E+03		8.18E+01		1.23E+02	0.00E+00	0.00E+00
24A8M64	5/26/2017	1.30E+06	1.94E+05	4.91E+02		6.13E+01		1.02E+02	0.00E+00	0.00E+00
24GC	5/29/2014	7.17E+05	8.29E+05	1.77E+06		6.82E+07		1.45E+10	2.82E+10	2.58E+10
24GC	8/23/2014	5.66E+05	7.22E+05	2.00E+06		8.08E+07		2.24E+10	2.60E+10	3.99E+10
24GC	6/11/2015	1.04E+06		2.46E+06	8.59E+06	2.48E+08	5.24E+10		3.44E+11	
24GC on M64	6/11/2015	7.00E+00		5.70E+01	5.08E+02	1.01E+03	4.98E+03		3.34E+04	
24GC on P4	6/11/2015	1.00E+00		1.00E+00	1.00E+00	3.60E+01	2.00E+02		3.77E+02	
24M128	2/13/2017	5.31E+05		3.43E+03		2.45E+02	1.23E+02	4.09E+01	4.09E+01	
24M128 on M64	2/13/2017	0.00E+00		0.00E+00		2.04E+01	0.00E+00	0.00E+00	0.00E+00	
24M128F on M64	2/13/2017	0.00E+00		0.00E+00		0.00E+00	0.00E+00	0.00E+00	0.00E+00	
24M16	5/20/2014	4.53E+05	5.68E+05	1.17E+05		4.03E+03		9.81E+04	4.13E+10	1.92E+10
24M16	6/10/2014	6.02E+05	6.34E+05	1.17E+05		2.15E+03		6.18E+03	3.10E+10	1.11E+11
24M16(2)P0.25	9/6/2017	9.73E+05	1.18E+04	8.18E+01	6.13E+01	2.04E+01		3.07E+02	2.47E+09	1.65E+11
24M16(2)P0.25	9/29/2017	1.16E+06	3.88E+02	5.11E+02		8.18E+01		3.74E+03	2.24E+10	1.37E+11
24M16(2)P1	9/6/2017	8.57E+05	2.39E+03	8.18E+01	2.04E+01	0.00E+00		6.13E+01	6.13E+08	9.78E+08
24M16(2)P1	9/29/2017	1.03E+06	3.68E+02	1.02E+02		4.09E+01		1.49E+03	1.58E+10	5.18E+10
24M16P0.25(2)	9/26/2017	1.73E+06	1.58E+06	2.66E+05	5.40E+02	3.27E+02		6.13E+01	3.23E+05	1.33E+11
24M16P0.25(2)	9/29/2017	1.16E+06	1.17E+06	7.88E+04		2.04E+02		6.13E+01	2.25E+02	9.02E+09
24M16P1(2)	9/26/2017	1.71E+06	1.51E+06	1.78E+05	0.00E+00	4.09E+01		0.00E+00	1.12E+05	2.42E+05
24M16P1(2)	9/29/2017	1.15E+06	1.15E+06	9.62E+04		4.09E+01		0.00E+00	0.00E+00	0.00E+00
24M16P1(2)	2/26/2018	4.51E+05	5.30E+05	5.65E+04		0.00E+00		0.00E+00	1.27E+03	1.54E+10
24M16P1(2)	3/13/2018	6.66E+05	6.46E+05	8.38E+04		2.04E+01		2.04E+01	2.14E+06	2.86E+10
24M16PB0.25	5/20/2014	4.26E+05	1.84E+02	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
24M16PB0.25	6/10/2014	6.37E+05	1.40E+05	2.17E+03		5.72E+02		8.38E+02	4.79E+10	6.00E+10

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
24M16PB0.25	7/9/2014	7.13E+05	1.68E+03	6.13E+01		8.18E+01		1.00E+00	1.00E+00	1.00E+00
24M16PB1	5/20/2014	4.30E+05	4.09E+01	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
24M16PB1	6/10/2014	6.38E+05	4.91E+04	2.04E+01		1.00E+00		1.00E+00	2.11E+05	5.11E+10
24M16PB1	7/9/2014	7.14E+05	2.86E+02	1.00E+00		6.13E+01		1.00E+00	1.00E+00	1.00E+00
24M4	5/20/2014	4.46E+05	5.92E+05	4.79E+05		1.71E+04		2.07E+06	3.95E+10	5.48E+10
24M4	6/10/2014	6.01E+05	7.29E+05	4.84E+05		1.08E+04		1.37E+03	2.79E+10	1.18E+11
24M4(2)P0.25	9/6/2017	9.73E+05	8.34E+03	2.04E+02	1.29E+03	2.04E+01		3.45E+03	4.39E+10	1.25E+11
24M4(2)P0.25	9/29/2017	8.31E+05	3.68E+02	3.68E+02		1.64E+02		5.31E+03	1.06E+10	1.74E+11
24M4(2)P1	9/6/2017	1.11E+06	2.90E+03	6.13E+01	0.00E+00	0.00E+00		2.25E+02	4.70E+08	3.59E+10
24M4(2)P1	9/29/2017	1.14E+06	1.64E+02	6.13E+01		6.13E+01		1.80E+03	3.00E+09	1.87E+10
24M4B1	7/9/2014	8.02E+05	4.09E+01	2.04E+01		1.00E+00		3.27E+02	6.54E+08	1.61E+11
24M4P0.25(2)	9/26/2017	2.27E+06	1.65E+06	9.83E+05	9.22E+03	7.23E+05		6.13E+08	3.11E+09	2.14E+10
24M4P0.25(2)	9/29/2017	1.15E+06	1.26E+06	6.33E+05		1.84E+02		1.80E+05	5.70E+09	2.62E+10
24M4P0.25(2)	2/26/2018	4.65E+05	5.97E+05	4.19E+05		5.01E+03		3.88E+02	3.70E+09	6.20E+10
24M4P1(2)	9/26/2017	1.80E+06	1.35E+06	8.35E+05	0.00E+00	0.00E+00		0.00E+00	1.83E+06	1.03E+11
24M4P1(2)	9/29/2017	1.08E+06	1.10E+06	7.38E+05		8.18E+01		8.18E+01	6.40E+06	4.07E+10
24M4PB0.25	5/20/2014	4.62E+05	2.86E+02	1.00E+00		1.00E+00		1.43E+02	2.72E+10	8.07E+10
24M4PB0.25	6/10/2014	5.61E+05	1.43E+05	4.23E+03		4.91E+02		1.01E+05	3.98E+10	2.15E+11
24M4PB0.25	7/9/2014	8.23E+05	2.86E+02	2.04E+01		1.00E+00		6.21E+03	4.05E+10	9.07E+10
24M4PB1	5/20/2014	4.55E+05	1.00E+00	1.00E+00		1.00E+00		1.00E+00	4.93E+10	9.97E+10
24M4PB1	6/10/2014	6.61E+05	1.04E+04	4.09E+01		1.00E+00		3.68E+02	3.84E+07	6.48E+10
24M64	5/20/2014	4.36E+05	9.40E+04	1.05E+04		2.17E+03		1.96E+03	2.36E+11	8.95E+10
24M64	6/10/2014	5.40E+05	3.19E+05	8.73E+03		8.79E+02		1.29E+03	8.83E+10	9.27E+10
24M64	2/13/2017	5.29E+05		1.28E+04		3.68E+02	2.66E+02	2.45E+02	1.58E+10	
24M64 on M64	2/13/2017	0.00E+00		0.00E+00		0.00E+00	0.00E+00	4.09E+01	1.50E+10	

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
24M64F on M64	2/13/2017	1.00E+00		0.00E+00		0.00E+00	3.00E+00	2.70E+01		
24M64PB0.25	5/20/2014	4.33E+05	1.02E+02	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
24M64PB0.25	6/10/2014	6.19E+05	9.76E+04	1.00E+00		1.00E+00		1.00E+00	3.88E+02	1.54E+11
24M64PB0.25	7/9/2014	8.34E+05	5.72E+02	2.04E+01		1.00E+00		1.00E+00	4.89E+05	7.95E+10
24M64PB1	5/20/2014	4.22E+05	1.00E+00	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
24M64PB1	6/10/2014	5.98E+05	3.02E+03	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
24M64PB1	7/9/2014	4.71E+05	2.04E+01	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
24PB0.06	6/10/2014	5.87E+05	1.10E+05	4.91E+04		8.71E+05		5.43E+09	4.00E+10	5.45E+10
24PB0.06	6/18/2014	3.37E+05	2.78E+04	1.19E+03		1.22E+04		1.86E+06	3.66E+10	1.35E+11
24PB0.125	6/18/2014	3.67E+05	1.00E+00	6.13E+01		1.23E+02		1.83E+04	4.96E+10	1.42E+11
24PB0.125	7/9/2014	7.51E+05	3.70E+03	9.40E+02		5.33E+03		2.22E+05	8.15E+10	1.39E+11
24PB0.25	5/20/2014	4.26E+05	9.69E+03	1.00E+00		1.00E+00		4.09E+02	1.12E+10	7.88E+10
24PB0.25	6/10/2014	6.72E+05	4.69E+04	2.04E+02		1.00E+00		1.19E+03	9.95E+09	4.51E+10
24PB1	5/20/2014	4.85E+05	8.18E+01	1.00E+00		1.00E+00		3.47E+02	4.71E+09	7.02E+10
24PB1	6/10/2014	5.81E+05	4.01E+03	1.00E+00		1.00E+00		7.77E+02	2.06E+10	6.99E+10
24PB16	2/13/2017	5.89E+05	4.09E+01	8.18E+01		0.00E+00		1.84E+05	6.63E+08	
24PB16 on PB4	2/13/2017	0.00E+00	0.00E+00	0.00E+00		0.00E+00		1.78E+05	4.74E+08	
24PB16F on PB4	2/13/2017	1.29E+03	7.80E+01	6.60E+01		1.61E+02		1.89E+03		
24PB2	5/29/2014	6.33E+05	1.00E+00	1.00E+00		1.00E+00		1.00E+00	1.79E+06	7.33E+10
24PB2	6/10/2014	6.26E+05	1.00E+00	1.00E+00		1.00E+00		3.88E+02	4.70E+08	1.10E+11
24PB2	8/23/2014	5.37E+05	1.00E+00	1.00E+00		4.09E+01		9.40E+02	4.95E+07	1.02E+10
24PB4	5/29/2014	7.29E+05	1.00E+00	1.00E+00		1.00E+00		1.00E+00	2.08E+06	2.53E+09
24PB4	6/10/2014	5.66E+05	1.00E+00	1.00E+00		1.00E+00		1.23E+02	7.23E+06	6.87E+10
24PB4	8/23/2014	5.04E+05	1.00E+00	1.00E+00		1.00E+00		1.23E+02	6.66E+06	2.12E+10

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
24PB8	2/13/2017	6.40E+05	1.02E+02	2.04E+01		2.86E+02		2.66E+05	4.19E+08	
24PB8 on PB4	2/13/2017	0.00E+00	0.00E+00	0.00E+00		8.18E+01		1.70E+05	6.87E+08	
24PB8F on PB4	2/13/2017	1.45E+03		9.00E+01		1.71E+02		2.23E+03		
29A16	7/21/2017	3.94E+05	6.57E+05	2.72E+05		4.31E+05		7.35E+07	9.20E+08	1.25E+09
29A16M16	7/21/2017	3.64E+05	4.95E+05	3.83E+04		2.25E+02		5.04E+03	1.90E+09	1.76E+09
29A16M4	7/21/2017	4.22E+05	8.38E+05	6.66E+04		2.17E+03		1.14E+04	3.80E+09	1.72E+09
29A16M64	7/21/2017	8.27E+05	2.04E+03	2.86E+02		4.09E+01		0.00E+00	1.96E+03	7.97E+08
29A8	10/18/2016	2.51E+06		2.42E+06		5.36E+09		2.22E+11	3.89E+10	7.20E+10
29A8	7/21/2017	6.33E+05	7.90E+05	3.74E+06		5.52E+08		4.81E+09	2.72E+09	1.00E+09
29A8M16	7/21/2017	5.05E+05	6.49E+05	4.57E+04		1.02E+03		5.31E+04	3.45E+09	1.88E+09
29A8M4	7/21/2017	3.24E+05	1.18E+06	6.13E+04		8.38E+02		1.96E+06	8.18E+08	1.22E+10
29A8M64	7/21/2017	5.95E+05	1.88E+04	2.45E+02		2.04E+01		6.13E+01	1.47E+09	7.93E+09
29GC	10/18/2016	3.01E+06		1.10E+07		2.76E+09		1.33E+11	5.38E+10	6.45E+10
29GC	7/21/2017	5.59E+05	1.48E+06	4.00E+06		9.61E+08		1.66E+10	4.65E+09	1.53E+09
29M16	10/18/2016	2.26E+06		3.09E+03		1.19E+03		1.45E+06	3.91E+11	1.50E+11
29M16	7/21/2017	3.12E+05	8.62E+05	2.35E+03		6.13E+02		5.40E+05	2.02E+09	2.68E+09
29M16A8	10/18/2016	2.76E+06		1.06E+05		1.39E+03		4.99E+05	9.88E+10	1.61E+11
29M16PB1	10/18/2016	1.93E+06		3.11E+03		3.88E+02		2.04E+01	0.00E+00	0.00E+00
29M4	7/21/2017	4.59E+05	1.09E+06	3.84E+03		9.94E+03		3.00E+06	3.94E+10	1.78E+09
29M64	7/21/2017	4.89E+05	2.62E+04	4.11E+03		6.13E+01		1.29E+03	1.74E+09	6.42E+09
29PB1	10/18/2016	1.76E+06		5.43E+04		4.26E+04		1.61E+05	2.69E+11	6.82E+10
32A16	7/21/2017	4.33E+05	2.31E+03	0.00E+00		0.00E+00		0.00E+00	0.00E+00	0.00E+00
32A16	2/13/2018	4.28E+05	1.04E+03	4.09E+01		6.54E+02		5.60E+04	7.45E+09	5.95E+09
32A16	2/26/2018	3.05E+05	6.95E+02	8.18E+01		4.91E+02		2.04E+02	1.82E+06	4.07E+09
32A16M16	7/21/2017	4.82E+05	6.13E+01	0.00E+00		0.00E+00		0.00E+00	0.00E+00	0.00E+00
32A16M16	2/13/2018	5.01E+05	3.27E+02	0.00E+00		6.13E+01		5.08E+03	3.58E+09	4.39E+09

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
32A16M16	2/26/2018	2.65E+05	4.70E+02	0.00E+00		0.00E+00		0.00E+00	0.00E+00	0.00E+00
32A16M4	7/21/2017	2.83E+05	9.20E+02	0.00E+00		0.00E+00		0.00E+00	0.00E+00	0.00E+00
32A16M4	2/13/2018	5.01E+05	6.74E+02	2.04E+01		1.43E+02		8.38E+04	5.15E+09	4.25E+09
32A16M4	2/26/2018	2.78E+05	9.40E+02	2.04E+01		0.00E+00		0.00E+00	0.00E+00	0.00E+00
32A8	7/21/2017	6.11E+05	2.70E+04	2.04E+01		0.00E+00		0.00E+00	0.00E+00	0.00E+00
32A8	2/13/2018	4.57E+05	1.31E+04	2.04E+02		1.84E+03		3.58E+06	3.99E+10	4.83E+09
32A8	2/26/2018	3.61E+05	3.33E+04	3.47E+02		6.57E+03		6.34E+06	4.11E+09	2.80E+09
32A8M16	7/21/2017	7.05E+05	3.25E+04	4.09E+01		0.00E+00		0.00E+00	0.00E+00	0.00E+00
32A8M16	2/13/2018	5.01E+05	2.22E+04	2.04E+01		4.50E+02		5.08E+05	4.20E+10	5.95E+10
32A8M16	2/26/2018	3.51E+05	3.24E+04	2.04E+01		0.00E+00		0.00E+00	0.00E+00	0.00E+00
32A8M4	7/21/2017	3.87E+05	3.00E+04	0.00E+00		0.00E+00		0.00E+00	0.00E+00	0.00E+00
32A8M4	2/13/2018	5.01E+05	2.60E+04	0.00E+00		7.97E+02		5.73E+05	4.96E+10	4.27E+10
32A8M4	2/26/2018	3.18E+05	3.78E+04	6.13E+01		0.00E+00		0.00E+00	0.00E+00	0.00E+00
32GC	7/21/2017	4.44E+05	9.15E+05	2.08E+06		1.98E+09		8.32E+10	5.23E+10	3.02E+10
32GC	2/13/2018	4.64E+05	8.19E+05	1.76E+06		1.74E+09		2.72E+10	7.42E+10	6.09E+10
32M16	7/21/2017	5.14E+05	8.68E+04	1.39E+03		4.50E+02		1.80E+03	3.50E+07	2.86E+10
32M16	2/13/2018	6.63E+05	2.68E+05	4.69E+03		1.88E+03		2.25E+06	7.39E+10	6.75E+10
32M4	7/21/2017	8.35E+05	8.82E+05	7.58E+03		1.12E+03		2.35E+05	4.66E+09	2.93E+10
32M4	2/13/2018	4.92E+05	6.72E+05	5.21E+03		2.33E+03		2.20E+06	7.92E+10	6.81E+10
32M64	7/21/2017	8.67E+05	1.48E+04	7.15E+02		1.02E+02		0.00E+00	0.00E+00	0.00E+00
349A8	10/18/2016	5.11E+05		8.18E+01		0.00E+00		0.00E+00	0.00E+00	0.00E+00
349GC	10/18/2016	1.45E+06		3.55E+06		4.79E+10		2.53E+11	1.81E+11	1.25E+11
349M4	10/18/2016	3.07E+05		3.39E+03		4.91E+02		1.02E+02	0.00E+00	0.00E+00
349M4A8	10/18/2016	9.17E+05		0.00E+00		0.00E+00		0.00E+00	0.00E+00	0.00E+00
349M4PB1	10/18/2016	1.45E+06		0.00E+00		0.00E+00		0.00E+00	0.00E+00	2.04E+01
349PB1	10/18/2016	6.78E+05		1.02E+02		1.82E+03		2.19E+05	2.22E+11	1.83E+11

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
34A16	5/29/2014	1.76E+05	1.00E+00	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
34A16	5/26/2017	8.62E+05	0.00E+00	0.00E+00		6.13E+01		0.00E+00	1.30E+06	3.86E+09
34A64	5/29/2014	2.86E+04	1.00E+00	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
34A8	5/26/2017	1.04E+06	0.00E+00	0.00E+00		0.00E+00		0.00E+00	3.73E+06	7.00E+09
34A8M16	5/26/2017	7.98E+05	0.00E+00	0.00E+00		0.00E+00		0.00E+00	0.00E+00	0.00E+00
34A8M4	5/26/2017	8.24E+05	0.00E+00	0.00E+00		0.00E+00		0.00E+00	0.00E+00	0.00E+00
34GC	1/26/2014	5.35E+05	1.02E+06	2.63E+06		5.07E+08		1.41E+09	4.43E+10	4.43E+11
34GC	2/15/2014	7.44E+05	7.16E+05	4.44E+06		1.36E+10		3.42E+10	4.12E+11	3.93E+12
34GC	3/30/2014	7.82E+05	1.53E+06	1.13E+07		3.25E+10		1.06E+11	5.43E+11	9.31E+10
34GC	6/11/2015	8.35E+05		4.01E+06	1.14E+07	2.85E+08	5.11E+10		5.86E+11	
34GC on M16	6/11/2015	1.00E+00		0.00E+00	3.71E+02	5.09E+03	2.98E+05		2.98E+05	
34GC on P4	6/11/2015	6.00E+00		3.20E+01	1.42E+02	1.08E+03	2.02E+03		1.74E+04	
34M16	1/26/2014	7.48E+05	8.31E+04	2.19E+03		4.29E+02		1.84E+02	6.88E+03	4.53E+06
34M16	7/9/2014	4.85E+05	5.25E+04	6.13E+01		1.43E+02		1.00E+00	2.04E+01	2.04E+01
34M16	2/13/2017	1.14E+06		1.27E+03		2.25E+02	2.04E+02	8.18E+01	4.09E+01	
34M16 on M16	2/13/2017	0.00E+00		0.00E+00		0.00E+00	0.00E+00	0.00E+00	0.00E+00	
34M16F on M16	2/13/2017	0.00E+00		0.00E+00		0.00E+00	0.00E+00	0.00E+00	0.00E+00	
34M16PB0.25	2/15/2014	5.87E+05	1.22E+04	1.33E+03		2.04E+01		1.00E+00	1.00E+00	1.00E+00
34M16PB0.25	7/9/2014	1.21E+06	7.03E+03	7.15E+02		2.45E+02		2.04E+01	1.00E+00	1.00E+00
34M16PB1	2/15/2014	6.72E+05	2.46E+04	2.79E+02		1.09E+02		1.00E+00	1.00E+00	1.00E+00
34M16PB1	7/9/2014	7.14E+05	4.81E+03	1.00E+00		1.00E+00		2.04E+01	1.00E+00	2.04E+01
34M4	1/26/2014	8.06E+05	5.94E+05	2.26E+04		1.21E+03		5.74E+03	1.06E+09	1.71E+10
34M4	7/9/2014	1.04E+06	5.86E+05	8.73E+03		9.61E+02		5.72E+04	8.39E+10	5.76E+10
34M4PB0.25	2/15/2014	6.02E+05	1.92E+04	1.20E+03		4.09E+01		1.00E+00	1.00E+00	1.00E+00
34M4PB0.25	7/9/2014	8.09E+05	4.50E+04	8.58E+02		3.47E+02		1.00E+00	6.77E+03	6.21E+10

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
34M4PB1	2/15/2014	7.34E+05	2.83E+04	2.59E+02		2.04E+01		1.00E+00	1.00E+00	1.00E+00
34M4PB1	7/9/2014	9.91E+05	6.13E+04	8.58E+02		2.04E+01		1.00E+00	6.82E+03	7.33E+10
34M64	1/26/2014	6.54E+05	3.39E+04	3.47E+02		6.13E+01		4.09E+01	1.43E+02	2.04E+01
34M64	7/9/2014	7.54E+05	2.98E+04	2.25E+02		4.09E+01		1.00E+00	1.00E+00	1.00E+00
34M8	2/13/2017	7.59E+05		2.08E+03		7.77E+02	3.47E+02	8.18E+01	2.04E+02	
34M8 on M16	2/13/2017	0.00E+00		0.00E+00		0.00E+00	0.00E+00	0.00E+00	0.00E+00	
34M8F on M16	2/13/2017	0.00E+00		0.00E+00		0.00E+00	0.00E+00	0.00E+00	0.00E+00	
34PB0.06	6/10/2014	6.82E+05	6.25E+05	1.89E+06		9.20E+08		7.61E+10	1.30E+11	7.47E+10
34PB0.06	6/18/2014	5.01E+05	6.26E+04	6.12E+03		5.87E+03		1.81E+06	9.88E+10	1.56E+11
34PB0.06	7/9/2014	7.87E+05	1.36E+05	6.44E+04		3.79E+04		1.19E+08	2.19E+11	8.18E+10
34PB0.125	6/18/2014	5.15E+05	2.15E+04	6.54E+02		3.88E+02		4.46E+05	6.48E+10	7.37E+10
34PB0.125	7/9/2014	9.30E+05	4.70E+04	4.24E+03		2.76E+03		1.18E+06	8.44E+10	9.84E+10
34PB0.25	1/26/2014	7.07E+05	2.52E+04	6.74E+02		1.92E+03		3.54E+06	4.19E+07	4.71E+10
34PB0.25	3/30/2014	7.23E+05	3.27E+02	1.43E+02		5.93E+02		4.16E+04	7.16E+10	7.93E+09
34PB0.25	8/23/2014	8.95E+05	3.47E+03	6.34E+02		2.92E+03		4.26E+04	7.88E+10	1.71E+10
34PB1	1/26/2014	6.54E+05	7.07E+03	2.86E+02		4.91E+02		6.16E+05	1.18E+08	1.49E+10
34PB1	3/30/2014	5.84E+05	1.00E+00	1.00E+00		2.66E+02		1.56E+04	1.67E+11	1.19E+10
34PB1	8/23/2014	8.43E+05	2.21E+03	1.64E+02		4.37E+03		2.00E+04	8.59E+10	1.78E+10
34PB16	2/13/2017	5.78E+05	2.25E+02	1.64E+02		2.04E+01		1.31E+03	5.26E+09	
34PB16 on PB4	2/13/2017	0.00E+00	2.04E+01	0.00E+00		2.04E+02		1.68E+03	3.47E+09	
34PB16F on PB4	2/13/2017	3.13E+02	6.00E+00	1.40E+01		7.70E+01		9.70E+02		
34PB2	5/29/2014	8.00E+05	6.13E+02	1.02E+02		8.99E+02		7.16E+04	1.29E+11	5.73E+10
34PB2	6/10/2014	6.92E+05	1.00E+03	2.45E+02		1.84E+02		2.25E+04	6.13E+10	1.57E+11
34PB32	2/13/2017	9.25E+05	4.09E+01	6.13E+01		0.00E+00		0.00E+00	1.23E+02	
34PB32 on PB4	2/13/2017	0.00E+00	0.00E+00	0.00E+00		0.00E+00		0.00E+00	6.13E+01	

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
34PB32F on PB4	2/13/2017	3.18E+02	4.00E+00	6.00E+00		3.00E+00		1.10E+01	4.60E+01	
34PB4	5/29/2014	7.79E+05	1.64E+02	6.13E+01		3.07E+02		3.09E+04	5.31E+10	1.67E+11
34PB4	6/10/2014	6.85E+05	7.97E+02	4.09E+01		1.02E+02		8.36E+03	9.78E+10	1.96E+11
37A16	7/21/2017	1.20E+06	1.77E+06	4.24E+06		2.86E+09		9.91E+09	5.82E+10	4.76E+10
37A16	2/13/2018	7.79E+05	9.82E+05	3.54E+06		2.10E+10		8.11E+10	1.03E+11	1.41E+11
37A16M16	7/21/2017	1.14E+06	3.94E+05	4.93E+03		8.18E+01		0.00E+00	3.50E+07	6.40E+09
37A16M16	2/13/2018	6.51E+05	4.13E+05	1.42E+04		1.02E+02		8.58E+04	3.10E+10	7.45E+09
37A16M4	7/21/2017	1.02E+06	7.29E+05	1.08E+04		8.18E+01		0.00E+00	8.71E+07	5.35E+10
37A16M4	2/13/2018	7.66E+05	5.95E+05	1.19E+04		8.18E+01		7.56E+04	5.13E+09	3.78E+09
37A8	7/21/2017	1.14E+06	1.20E+06	2.92E+06		1.39E+10		1.16E+10	4.86E+10	5.56E+10
37A8	2/13/2018	7.22E+05	1.05E+06	2.97E+06		5.13E+09		1.14E+11	1.20E+11	8.91E+10
37A8M16	7/21/2017	7.39E+05	3.25E+06	1.82E+03		6.13E+01		2.04E+01	1.77E+07	9.07E+10
37A8M16	2/13/2018	8.11E+05	4.27E+05	9.55E+03		2.45E+02		2.68E+05	2.99E+10	4.33E+09
37A8M4	7/21/2017	1.05E+06	1.07E+06	5.83E+03		2.25E+02		2.72E+05	5.19E+09	8.16E+10
37A8M4	2/13/2018	7.65E+05	1.00E+06	3.76E+03		2.86E+02		1.22E+06	1.06E+11	1.11E+11
37GC	7/21/2017	9.78E+05	1.50E+06	5.51E+06		4.43E+10		6.39E+10	1.12E+11	3.91E+10
37GC	2/13/2018	7.23E+05	1.08E+06	2.84E+06		2.35E+10		9.23E+10	6.72E+10	6.15E+10
37M16	7/21/2017	9.44E+05	6.44E+05	3.19E+03		1.90E+03		1.81E+06	5.33E+10	1.00E+10
37M16	2/13/2018	7.97E+05	5.06E+05	4.46E+03		4.70E+02		5.84E+05	7.42E+09	1.13E+11
37M4	7/21/2017	1.08E+06	9.69E+05	3.82E+03		2.35E+03		7.44E+08	4.53E+10	6.57E+10
37M4	2/13/2018	8.77E+05	9.33E+05	1.84E+03		1.21E+03		4.55E+06	9.27E+10	7.26E+10
40A16	9/23/2014	9.65E+05	1.57E+05	3.80E+03		9.40E+02		1.00E+02	2.70E+06	1.17E+09
40A16	9/29/2014	4.54E+05	3.19E+05	4.33E+03		6.13E+02		3.27E+02	1.00E+05	9.20E+06
40A16	12/16/2014	2.94E+05	1.09E+05	2.13E+04		3.23E+05		1.21E+09	6.63E+10	3.14E+10
40A16	1/6/2015	3.78E+05	2.17E+05	8.04E+05		6.76E+07		1.08E+10	9.93E+10	3.74E+10

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
40A8	9/23/2014	2.50E+06	5.22E+05	1.00E+05		1.13E+04		4.70E+02	1.22E+06	5.11E+10
40A8	9/29/2014	5.33E+05	8.18E+05	1.08E+05		1.47E+03		2.86E+02	3.22E+06	3.08E+08
40GC	9/17/2014	5.46E+05	1.51E+06	3.43E+06		4.92E+08		3.39E+10	5.76E+10	1.63E+10
40GC	10/21/2014	5.31E+05	6.08E+05	1.78E+06		1.83E+08		4.68E+10	2.72E+10	4.29E+09
40M16	9/17/2014	6.31E+05	1.13E+06	9.61E+04		1.35E+04		1.03E+04	1.00E+02	1.00E+02
40M16	10/21/2014	5.36E+05	3.41E+05	1.98E+03		4.29E+02		1.00E+02	1.00E+02	1.00E+02
40M16A8	9/29/2014	7.67E+05	3.05E+05	1.88E+03		1.10E+03		1.23E+02	1.00E+02	1.00E+02
40M16A8	12/9/2014	3.92E+05	8.80E+04	1.66E+04		2.13E+03		1.00E+02	1.00E+02	1.00E+02
40M16PB0.25	9/23/2014	9.40E+05	1.45E+05	1.00E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
40M16PB0.25	10/28/2014	3.39E+05	4.09E+03	7.97E+02		2.86E+02		1.00E+02	1.00E+02	1.00E+02
40M4	9/17/2014	1.10E+06	7.38E+05	2.00E+05		1.14E+03		1.21E+05	2.41E+09	6.62E+10
40M4	10/21/2014	6.63E+05	5.43E+05	1.32E+04		1.59E+03		4.60E+04	1.94E+10	2.82E+10
40M4A16	9/29/2014	8.00E+05	5.76E+05	6.13E+04		1.64E+02		1.00E+02	1.00E+02	1.00E+02
40M4A16	12/9/2014	3.86E+05	6.65E+04	6.75E+03		1.10E+05		1.64E+02	1.00E+02	1.00E+02
40M4A8	9/29/2014	8.98E+05	6.67E+05	6.06E+03		1.02E+03		1.00E+02	1.00E+02	1.00E+02
40M4A8	12/9/2014	4.03E+05	1.12E+05	3.35E+04		4.21E+03		1.53E+03	5.48E+03	6.51E+10
40M4A8	12/16/2014	3.56E+05	2.21E+05	4.19E+04		1.96E+03		1.19E+03	1.00E+02	1.00E+02
40M4PB0.25	9/23/2014	1.73E+06	3.50E+04	1.00E+02		1.00E+02		1.00E+02	3.28E+04	6.93E+09
40M4PB0.25	10/28/2014	3.07E+05	2.15E+03	1.00E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
40M4PB0.25	12/9/2014	4.17E+05	1.90E+04	1.00E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
40M4PB0.25	12/16/2014	3.80E+05	8.19E+03	4.70E+02		1.23E+02		1.00E+02	1.00E+02	1.00E+02
40M4PB1	9/23/2014	1.05E+06	1.84E+03	1.00E+02		1.00E+02		1.00E+02	1.00E+02	1.61E+10
40M4PB1	10/28/2014	1.76E+05	2.45E+02	1.00E+02		1.00E+02		1.00E+02	1.00E+02	1.00E+02
40M4PB1	12/9/2014	4.11E+05	1.66E+04	1.00E+02		1.00E+02		1.64E+02	1.00E+02	1.00E+02
40PB0.25	9/17/2014	6.15E+05	6.54E+02	2.45E+02		8.55E+03		1.16E+05	6.00E+10	3.79E+10
40PB0.25	10/21/2014	4.99E+05	3.27E+04	1.00E+02		1.00E+02		6.73E+03	1.20E+10	1.89E+10

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
40PB1	9/17/2014	9.55E+05	1.02E+02	1.00E+02		4.09E+02		4.88E+04	8.07E+10	9.40E+10
40PB1	10/21/2014	5.68E+05	1.24E+04	1.00E+02		1.00E+02		5.11E+03	2.32E+10	9.53E+10
44A16	5/29/2014	5.45E+05	4.61E+03	1.00E+00		4.09E+01		1.00E+00	1.00E+00	1.00E+00
44A16	5/26/2017	5.87E+05	1.10E+05	1.06E+03		6.95E+04		8.06E+10	3.34E+11	6.72E+10
44A64	5/29/2014	4.94E+05	8.18E+01	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
44A8	5/26/2017	6.16E+05	5.53E+04	1.21E+03		1.04E+05		1.59E+10	2.42E+11	6.08E+10
44A8M16	5/26/2017	6.02E+05	2.87E+04	1.23E+02		2.04E+01		0.00E+00	0.00E+00	0.00E+00
44A8M4	5/26/2017	7.65E+05	1.97E+04	1.02E+02		0.00E+00		0.00E+00	0.00E+00	0.00E+00
44A8M64	5/26/2017	5.04E+05	7.56E+04	2.66E+02		6.13E+01		0.00E+00	0.00E+00	0.00E+00
44GC	1/26/2014	6.71E+05	4.07E+05	1.63E+06		9.31E+07		7.23E+08	1.04E+11	8.31E+11
44GC	2/15/2014	6.97E+05	6.86E+05	2.48E+06		1.80E+09		5.13E+10	1.04E+11	1.19E+12
44GC	3/30/2014	5.16E+05	1.08E+06	4.29E+06		4.37E+09		3.05E+10	3.11E+11	2.01E+11
44GC	6/11/2015	8.27E+05		4.93E+06	1.81E+07	4.32E+08	4.76E+10		5.72E+11	
44GC on M64	6/11/2015	4.39E+03		6.95E+04	2.58E+05	7.36E+06	4.29E+08		4.50E+08	
44GC on P4	6/11/2015	2.00E+00		2.50E+01	8.60E+01	4.09E+02	7.56E+02		1.08E+04	
44M128	2/13/2017	8.30E+05		2.64E+04		6.48E+03	3.92E+03	1.66E+03	1.69E+10	
44M128 on M64	2/13/2017	1.41E+04		6.64E+03		1.29E+03	4.91E+02	4.91E+02	5.00E+09	
44M128F on M64	2/13/2017	TNTC		TNTC		4.50E+02	1.89E+02	2.10E+02		
44M16	1/26/2014	7.21E+05	6.97E+05	1.73E+06		9.92E+04		2.05E+08	1.90E+10	1.22E+10
44M16	5/20/2014	4.52E+05	7.32E+05	1.87E+06		2.14E+06		4.04E+10	5.93E+10	1.69E+10
44M16(2)P0.25	9/6/2017	4.29E+05	3.29E+03	6.13E+02	1.64E+02	4.09E+01		6.74E+02	3.30E+10	5.07E+10
44M16(2)P0.25	9/29/2017	7.72E+05	1.90E+03	6.74E+02		1.64E+02		3.27E+02	6.82E+09	5.47E+10
44M16(2)P1	9/6/2017	9.11E+05	1.04E+03	1.43E+02	1.02E+02	2.04E+01		0.00E+00	2.86E+08	1.56E+11
44M16(2)P1	9/29/2017	7.62E+05	4.50E+02	2.25E+02		0.00E+00		3.47E+02	7.47E+09	4.64E+10
44M16P0.25(2)	9/26/2017	6.87E+05	9.13E+05	2.29E+06	2.04E+01	0.00E+00		1.29E+03	7.77E+08	1.80E+10

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
44M16P0.25(2)	9/29/2017	7.61E+05	1.00E+06	1.80E+06		2.04E+02		4.70E+03	3.70E+10	3.70E+10
44M16P1(2)	9/26/2017	6.16E+05	8.83E+05	2.82E+06	0.00E+00	3.59E+03		2.30E+04	2.70E+10	4.52E+10
44M16P1(2)	9/29/2017	7.45E+05	9.24E+05	1.65E+06		8.18E+01		4.62E+03	3.15E+10	3.05E+10
44M16PB0.25	2/15/2014	5.66E+05	5.17E+03	2.86E+02		2.04E+01		2.78E+03	5.39E+10	1.77E+12
44M16PB0.25	3/30/2014	5.68E+05	1.00E+00	1.00E+00		6.13E+01		2.84E+03	3.61E+10	5.20E+10
44M16PB0.25	5/14/2014	8.35E+05	1.61E+03	1.43E+02		6.13E+01		2.06E+03	1.68E+11	1.64E+10
44M16PB1	2/15/2014	5.71E+05	1.24E+03	1.00E+00		1.00E+00		2.45E+02	2.43E+09	1.82E+12
44M16PB1	3/30/2014	6.09E+05	1.00E+00	1.00E+00		1.00E+00		8.18E+01	2.60E+04	1.27E+11
44M16PB1	4/19/2014	8.25E+05	6.13E+01	2.25E+02		2.04E+01		1.23E+02	2.58E+06	5.06E+11
44M16PB1	5/14/2014	7.52E+05	1.84E+02	1.00E+00		2.04E+01		1.25E+03	2.21E+10	8.75E+09
44M16PB1	8/23/2014	7.32E+05	1.00E+00	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
44M16PB1G	4/19/2014	8.78E+05	1.00E+00	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
44M16PB4	5/20/2014	4.86E+05	1.00E+00	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
44M16PB4	7/9/2014	6.09E+05	2.04E+01	1.00E+00		1.00E+00		1.84E+02	2.10E+05	1.12E+11
44M16PB4	8/23/2014	4.96E+05	1.00E+00	1.00E+00		1.00E+00		1.00E+00	6.87E+04	1.51E+10
44M4	1/26/2014	8.06E+05	6.80E+05	1.97E+06		5.56E+05		1.16E+09	2.71E+10	1.89E+10
44M4	5/14/2014	1.14E+06	1.34E+06	4.97E+06		3.66E+09		9.15E+10	1.21E+11	2.26E+10
44M4(2)P0.25	9/6/2017	4.31E+05	4.11E+03	8.38E+02	3.88E+02	4.09E+01		3.68E+02	6.56E+10	1.98E+10
44M4(2)P0.25	9/29/2017	7.36E+05	1.10E+03	4.50E+02		1.02E+02		4.09E+02	1.28E+10	2.93E+10
44M4(2)P1	9/6/2017	4.00E+05	1.14E+03	2.66E+02	0.00E+00	2.04E+01		1.23E+02	1.33E+09	5.32E+10
44M4(2)P1	9/29/2017	7.32E+05	5.72E+02	1.23E+02		4.09E+01		3.68E+02	1.20E+10	4.55E+10
44M4P0.25(2)	9/26/2017	7.26E+05	1.08E+06	3.61E+06	3.40E+02	2.43E+03		7.61E+05	2.50E+10	9.04E+09
44M4P0.25(2)	9/29/2017	6.15E+05	9.64E+05	2.22E+06		2.66E+02		5.81E+03	7.20E+10	5.81E+10
44M4P1(2)	9/26/2017	8.36E+05	9.41E+05	4.04E+06	0.00E+00	3.27E+03			3.53E+10	3.12E+10
44M4P1(2)	9/29/2017	6.73E+05	9.65E+05	2.25E+06		2.25E+02		2.25E+02	1.78E+05	1.02E+11
44M4PB0.25	2/15/2014	5.00E+05	2.58E+03	3.54E+02		6.80E+00		7.21E+02	4.19E+10	8.02E+11

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
44M4PB0.25	3/30/2014	4.47E+05	1.00E+00	1.00E+00		1.02E+02		1.80E+03	4.20E+10	6.52E+08
44M4PB0.25	5/14/2014	9.67E+05	3.31E+03	4.50E+02		4.09E+01		4.68E+03	2.23E+11	2.62E+10
44M4PB1	2/15/2014	5.44E+05	6.53E+03	6.13E+01		1.00E+00		6.80E+00	1.96E+04	2.76E+12
44M4PB1	3/30/2014	4.26E+05	1.00E+00	1.00E+00		2.04E+01		1.00E+00	1.12E+04	1.28E+11
44M4PB1	4/19/2014	1.00E+06	1.02E+02	4.09E+01		2.04E+02		4.91E+02	1.35E+10	8.07E+11
44M4PB1	5/14/2014	7.80E+05	4.50E+02	1.64E+02		2.25E+02		1.12E+03	8.83E+10	2.98E+10
44M4PB1	8/23/2014	8.30E+05	4.09E+01	1.00E+00		1.00E+00		1.00E+00	3.70E+07	1.89E+10
44M4PB1G	4/19/2014	8.23E+05	2.04E+01	1.00E+00		1.43E+02		5.11E+02	1.13E+10	8.18E+06
44M4PB4	5/20/2014	5.32E+05	1.00E+00	1.00E+00		1.00E+00		1.00E+00	4.09E+01	1.00E+00
44M4PB4	7/9/2014	8.20E+05	1.00E+00	1.00E+00		1.00E+00		1.02E+02	2.68E+05	1.27E+11
44M4PB4	8/23/2014	9.69E+05	4.09E+01	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
44M64	1/26/2014	7.36E+05	9.32E+05	6.86E+05		1.88E+05		3.63E+05	5.80E+08	2.06E+10
44M64	5/14/2014	1.11E+06	1.38E+06	3.05E+05		4.01E+05		6.40E+05	5.77E+10	1.01E+10
44M64	2/13/2017	8.75E+05		1.86E+05		1.27E+05	1.25E+05	2.06E+05	9.93E+10	
44M64 on M64	2/13/2017	1.66E+04		1.72E+04		1.25E+04	1.54E+04	4.70E+04	5.35E+09	
44M64(2)P0.25	9/6/2017	4.20E+05	2.17E+03	3.88E+02	1.02E+02	6.13E+01		2.04E+02	7.54E+09	1.21E+11
44M64(2)P0.25	9/29/2017	6.58E+05	1.61E+03	4.29E+02		1.43E+02		0.00E+00	6.06E+03	9.83E+10
44M64(2)P0.25	2/26/2018	6.30E+05	2.47E+03	6.54E+02		2.04E+02		6.13E+01	0.00E+00	0.00E+00
44M64(2)P1	9/6/2017	5.84E+05	9.61E+02	2.04E+02	2.04E+01	2.04E+01		0.00E+00	0.00E+00	0.00E+00
44M64(2)P1	9/29/2017	7.01E+05	7.77E+02	2.45E+02		2.04E+01		2.04E+01	6.13E+02	1.44E+04
44M64(2)P1	2/26/2018	4.62E+05	8.18E+02	1.64E+02		2.04E+01		6.13E+01	5.63E+04	1.07E+10
44M64F on M64	2/13/2017	TNTC								
44M64P0.25(2)	9/26/2017	8.71E+05	1.02E+06	2.10E+05	2.04E+01	2.04E+01		2.04E+01	0.00E+00	0.00E+00
44M64P0.25(2)	9/29/2017	8.27E+05	8.12E+05	7.56E+04		1.23E+02		2.04E+01	0.00E+00	0.00E+00
44M64P1	2/26/2018	4.86E+05	1.02E+03	2.66E+02		1.02E+02		6.13E+01	0.00E+00	0.00E+00

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
44M64P1	2/26/2018	4.68E+05	2.45E+02	0.00E+00		2.04E+01		2.04E+01	1.61E+03	6.68E+04
44M64P1(2)	9/26/2017	1.01E+06	1.15E+06	2.66E+05	0.00E+00	2.04E+01		0.00E+00	1.00E+02	0.00E+00
44M64P1(2)	9/29/2017	8.04E+05	3.43E+05	6.54E+04		4.09E+01		4.09E+01	0.00E+00	0.00E+00
44M64PB0.25	2/15/2014	5.40E+05	4.30E+03	4.29E+02		6.80E+00		4.91E+02	2.10E+10	1.78E+12
44M64PB0.25	3/30/2014	5.23E+05	1.00E+00	1.00E+00		1.00E+00		1.23E+02	3.92E+09	1.00E+10
44M64PB0.25	5/14/2014	6.99E+05	1.84E+03	2.86E+02		1.00E+00		1.23E+02	8.58E+09	4.70E+10
44M64PB1	2/15/2014	5.14E+05	9.53E+01	1.00E+00		6.80E+00		3.40E+01	5.07E+09	1.80E+12
44M64PB1	3/30/2014	7.37E+04	1.00E+00	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
44M64PB1	4/19/2014	9.28E+05	6.13E+01	1.00E+00		1.00E+00		1.00E+00	2.25E+02	2.69E+05
44M64PB1	5/14/2014	8.81E+05	1.00E+00	1.00E+00		1.00E+00		1.00E+00	8.97E+04	2.06E+09
44M64PB1	8/23/2014	5.57E+05	2.04E+01	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
44M64PB1G	4/19/2014	7.32E+05	1.00E+00	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
44M64PB4	5/20/2014	5.69E+05	1.00E+00	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
44M64PB4	7/9/2014	6.45E+05	1.00E+00	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
44PB0.06	6/10/2014	6.04E+05	6.95E+04	4.70E+04		1.42E+06		6.21E+10	4.91E+10	2.06E+10
44PB0.06	6/18/2014	5.29E+05	7.15E+02	3.19E+03		2.18E+04		8.18E+05	9.18E+10	4.28E+10
44PB0.125	6/18/2014	5.42E+05	1.64E+02	2.86E+02		1.76E+03		6.73E+04	1.61E+11	4.92E+10
44PB0.125	7/9/2014	7.16E+05	2.00E+03	1.68E+04		2.82E+03		5.52E+04	7.37E+10	1.41E+11
44PB0.25	1/26/2014	7.26E+05	1.29E+04	1.08E+03		2.66E+02		4.78E+06	4.45E+08	4.06E+09
44PB0.25	3/30/2014	2.68E+05	1.00E+00	1.00E+00		1.00E+00		1.00E+03	4.30E+10	2.43E+10
44PB0.25	4/19/2014	8.53E+05	5.72E+02	1.23E+02		1.23E+02		9.81E+02	1.05E+10	9.97E+11
44PB0.25	5/14/2014	8.93E+05	1.74E+03	6.74E+02		1.64E+02		2.76E+03	1.76E+11	3.44E+10
44PB0.25G	4/19/2014	8.71E+05	3.47E+02	1.84E+02		1.84E+02		2.68E+03	8.10E+09	3.27E+09
44PB1	1/26/2014	7.00E+05	8.86E+03	1.00E+03		1.02E+02		1.99E+06	4.94E+08	4.37E+09
44PB1	3/30/2014	4.48E+05	1.00E+00	1.00E+00		4.09E+01		5.11E+02	8.29E+09	8.94E+10
44PB1	4/19/2014	8.13E+05	4.09E+01	2.04E+01		2.25E+02		7.15E+02	8.23E+09	3.09E+11

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
44PB1	5/14/2014	9.82E+05	7.15E+02	1.84E+02		1.84E+02		2.55E+03	1.11E+11	1.67E+10
44PB1G	4/19/2014	8.80E+05	8.18E+01	1.00E+00		1.00E+00		1.00E+00	2.74E+03	5.29E+10
44PB2	5/29/2014	6.47E+05	8.18E+01	1.00E+00		1.00E+00		1.64E+02	2.27E+11	3.74E+10
44PB2	6/10/2014	6.18E+05	8.18E+01	2.04E+02		1.00E+00		1.00E+00	1.21E+06	1.27E+10
44PB2	8/23/2014	4.75E+05	1.00E+00	1.00E+00		1.00E+00		1.00E+00	5.38E+04	2.98E+09
44PB4	5/20/2014	5.56E+05	1.00E+00	1.00E+00		1.00E+00		1.00E+00	1.00E+00	1.00E+00
44PB4	5/29/2014	6.53E+05	4.09E+01	1.00E+00		1.00E+00		5.72E+02	2.30E+10	3.77E+10
44PB4	6/10/2014	5.87E+05	2.04E+01	2.04E+01		1.00E+00		2.04E+01	6.52E+05	4.00E+10
44PB4	7/9/2014	7.75E+05	2.86E+02	1.00E+00		2.04E+01		1.02E+02	2.13E+07	3.17E+07
44PB4	8/23/2014	1.10E+06	1.00E+00	1.00E+00		1.00E+00		1.00E+00	2.68E+07	5.87E+10
44PB4	2/13/2017	9.48E+05	2.66E+02	6.13E+01		0.00E+00		9.81E+02	7.44E+10	
44PB4 on PB4	2/13/2017	0.00E+00	0.00E+00	0.00E+00		6.13E+01		6.54E+02	1.31E+10	
44PB4F on PB4	2/13/2017	9.20E+02	7.10E+01	8.20E+01		2.10E+02		1.12E+03		
44PB8	2/13/2017	1.02E+06	4.09E+01	0.00E+00		1.02E+02		2.04E+02	1.97E+10	
44PB8 on PB4	2/13/2017	0.00E+00	0.00E+00	0.00E+00		0.00E+00		2.04E+02	8.36E+09	
44PB8F on PB4	2/13/2017	TNTC	5.90E+01	8.90E+01		1.47E+02		1.71E+03		
45A8	5/26/2017	6.53E+05	2.70E+05	4.07E+03		2.66E+02		0.00E+00	6.79E+06	1.94E+09
45A8M16	5/26/2017	4.99E+05	1.04E+05	2.15E+03		2.04E+02		2.00E+01	0.00E+00	0.00E+00
45A8M4	5/26/2017	6.29E+05	7.13E+04	1.86E+03		1.02E+02		0.00E+00	0.00E+00	0.00E+00
45A8M64	5/26/2017	9.20E+05	1.20E+04	2.40E+02		4.09E+01		0.00E+00	0.00E+00	0.00E+00
123A8	10/18/2016	1.23E+06		2.19E+03		1.36E+04		7.45E+05	2.89E+10	1.65E+11
123GC	10/18/2016	1.23E+06		1.61E+07		7.82E+09		1.81E+11	1.22E+11	2.70E+11
123M16	10/18/2016	1.43E+06		1.91E+06		5.11E+08		1.37E+11	1.18E+11	1.37E+11
123M16A8	10/18/2016	1.68E+06		7.56E+02		0.00E+00		0.00E+00	0.00E+00	0.00E+00
123M16PB1	10/18/2016	1.41E+06		2.54E+05		1.49E+03		6.48E+06	7.81E+10	1.44E+11
123PB1	10/18/2016	1.43E+06		1.21E+05		7.36E+08		3.36E+10	1.25E+11	9.39E+10

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
126A8	10/18/2016	1.78E+06		5.48E+04		1.84E+03		8.65E+05	1.20E+11	4.18E+10
126GC	10/18/2016	1.84E+06		7.68E+06		3.63E+10		2.11E+11	1.06E+11	1.24E+11
126M16	10/18/2016	1.90E+06		4.71E+04		1.82E+03		1.57E+05	1.00E+11	1.35E+11
126M16A8	10/18/2016	1.23E+06		3.74E+03		0.00E+00		0.00E+00	0.00E+00	0.00E+00
126M16PB1	10/18/2016	1.96E+06		2.35E+03		4.91E+02		8.79E+02	1.19E+11	1.08E+11
126PB1	10/18/2016	1.78E+06		3.15E+06		6.24E+08		1.20E+11	1.99E+11	9.75E+10
169A16	2/13/2018	6.76E+05	1.13E+06	3.67E+06		4.31E+09		1.11E+11	1.08E+10	5.06E+09
169A16	2/26/2018	7.02E+05	3.96E+04	3.11E+03		1.25E+03		1.43E+02	1.34E+04	5.43E+10
169A16	2/26/2018	5.43E+05	4.64E+04	2.00E+03		1.17E+03		1.23E+02	4.09E+02	1.98E+06
169A16	3/13/2018	8.11E+05	8.37E+04	2.39E+03		9.20E+02		1.02E+02	4.09E+01	0.00E+00
169A16M16	2/13/2018	6.72E+05	3.02E+04	3.07E+03		7.97E+02		6.13E+01	0.00E+00	0.00E+00
169A16M16	2/26/2018	4.62E+05	3.53E+04	2.58E+03		5.52E+02		0.00E+00	0.00E+00	0.00E+00
169A16M4	2/13/2018	5.93E+05	4.25E+04	4.84E+03		1.27E+03		8.18E+01	0.00E+00	0.00E+00
169A16M4	2/26/2018	4.47E+05	4.31E+04	3.15E+03		7.77E+02		6.13E+01	0.00E+00	0.00E+00
169A8	10/18/2016	8.99E+05		9.10E+04		4.09E+04		8.46E+04	2.53E+11	1.20E+11
169A8	2/13/2018	7.23E+05	2.43E+05	3.19E+04		1.14E+04		1.92E+04	1.27E+11	2.49E+09
169A8	2/26/2018	4.65E+06	1.66E+05	5.52E+04		1.24E+04		4.29E+03	1.21E+11	5.73E+09
169A8M16	2/13/2018	6.17E+05	9.04E+04	1.88E+04		3.27E+03		3.88E+02	0.00E+00	0.00E+00
169A8M16	2/26/2018	4.61E+05	1.18E+05	5.93E+04		1.22E+04		1.67E+04	7.64E+10	1.12E+10
169A8M16	3/13/2018	7.41E+05	2.29E+05	6.74E+04		2.86E+03		8.18E+01	0.00E+00	0.00E+00
169A8M4	10/18/2016	1.17E+06		1.41E+05		7.82E+03		7.56E+02	0.00E+00	0.00E+00
169A8M4	2/13/2018	6.19E+05	2.11E+05	4.49E+04		8.89E+03		8.18E+02	0.00E+00	0.00E+00
169A8M4	2/26/2018	4.60E+05	1.72E+05	4.02E+04		1.22E+04		1.52E+07	4.09E+01	1.76E+05
169A8M4	3/13/2018	7.47E+05	3.90E+05	1.23E+05		8.34E+03		4.70E+02	2.04E+01	0.00E+00
169GC	10/18/2016	1.41E+06		1.23E+07		7.02E+10		6.82E+10	9.58E+10	1.46E+11
169GC	2/13/2018	8.77E+05	1.15E+06	3.63E+06		5.24E+09		2.19E+11	5.98E+10	7.55E+09

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
169GC	2/26/2018	5.49E+05	6.08E+05	1.04E+07		3.10E+08		1.31E+11	3.13E+10	3.19E+09
169M16	2/13/2018	6.38E+05	1.45E+05	5.62E+03		1.37E+03		1.43E+02	1.29E+10	2.99E+10
169M16	2/26/2018	4.83E+05	1.15E+05	2.92E+03		1.23E+03		2.66E+02	0.00E+00	0.00E+00
169M16	3/13/2018	7.56E+05	1.98E+05	2.19E+03		5.52E+02		1.23E+02	0.00E+00	0.00E+00
169M4	10/18/2016	1.31E+06		1.19E+05		4.59E+04		1.89E+10	1.16E+11	1.49E+11
169M4	2/13/2018	1.49E+06	1.29E+06	2.34E+04		2.44E+04		7.38E+07	2.09E+10	1.69E+10
169M4	2/26/2018	5.32E+05	5.81E+05	5.03E+03		2.98E+03		3.94E+06	1.48E+10	9.11E+09
169M4PB1	10/18/2016	1.37E+06		1.64E+02		0.00E+00		0.00E+00	0.00E+00	0.00E+00
169PB1	10/18/2016	1.53E+06		2.04E+01		0.00E+00		6.74E+02	2.12E+11	8.07E+10
256A8	10/18/2016	9.82E+05		1.25E+04		8.18E+01		0.00E+00	0.00E+00	0.00E+00
256GC	10/18/2016	1.70E+06		1.51E+07		5.93E+10		2.13E+11	1.23E+11	1.44E+11
256M16	10/18/2016	1.82E+06		2.06E+04		7.97E+02		1.23E+02	1.95E+11	1.34E+11
256M16A8	10/18/2016	1.17E+06		1.76E+03		2.04E+01		0.00E+00	0.00E+00	0.00E+00
256M16PB1	10/18/2016	1.70E+06		2.04E+01		0.00E+00		2.04E+01	1.92E+03	1.55E+11
256PB1	10/18/2016	1.05E+06		2.17E+03		2.60E+04		7.23E+06	2.34E+11	1.21E+11
266A8	10/18/2016	1.41E+06		5.00E+04		5.31E+04		6.10E+05	2.07E+11	2.09E+11
266GC	10/18/2016	1.57E+06		1.68E+07		1.80E+11		1.12E+11	1.84E+11	2.22E+11
266GC	9/6/2017	6.98E+05	1.26E+06	5.59E+06	1.24E+08	1.21E+09		8.05E+10	1.56E+11	3.05E+10
266GC	9/26/2017	1.69E+06	2.68E+06	2.26E+07	1.59E+09	3.29E+10		1.13E+11	6.82E+10	3.14E+10
266M16	9/6/2017	9.41E+05	1.62E+05	5.93E+02	2.66E+02	0.00E+00		0.00E+00	0.00E+00	0.00E+00
266M16	9/26/2017	1.37E+06	2.10E+05	6.00E+02	8.18E+01	6.14E+01		4.09E+01	2.56E+01	1.02E+01
266M16(2)P0.25	9/6/2017	1.14E+06	2.94E+03	2.86E+02	6.13E+01	4.09E+01		0.00E+00	0.00E+00	0.00E+00
266M16(2)P1	9/6/2017	1.03E+06	1.78E+03	4.09E+02	8.18E+01	2.04E+01		1.02E+02	0.00E+00	0.00E+00
266M16P0.25	9/26/2017	1.53E+06	3.20E+02	3.92E+01	6.13E+01	8.18E+01		0.00E+00	0.00E+00	0.00E+00
266M16P0.25(2)	9/26/2017	1.66E+06	1.68E+05	3.00E+02	0.00E+00	0.00E+00		4.09E+01	0.00E+00	0.00E+00
266M16P1	9/26/2017	1.56E+06	8.00E+01	0.00E+00	1.02E+02	6.13E+01		2.04E+01	0.00E+00	0.00E+00

Table E.1: Time-kill Data for Carbapenem-resistant *Enterobacteriaceae*

Time-kill Flask	Date	Time (h)								
		0	1	2	3	4	6	8	24	48
266M16P1(2)	9/26/2017	1.51E+06	1.64E+05	1.40E+02	0.00E+00	0.00E+00		6.13E+01	0.00E+00	0.00E+00
266M4	10/18/2016	1.25E+06		2.49E+03		9.81E+02		3.63E+06	3.33E+11	2.39E+11
266M4	9/6/2017	9.84E+05	7.05E+05	1.61E+03	7.77E+02	1.00E+03		2.27E+05	2.96E+11	4.73E+10
266M4	9/26/2017	1.69E+06	1.26E+06	2.00E+03	1.93E+03	1.82E+03		1.10E+07	2.40E+10	7.16E+10
266M4(2)P0.25	9/6/2017	8.86E+05	9.40E+03	2.04E+02	4.09E+01	6.34E+02		6.59E+03	2.17E+11	5.01E+09
266M4(2)P1	9/6/2017	8.13E+05	1.88E+03	2.86E+02	1.02E+02	4.09E+01		4.09E+01	6.37E+10	1.01E+10
266M4A8	10/18/2016	1.55E+06		4.42E+04		5.17E+03		2.45E+02	0.00E+00	0.00E+00
266M4P0.25	9/26/2017	1.37E+06	3.60E+02	8.00E+01	2.04E+01	0.00E+00		0.00E+00	4.09E+01	0.00E+00
266M4P0.25(2)	9/26/2017	1.53E+06	1.05E+06	3.28E+03	0.00E+00	0.00E+00		0.00E+00	4.09E+01	2.04E+01
266M4P1	9/26/2017	1.70E+06	1.97E+03	4.40E+02	0.00E+00	0.00E+00		0.00E+00	6.13E+01	0.00E+00
266M4P1(2)	9/26/2017	1.63E+06	1.20E+06	3.96E+03	0.00E+00	2.04E+01		2.04E+01	0.00E+00	0.00E+00
266M4PB1	10/18/2016	9.40E+05		2.74E+03		1.02E+02		0.00E+00	0.00E+00	0.00E+00
266P0.25	9/6/2017	9.95E+05	1.17E+03	4.91E+02	1.02E+02	1.43E+03		1.20E+04	6.17E+10	3.74E+10
266P0.25	9/26/2017	1.51E+06	1.50E+03	1.52E+03	3.51E+03	3.82E+03		1.68E+05	9.47E+10	8.22E+10
266P1	9/6/2017	1.07E+06	1.61E+03	2.04E+02	2.25E+02	4.29E+02		8.83E+04	1.06E+11	2.83E+10
266P1	9/26/2017	1.56E+06	1.56E+03	1.74E+03	2.74E+03	3.27E+03		3.56E+04	3.65E+10	1.00E+11
266PB1	10/18/2016	6.95E+05		1.22E+04		7.97E+04		4.09E+04	9.40E+10	1.44E+11
369A8	10/18/2016	9.81E+05		7.56E+02		8.18E+01		0.00E+00	2.04E+01	0.00E+00
369GC	10/18/2016	1.57E+06		5.07E+06		6.98E+10		2.91E+11	1.88E+11	1.95E+11
369M4	10/18/2016	2.06E+06		9.23E+04		1.67E+04		4.79E+03	3.07E+02	2.04E+03
369M4A8	10/18/2016	7.36E+05		5.31E+02		0.00E+00		0.00E+00	0.00E+00	0.00E+00
369M4PB1	10/18/2016	1.98E+06		4.57E+04		1.57E+04		2.11E+03	6.13E+01	2.04E+01
369PB1	10/18/2016	1.47E+06		5.50E+05		3.39E+05		4.66E+10	1.57E+11	1.20E+11

Appendix F:

Model Input Data for Mathematical Models

Tables F.1-F.22 contain the model input data extracted from Appendix E for each model fit.

Table F.1: Isolate 34 Growth Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	2.63E+06	6.42	14.78	1	0	1/26/2014
2	5.07E+08	8.71	20.04	1		
22	4.43E+10	10.65	24.51	1		
46	4.43E+11	11.65	26.82	1		
0	4.44E+06	6.65	15.31	2	0	2/15/2014
2	1.36E+10	10.13	23.33	2		
6	3.42E+10	10.53	24.26	2		
22	4.12E+11	11.61	26.74	2		
46	3.93E+12	12.59	29.00	2		
0	1.13E+07	7.05	16.24	3	0	3/30/2014
2	3.25E+10	10.51	24.20	3		
6	1.06E+11	11.03	25.39	3		
22	5.43E+11	11.73	27.02	3		
46	9.31E+10	10.97	25.26	3		
0	1.14E+07	7.06	16.25	4	0	6/11/2015
1	2.85E+08	8.45	19.47	4		
3	5.11E+10	10.71	24.66	4		
21	5.86E+11	11.77	27.10	4		
0	3.71E+02	2.57	5.92	5	0	6/11/2015
1	5.09E+03	3.71	8.54	5		
3	2.98E+05	5.47	12.60	5		
21	2.98E+05	5.47	12.60	5		
0	1.42E+02	2.15	4.96	6	0	6/11/2015
1	1.08E+03	3.03	6.98	6		
3	2.02E+03	3.31	7.61	6		
21	1.74E+04	4.24	9.76	6		

Table F.2: Isolate 34 Meropenem Initial Killing Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	2.63E+06	6.42	14.78	1		1/26/2014
2	5.07E+08	8.71	20.04	1		
22	4.43E+10	10.65	24.51	1		
46	4.43E+11	11.65	26.82	1		
0	4.44E+06	6.65	15.31	2		2/15/2014
2	1.36E+10	10.13	23.33	2		
6	3.42E+10	10.53	24.26	2		
22	4.12E+11	11.61	26.74	2		
46	3.93E+12	12.59	29.00	2		
0	1.13E+07	7.05	16.24	3		3/30/2014
2	3.25E+10	10.51	24.20	3		
6	1.06E+11	11.03	25.39	3		
22	5.43E+11	11.73	27.02	3		
46	9.31E+10	10.97	25.26	3		
0	1.14E+07	7.06	16.25	4		6/11/2015
1	2.85E+08	8.45	19.47	4		
3	5.11E+10	10.71	24.66	4		
21	5.86E+11	11.77	27.10	4		
0	8.06E+05	5.91	13.60	5	4	1/26/2014
1	5.94E+05	5.77	13.29	5		
2	2.26E+04	4.35	10.03	5		
4	1.21E+03	3.08	7.10	5		
0	7.48E+05	5.87	13.53	6	16	1/26/2014
1	8.31E+04	4.92	11.33	6		
2	2.19E+03	3.34	7.69	6		
0	6.54E+05	5.82	13.39	7	64	1/26/2014
1	3.39E+04	4.53	10.43	7		
2	3.47E+02	2.54	5.85	7		
0	1.04E+06	6.02	13.85	8	4	7/9/2014
1	5.86E+05	5.77	13.28	8		
2	8.73E+03	3.94	9.07	8		
4	9.61E+02	2.98	6.87	8		
0	4.85E+05	5.69	13.09	9	16	7/9/2014
1	5.25E+04	4.72	10.87	9		
0	7.54E+05	5.88	13.53	10	64	7/9/2014
1	2.98E+04	4.47	10.30	10		
2	2.25E+02	2.35	5.42	10		
0	7.59E+05	5.88	13.54	11	8	2/13/2017
2	2.08E+03	3.32	7.64	11		
0	1.14E+06	6.06	13.95	12	16	2/13/2017

Table F.2: Isolate 34 Meropenem Initial Killing Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
2	1.27E+03	3.10	7.15	12		

Table F.3: Isolate 34 Polymyxin B Initial Killing Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	2.63E+06	6.42	14.78	1	0	1/26/2014
22	4.43E+10	10.65	24.51	1		
46	4.43E+11	11.65	26.82	1		
0	4.44E+06	6.65	15.31	2	0	2/15/2014
2	1.36E+10	10.13	23.33	2		
6	3.42E+10	10.53	24.26	2		
22	4.12E+11	11.61	26.74	2		
46	3.93E+12	12.59	29.00	2		
0	1.13E+07	7.05	16.24	3	0	3/30/2014
2	3.25E+10	10.51	24.20	3		
6	1.06E+11	11.03	25.39	3		
22	5.43E+11	11.73	27.02	3		
46	9.31E+10	10.97	25.26	3		
0	1.14E+07	7.06	16.25	4	0	6/11/2015
1	2.85E+08	8.45	19.47	4		
3	5.11E+10	10.71	24.66	4		
21	5.86E+11	11.77	27.10	4		
0	7.07E+05	5.85	13.47	5	0.25	1/24/2014
1	2.52E+04	4.40	10.13	5		
2	6.74E+02	2.83	6.51	5		
0	6.54E+05	5.82	13.39	6	1	1/24/2014
1	7.07E+03	3.85	8.86	6		
2	2.86E+02	2.46	5.66	6		
0	7.23E+05	5.86	13.49	7	0.25	3/30/2015
1	3.27E+02	2.51	5.79	7		
0	8.00E+05	5.90	13.59	8	2	5/29/2014
1	6.13E+02	2.79	6.42	8		
0	7.79E+05	5.89	13.57	9	4	5/29/2014
1	1.64E+02	2.21	5.10	9		
0	6.92E+05	5.84	13.45	10	2	6/10/2014
1	1.00E+03	3.00	6.91	10		
0	6.85E+05	5.84	13.44	11	4	6/10/2014
1	7.97E+02	2.90	6.68	11		
0	5.01E+05	5.70	13.12	12	0.06	6/18/2014
1	6.26E+04	4.80	11.04	12		
2	6.12E+03	3.79	8.72	12		
0	5.15E+05	5.71	13.15	13	0.125	6/18/2014
1	2.15E+04	4.33	9.98	13		
2	6.54E+02	2.82	6.48	13		
0	7.87E+05	5.90	13.58	14	0.06	7/9/2014

Table F.3: Isolate 34 Polymyxin B Initial Killing Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
1	1.36E+05	5.13	11.82	14		
2	6.44E+04	4.81	11.07	14		
0	9.30E+05	5.97	13.74	15	0.125	7/9/2014
1	4.70E+04	4.67	10.76	15		
2	4.24E+03	3.63	8.35	15		
0	8.95E+05	5.95	13.70	16	0.25	8/23/2014
1	3.47E+03	3.54	8.15	16		
2	6.34E+02	2.80	6.45	16		
0	8.43E+05	5.93	13.64	17	1	8/23/2014
1	2.21E+03	3.34	7.70	17		
2	1.64E+02	2.21	5.10	17		

Table F.4: Isolate 34 Meropenem Monotherapy Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	2.63E+06	6.42	14.78	1	0	1/26/2014
2	5.07E+08	8.71	20.04	1		
22	4.43E+10	10.65	24.51	1		
46	4.43E+11	11.65	26.82	1		
0	4.44E+06	6.65	15.31	2	0	2/15/2014
2	1.36E+10	10.13	23.33	2		
6	3.42E+10	10.53	24.26	2		
22	4.12E+11	11.61	26.74	2		
46	3.93E+12	12.59	29.00	2		
0	1.13E+07	7.05	16.24	3	0	3/30/2014
2	3.25E+10	10.51	24.20	3		
6	1.06E+11	11.03	25.39	3		
22	5.43E+11	11.73	27.02	3		
46	9.31E+10	10.97	25.26	3		
0	1.14E+07	7.06	16.25	4	0	6/11/2015
1	2.85E+08	8.45	19.47	4		
3	5.11E+10	10.71	24.66	4		
21	5.86E+11	11.77	27.10	4		
0	3.71E+02	2.57	5.92	5	0	6/11/2015
1	5.09E+03	3.71	8.54	5		
3	2.98E+05	5.47	12.60	5		
0	8.06E+05	5.91	13.60	6	4	1/26/2014
1	5.94E+05	5.77	13.29	6		
2	2.26E+04	4.35	10.03	6		
4	1.21E+03	3.08	7.10	6		
8	5.74E+03	3.76	8.65	6		
24	1.06E+09	9.03	20.78	6		
48	1.71E+10	10.23	23.56	6		
0	7.48E+05	5.87	13.53	7	16	1/26/2014
1	8.31E+04	4.92	11.33	7		
2	2.19E+03	3.34	7.69	7		
4	4.29E+02	2.63	6.06	7		
0	6.54E+05	5.82	13.39	8	64	1/26/2014
1	3.39E+04	4.53	10.43	8		
2	3.47E+02	2.54	5.85	8		
0	1.04E+06	6.02	13.85	9	4	7/9/2014
1	5.86E+05	5.77	13.28	9		
2	8.73E+03	3.94	9.07	9		
4	9.61E+02	2.98	6.87	9		
8	5.72E+04	4.76	10.95	9		

Table F.4: Isolate 34 Meropenem Monotherapy Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
24	8.39E+10	10.92	25.15	9		
48	5.76E+10	10.76	24.78	9		
0	4.85E+05	5.69	13.09	10	16	7/9/2014
1	5.25E+04	4.72	10.87	10		
4	1.43E+02	2.16	4.96	10		
0	7.54E+05	5.88	13.53	11	64	7/9/2014
1	2.98E+04	4.47	10.30	11		
2	2.25E+02	2.35	5.42	11		
0	7.59E+05	5.88	13.54	12	8	2/13/2017
2	2.08E+03	3.32	7.64	12		
4	7.77E+02	2.89	6.66	12		
6	3.47E+02	2.54	5.85	12		
24	2.04E+02	2.31	5.32	12		
0	1.14E+06	6.06	13.95	13	16	2/13/2017
2	1.27E+03	3.10	7.15	13		
4	2.25E+02	2.35	5.42	13		
6	2.04E+02	2.31	5.32	13		

Table F.5: Isolate 34 Polymyxin B Monotherapy Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	2.63E+06	6.42	14.78	1	0	1/26/2014
22	4.43E+10	10.65	24.51	1		
46	4.43E+11	11.65	26.82	1		
0	4.44E+06	6.65	15.31	2	0	2/15/2014
2	1.36E+10	10.13	23.33	2		
6	3.42E+10	10.53	24.26	2		
22	4.12E+11	11.61	26.74	2		
46	3.93E+12	12.59	29.00	2		
0	1.13E+07	7.05	16.24	3	0	3/30/2014
2	3.25E+10	10.51	24.20	3		
6	1.06E+11	11.03	25.39	3		
22	5.43E+11	11.73	27.02	3		
46	9.31E+10	10.97	25.26	3		
0	1.14E+07	7.06	16.25	4	0	6/11/2015
1	2.85E+08	8.45	19.47	4		
3	5.11E+10	10.71	24.66	4		
21	5.86E+11	11.77	27.10	4		
0	1.42E+02	2.15	4.96	5	0	6/11/2015
1	1.08E+03	3.03	6.98	5		
3	2.02E+03	3.31	7.61	5		
21	1.74E+04	4.24	9.76	5		
0	7.07E+05	5.85	13.47	6	0.25	1/26/2014
1	2.52E+04	4.40	10.13	6		
2	6.74E+02	2.83	6.51	6		
4	1.92E+03	3.28	7.56	6		
48	4.71E+10	10.67	24.58	6		
0	6.54E+05	5.82	13.39	7	1	1/26/2014
1	7.07E+03	3.85	8.86	7		
2	2.86E+02	2.46	5.66	7		
4	4.91E+02	2.69	6.20	7		
48	1.49E+10	10.17	23.42	7		
0	7.23E+05	5.86	13.49	8	0.25	3/30/2015
1	3.27E+02	2.51	5.79	8		
2	1.43E+02	2.16	4.96	8		
4	5.93E+02	2.77	6.39	8		
8	4.16E+04	4.62	10.64	8		
24	7.16E+10	10.85	24.99	8		
48	7.93E+09	9.90	22.79	8		
0	5.84E+05	5.77	13.28	9	1	3/30/2015
4	2.66E+02	2.42	5.58	9		

Table F.5: Isolate 34 Polymyxin B Monotherapy Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
8	1.56E+04	4.19	9.66	9		
24	1.67E+11	11.22	25.84	9		
48	1.19E+10	10.08	23.20	9		
0	8.00E+05	5.90	13.59	10	2	5/29/2014
1	6.13E+02	2.79	6.42	10		
2	1.02E+02	2.01	4.62	10		
4	8.99E+02	2.95	6.80	10		
8	7.16E+04	4.85	11.18	10		
24	1.29E+11	11.11	25.58	10		
48	5.73E+10	10.76	24.77	10		
0	7.79E+05	5.89	13.57	11	4	5/29/2014
1	1.64E+02	2.21	5.10	11		
4	3.07E+02	2.49	5.73	11		
8	3.09E+04	4.49	10.34	11		
24	5.31E+10	10.73	24.70	11		
48	1.67E+11	11.22	25.84	11		
0	6.92E+05	5.84	13.45	12	2	6/10/2014
1	1.00E+03	3.00	6.91	12		
2	2.45E+02	2.39	5.50	12		
4	1.84E+02	2.26	5.21	12		
8	2.25E+04	4.35	10.02	12		
24	6.13E+10	10.79	24.84	12		
48	1.57E+11	11.20	25.78	12		
0	6.85E+05	5.84	13.44	13	4	6/10/2014
1	7.97E+02	2.90	6.68	13		
4	1.02E+02	2.01	4.62	13		
8	8.36E+03	3.92	9.03	13		
24	9.78E+10	10.99	25.31	13		
48	1.96E+11	11.29	26.00	13		
0	5.01E+05	5.70	13.12	14	0.06	6/18/2014
1	6.26E+04	4.80	11.04	14		
2	6.12E+03	3.79	8.72	14		
4	5.87E+03	3.77	8.68	14		
8	1.81E+06	6.26	14.41	14		
24	9.88E+10	10.99	25.32	14		
48	1.56E+11	11.19	25.77	14		
0	5.15E+05	5.71	13.15	15	0.125	6/18/2014
1	2.15E+04	4.33	9.98	15		
2	6.54E+02	2.82	6.48	15		
4	3.88E+02	2.59	5.96	15		

Table F.5: Isolate 34 Polymyxin B Monotherapy Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
8	4.46E+05	5.65	13.01	15		
24	6.48E+10	10.81	24.89	15		
48	7.37E+10	10.87	25.02	15		
0	7.87E+05	5.90	13.58	16	0.06	7/9/2014
1	1.36E+05	5.13	11.82	16		
2	6.44E+04	4.81	11.07	16		
4	3.79E+04	4.58	10.54	16		
8	1.19E+08	8.08	18.59	16		
24	2.19E+11	11.34	26.11	16		
48	8.18E+10	10.91	25.13	16		
0	9.30E+05	5.97	13.74	17	0.125	7/9/2014
1	4.70E+04	4.67	10.76	17		
2	4.24E+03	3.63	8.35	17		
4	2.76E+03	3.44	7.92	17		
8	1.18E+06	6.07	13.98	17		
24	8.44E+10	10.93	25.16	17		
48	9.84E+10	10.99	25.31	17		
0	8.95E+05	5.95	13.70	18	0.25	8/23/2014
1	3.47E+03	3.54	8.15	18		
2	6.34E+02	2.80	6.45	18		
4	2.92E+03	3.47	7.98	18		
8	4.26E+04	4.63	10.66	18		
24	7.88E+10	10.90	25.09	18		
48	1.71E+10	10.23	23.56	18		
0	8.43E+05	5.93	13.64	19	1	8/23/2014
1	2.21E+03	3.34	7.70	19		
2	1.64E+02	2.21	5.10	19		
4	4.37E+03	3.64	8.38	19		
8	2.00E+04	4.30	9.90	19		
24	8.59E+10	10.93	25.18	19		
48	1.78E+10	10.25	23.60	19		

Table F.6: Isolate 22 Growth Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	3.14E+06	6.50	14.96	1	0	5/29/2014
2	4.21E+09	9.62	22.16	1		
6	1.11E+11	11.05	25.43	1		
22	5.23E+10	10.72	24.68	1		
46	9.93E+10	11.00	25.32	1		
0	2.51E+06	6.40	14.74	2	0	8/23/2014
2	5.15E+09	9.71	22.36	2		
6	1.06E+11	11.03	25.39	2		
22	6.69E+10	10.83	24.93	2		
46	2.45E+09	9.39	21.62	2		
0	6.23E+06	6.79	15.64	3	0	6/11/2015
1	1.35E+08	8.13	18.72	3		
3	3.21E+10	10.51	24.19	3		
21	1.43E+11	11.16	25.69	3		
0	7.93E+02	2.90	6.68	4	0	6/11/2015
1	8.75E+03	3.94	9.08	4		
3	9.69E+04	4.99	11.48	4		
0	2.54E+02	2.40	5.54	5	0	6/11/2015
1	1.58E+03	3.20	7.37	5		
21	9.18E+03	3.96	9.12	5		

Table F.7: Isolate 22 Meropenem Initial Killing Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	3.14E+06	6.50	14.96	1	0	5/29/2014
2	4.21E+09	9.62	22.16	1		
6	1.11E+11	11.05	25.43	1		
22	5.23E+10	10.72	24.68	1		
46	9.93E+10	11.00	25.32	1		
0	2.51E+06	6.40	14.74	2	0	8/23/2014
2	5.15E+09	9.71	22.36	2		
6	1.06E+11	11.03	25.39	2		
22	6.69E+10	10.83	24.93	2		
46	2.45E+09	9.39	21.62	2		
0	6.23E+06	6.79	15.64	3	0	6/11/2015
1	1.35E+08	8.13	18.72	3		
3	3.21E+10	10.51	24.19	3		
21	1.43E+11	11.16	25.69	3		
0	1.70E+06	6.23	14.35	4	4	5/14/2014
1	4.70E+04	4.67	10.76	4		
0	1.26E+06	6.10	14.05	5	16	5/14/2014
1	2.43E+04	4.39	10.10	5		
0	9.34E+05	5.97	13.75	6	64	5/14/2014
1	1.43E+04	4.16	9.57	6		
2	2.86E+02	2.46	5.66	6		
0	6.11E+05	5.79	13.32	7	4	5/20/2014
1	3.78E+03	3.58	8.24	7		
0	3.52E+05	5.55	12.77	8	16	5/20/2014
1	6.70E+03	3.83	8.81	8		
0	5.28E+05	5.72	13.18	9	64	5/20/2014
1	4.19E+03	3.62	8.34	9		
0	7.82E+05	5.89	13.57	10	16	2/13/2017
2	1.22E+04	4.09	9.41	10		
4	1.84E+02	2.26	5.21	10		
0	8.00E+05	5.90	13.59	11	32	2/13/2017
2	1.23E+03	3.09	7.11	11		

Table F.8: Isolate 22 Polymyxin B Initial Killing Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	3.14E+06	6.50	14.96	1	0	5/29/2014
2	4.21E+09	9.62	22.16	1		
6	1.11E+11	11.05	25.43	1		
22	5.23E+10	10.72	24.68	1		
46	9.93E+10	11.00	25.32	1		
0	2.51E+06	6.40	14.74	2	0	8/23/2014
2	5.15E+09	9.71	22.36	2		
6	1.06E+11	11.03	25.39	2		
22	6.69E+10	10.83	24.93	2		
46	2.45E+09	9.39	21.62	2		
0	6.23E+06	6.79	15.64	3	0	6/11/2015
1	1.35E+08	8.13	18.72	3		
3	3.21E+10	10.51	24.19	3		
21	1.43E+11	11.16	25.69	3		
0	1.34E+06	6.13	14.11	4	0.25	5/14/2014
1	1.78E+03	3.25	7.48	4		
0	5.27E+05	5.72	13.17	5	0.06	6/10/2014
1	9.81E+04	4.99	11.49	5		
0	4.56E+05	5.66	13.03	6	0.06	6/18/2014
1	4.48E+03	3.65	8.41	6		
0	4.93E+05	5.69	13.11	7	0.125	6/18/2014
1	3.07E+02	2.49	5.73	7		
0	6.91E+05	5.84	13.45	8	0.125	7/9/2014
1	1.64E+02	2.21	5.10	8		

Table F.9: Isolate 22 Meropenem Monotherapy Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	3.14E+06	6.50	14.96	1	0	5/29/2014
2	4.21E+09	9.62	22.16	1		
6	1.11E+11	11.05	25.43	1		
22	5.23E+10	10.72	24.68	1		
46	9.93E+10	11.00	25.32	1		
0	2.51E+06	6.40	14.74	2	0	8/23/2014
2	5.15E+09	9.71	22.36	2		
6	1.06E+11	11.03	25.39	2		
22	6.69E+10	10.83	24.93	2		
46	2.45E+09	9.39	21.62	2		
0	6.23E+06	6.79	15.64	3	0	6/11/2015
1	1.35E+08	8.13	18.72	3		
3	3.21E+10	10.51	24.19	3		
21	1.43E+11	11.16	25.69	3		
0	7.93E+02	2.90	6.68	4	0	6/11/2015
1	8.75E+03	3.94	9.08	4		
3	9.69E+04	4.99	11.48	4		
0	1.70E+06	6.23	14.35	5	4	5/14/2014
1	4.70E+04	4.67	10.76	5		
3	1.68E+03	3.23	7.43	5		
7	1.37E+05	5.14	11.83	5		
23	1.19E+11	11.08	25.50	5		
47	7.98E+10	10.90	25.10	5		
0	1.26E+06	6.10	14.05	6	16	5/14/2014
1	2.43E+04	4.39	10.10	6		
3	2.02E+03	3.31	7.61	6		
7	4.09E+04	4.61	10.62	6		
23	9.27E+10	10.97	25.25	6		
47	4.13E+09	9.62	22.14	6		
0	9.34E+05	5.97	13.75	7	64	5/14/2014
1	1.43E+04	4.16	9.57	7		
2	2.86E+02	2.46	5.66	7		
0	6.11E+05	5.79	13.32	8	4	5/20/2014
1	3.78E+03	3.58	8.24	8		
3	2.86E+02	2.46	5.66	8		
7	5.23E+04	4.72	10.86	8		
23	1.03E+11	11.01	25.36	8		
47	1.01E+11	11.00	25.34	8		
0	3.52E+05	5.55	12.77	9	16	5/20/2014
1	6.70E+03	3.83	8.81	9		

Table F.9: Isolate 22 Meropenem Monotherapy Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
3	1.02E+02	2.01	4.62	9		
7	2.08E+03	3.32	7.64	9		
23	6.68E+10	10.82	24.92	9		
47	5.12E+10	10.71	24.66	9		
0	5.28E+05	5.72	13.18	10	64	5/20/2014
1	4.19E+03	3.62	8.34	10		
0	7.82E+05	5.89	13.57	11	16	2/13/2017
2	1.22E+04	4.09	9.41	11		
4	1.84E+02	2.26	5.21	11		
6	1.64E+02	2.21	5.10	12		
8	5.00E+03	3.70	8.52	12		
24	2.86E+10	10.46	24.08	12		
4	6.00E+00	0.78	1.79	12	16	2/13/2017
6	1.54E+02	2.19	5.04	12		
8	4.94E+03	3.69	8.51	12	16	2/13/2017
24	3.19E+10	10.50	24.19	12		
0	8.00E+05	5.90	13.59	13	32	2/13/2017
2	1.23E+03	3.09	7.11	13		
4	1.23E+02	2.09	4.81	13		
6	1.43E+02	2.16	4.96	13		

Table F.10: Isolate 22 Polymyxin B Monotherapy Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	3.14E+06	6.50	14.96	1	0	5/29/2014
2	4.21E+09	9.62	22.16	1		
6	1.11E+11	11.05	25.43	1		
22	5.23E+10	10.72	24.68	1		
46	9.93E+10	11.00	25.32	1		
0	2.51E+06	6.40	14.74	2	0	8/23/2014
2	5.15E+09	9.71	22.36	2		
6	1.06E+11	11.03	25.39	2		
22	6.69E+10	10.83	24.93	2		
46	2.45E+09	9.39	21.62	2		
0	6.23E+06	6.79	15.64	3	0	6/11/2015
1	1.35E+08	8.13	18.72	3		
3	3.21E+10	10.51	24.19	3		
21	1.43E+11	11.16	25.69	3		
0	2.54E+02	2.40	5.54	4	0	6/11/2015
1	1.58E+03	3.20	7.37	4		
21	9.18E+03	3.96	9.12	4		
0	1.34E+06	6.13	14.11	5	0.25	5/14/2014
1	1.78E+03	3.25	7.48	5		
8	3.94E+03	3.60	8.28	5		
24	2.21E+11	11.34	26.12	5		
48	2.65E+10	10.42	24.00	5		
0	1.43E+06	6.16	14.17	6	1	5/14/2014
8	2.25E+02	2.35	5.42	6		
24	5.95E+10	10.77	24.81	6		
48	1.04E+11	11.02	25.37	6		
0	5.01E+05	5.70	13.12	7	0.25	5/20/2014
8	2.23E+03	3.35	7.71	7		
24	1.35E+11	11.13	25.63	7		
48	9.15E+10	10.96	25.24	7		
0	5.53E+05	5.74	13.22	8	1	5/20/2014
8	8.18E+02	2.91	6.71	8		
24	1.08E+11	11.03	25.41	8		
48	1.23E+11	11.09	25.54	8		
0	6.68E+05	5.82	13.41	9	2	5/29/2014
8	2.41E+03	3.38	7.79	9		
24	6.37E+10	10.80	24.88	9		
48	1.21E+11	11.08	25.52	9		
0	6.60E+05	5.82	13.40	10	4	5/29/2014
8	2.86E+02	2.46	5.66	10		

Table F.10: Isolate 22 Polymyxin B Monotherapy Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
24	2.11E+10	10.32	23.77	10		
48	1.13E+11	11.05	25.45	10		
0	5.27E+05	5.72	13.17	11	0.06	6/10/2014
1	9.81E+04	4.99	11.49	11		
0	5.64E+05	5.75	13.24	12	2	6/10/2014
8	1.37E+03	3.14	7.22	12		
24	1.29E+11	11.11	25.58	12		
48	8.03E+10	10.90	25.11	12		
0	5.00E+05	5.70	13.12	13	4	6/10/2014
8	5.31E+02	2.73	6.27	13		
24	2.38E+10	10.38	23.89	13		
48	1.52E+11	11.18	25.75	13		
0	4.56E+05	5.66	13.03	14	0.06	6/18/2014
1	4.48E+03	3.65	8.41	14		
0	4.93E+05	5.69	13.11	15	0.125	6/18/2014
1	3.07E+02	2.49	5.73	15		
4	2.25E+02	2.35	5.42	15		
8	3.00E+05	5.48	12.61	15		
24	1.29E+11	11.11	25.58	15		
48	6.66E+10	10.82	24.92	15		
0	6.91E+05	5.84	13.45	16	0.125	7/9/2014
1	1.64E+02	2.21	5.10	16		
2	1.84E+02	2.26	5.21	16		
4	4.50E+02	2.65	6.11	16		
8	8.58E+04	4.93	11.36	16		
24	1.26E+11	11.10	25.56	16		
48	8.75E+10	10.94	25.19	16		

Table F.11: Isolate 24 Growth Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	1.77E+06	6.25	14.39	1	0	5/29/2014
2	6.82E+07	7.83	18.04	1		
6	1.45E+10	10.16	23.40	1		
22	2.82E+10	10.45	24.06	1		
46	2.58E+10	10.41	23.97	1		
0	2.00E+06	6.30	14.51	2	0	8/23/2014
2	8.08E+07	7.91	18.21	2		
6	2.24E+10	10.35	23.83	2		
22	2.60E+10	10.41	23.98	2		
46	3.99E+10	10.60	24.41	2		
0	2.46E+06	6.39	14.72	3	0	6/11/2015
1	8.59E+06	6.93	15.97	3		
2	2.48E+08	8.39	19.33	3		
4	5.24E+10	10.72	24.68	3		
22	3.44E+11	11.54	26.56	3		
0	5.70E+01	1.76	4.04	4	0	6/11/2015
1	5.08E+02	2.71	6.23	4		
2	1.01E+03	3.00	6.92	4		
4	4.98E+03	3.70	8.51	4		
22	3.34E+04	4.52	10.42	4		
2	3.60E+01	1.56	3.58	5	0	6/11/2015
4	2.00E+02	2.30	5.30	5		
22	3.77E+02	2.58	5.93	5		

Table F.12: Isolate 24 Meropenem Initial Killing Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	1.77E+06	6.25	14.39	1	0	5/29/2014
2	6.82E+07	7.83	18.04	1		
6	1.45E+10	10.16	23.40	1		
22	2.82E+10	10.45	24.06	1		
46	2.58E+10	10.41	23.97	1		
0	2.00E+06	6.30	14.51	2	0	8/23/2014
2	8.08E+07	7.91	18.21	2		
6	2.24E+10	10.35	23.83	2		
22	2.60E+10	10.41	23.98	2		
46	3.99E+10	10.60	24.41	2		
0	2.46E+06	6.39	14.72	3	0	6/11/2015
1	8.59E+06	6.93	15.97	3		
2	2.48E+08	8.39	19.33	3		
4	5.24E+10	10.72	24.68	3		
22	3.44E+11	11.54	26.56	3		
0	4.79E+05	5.68	13.08	4	4	5/20/2014
2	1.71E+04	4.23	9.75	4		
0	5.68E+05	5.75	13.25	5	16	5/20/2014
1	1.17E+05	5.07	11.67	5		
3	4.03E+03	3.61	8.30	5		
0	4.36E+05	5.64	12.99	6	64	5/20/2014
1	9.40E+04	4.97	11.45	6		
2	1.05E+04	4.02	9.26	6		
4	2.17E+03	3.34	7.68	6		
0	4.84E+05	5.68	13.09	7	4	6/10/2014
2	1.08E+04	4.03	9.29	7		
0	6.34E+05	5.80	13.36	8	16	6/10/2014
1	1.17E+05	5.07	11.67	8		
3	2.15E+03	3.33	7.67	8		
0	5.40E+05	5.73	13.20	9	64	6/10/2014
1	3.19E+05	5.50	12.67	9		
2	8.73E+03	3.94	9.07	9		
4	8.79E+02	2.94	6.78	9		
0	5.29E+05	5.72	13.18	10	64	2/13/2017
2	1.28E+04	4.11	9.46	10		
4	3.68E+02	2.57	5.91	10		
0	5.31E+05	5.73	13.18	11	128	2/13/2017
2	3.43E+03	3.54	8.14	11		
4	2.45E+02	2.39	5.50	11		

Table F.13: Isolate 24 Polymyxin B Initial Killing Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	1.77E+06	6.25	14.39	1	0	5/29/2014
2	6.82E+07	7.83	18.04	1		
6	1.45E+10	10.16	23.40	1		
22	2.82E+10	10.45	24.06	1		
46	2.58E+10	10.41	23.97	1		
0	2.00E+06	6.30	14.51	2	0	8/23/2014
2	8.08E+07	7.91	18.21	2		
6	2.24E+10	10.35	23.83	2		
22	2.60E+10	10.41	23.98	2		
46	3.99E+10	10.60	24.41	2		
0	2.46E+06	6.39	14.72	3	0	6/11/2015
1	8.59E+06	6.93	15.97	3		
2	2.48E+08	8.39	19.33	3		
4	5.24E+10	10.72	24.68	3		
22	3.44E+11	11.54	26.56	3		
0	4.26E+05	5.63	12.96	4	0.25	5/20/2014
1	9.69E+03	3.99	9.18	4		
0	5.87E+05	5.77	13.28	5	0.0625	6/10/2014
1	1.10E+05	5.04	11.61	5		
0	6.72E+05	5.83	13.42	6	0.25	6/10/2014
1	4.69E+04	4.67	10.76	6		
2	2.04E+02	2.31	5.32	6		
0	5.81E+05	5.76	13.27	7	1	6/10/2014
1	4.01E+03	3.60	8.30	7		
0	3.37E+05	5.53	12.73	8	0.0625	6/18/2014
1	2.78E+04	4.44	10.23	8		
0	7.51E+05	5.88	13.53	9	0.125	7/9/2014
1	3.70E+03	3.57	8.22	9		

Table F.14: Isolate 24 Meropenem Monotherapy Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	1.77E+06	6.25	14.39	1	0	5/29/2014
2	6.82E+07	7.83	18.04	1		
6	1.45E+10	10.16	23.40	1		
22	2.82E+10	10.45	24.06	1		
46	2.58E+10	10.41	23.97	1		
0	2.00E+06	6.30	14.51	2	0	8/23/2014
2	8.08E+07	7.91	18.21	2		
6	2.24E+10	10.35	23.83	2		
22	2.60E+10	10.41	23.98	2		
46	3.99E+10	10.60	24.41	2		
0	2.46E+06	6.39	14.72	3	0	6/11/2015
1	8.59E+06	6.93	15.97	3		
2	2.48E+08	8.39	19.33	3		
4	5.24E+10	10.72	24.68	3		
22	3.44E+11	11.54	26.56	3		
0	5.70E+01	1.76	4.04	4	0	6/11/2015
1	5.08E+02	2.71	6.23	4		
2	1.01E+03	3.00	6.92	4		
4	4.98E+03	3.70	8.51	4		
22	3.34E+04	4.52	10.42	4		
0	4.79E+05	5.68	13.08	5	4	5/20/2014
2	1.71E+04	4.23	9.75	5		
6	2.07E+06	6.32	14.54	5		
22	3.95E+10	10.60	24.40	5		
46	5.48E+10	10.74	24.73	5		
0	5.68E+05	5.75	13.25	6	16	5/20/2014
1	1.17E+05	5.07	11.67	6		
3	4.03E+03	3.61	8.30	6		
7	9.81E+04	4.99	11.49	6		
23	4.13E+10	10.62	24.44	6		
47	1.92E+10	10.28	23.68	6		
0	4.36E+05	5.64	12.99	7	64	5/20/2014
1	9.40E+04	4.97	11.45	7		
2	1.05E+04	4.02	9.26	7		
4	2.17E+03	3.34	7.68	7		
8	1.96E+03	3.29	7.58	7		
24	2.36E+11	11.37	26.19	7		
48	8.95E+10	10.95	25.22	7		
0	4.84E+05	5.68	13.09	8	4	6/10/2014
2	1.08E+04	4.03	9.29	8		

Table F.14: Isolate 24 Meropenem Monotherapy Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
6	1.37E+03	3.14	7.22	8		
22	2.79E+10	10.45	24.05	8		
46	1.18E+11	11.07	25.49	8		
0	6.34E+05	5.80	13.36	9	16	6/10/2014
1	1.17E+05	5.07	11.67	9		
3	2.15E+03	3.33	7.67	9		
7	6.18E+03	3.79	8.73	9		
23	3.10E+10	10.49	24.16	9		
47	1.11E+11	11.05	25.43	9		
0	5.40E+05	5.73	13.20	10	64	6/10/2014
1	3.19E+05	5.50	12.67	10		
2	8.73E+03	3.94	9.07	10		
4	8.79E+02	2.94	6.78	10		
8	1.29E+03	3.11	7.16	10		
24	8.83E+10	10.95	25.20	10		
48	9.27E+10	10.97	25.25	10		
0	5.29E+05	5.72	13.18	11	64	2/13/2017
2	1.28E+04	4.11	9.46	11		
4	3.68E+02	2.57	5.91	11		
6	2.66E+02	2.42	5.58	11		
8	2.45E+02	2.39	5.50	11		
24	1.58E+10	10.20	23.48	11		
0	5.31E+05	5.73	13.18	12	128	2/13/2017
2	3.43E+03	3.54	8.14	12		
4	2.45E+02	2.39	5.50	12		
6	1.23E+02	2.09	4.81	12		
8	4.09E+01	1.61	3.71	13	64	2/13/2017
24	1.50E+10	10.18	23.43	13		
6	3.00E+00	0.48	1.10	13	64	2/13/2017
8	2.70E+01	1.43	3.30	13		

Table F.15: Isolate 24 Polymyxin B Monotherapy Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	1.77E+06	6.25	14.39	1	0	5/29/2014
2	6.82E+07	7.83	18.04	1		
6	1.45E+10	10.16	23.40	1		
22	2.82E+10	10.45	24.06	1		
46	2.58E+10	10.41	23.97	1		
0	2.00E+06	6.30	14.51	2	0	8/23/2014
2	8.08E+07	7.91	18.21	2		
6	2.24E+10	10.35	23.83	2		
22	2.60E+10	10.41	23.98	2		
46	3.99E+10	10.60	24.41	2		
0	2.46E+06	6.39	14.72	3	0	6/11/2015
1	8.59E+06	6.93	15.97	3		
2	2.48E+08	8.39	19.33	3		
4	5.24E+10	10.72	24.68	3		
22	3.44E+11	11.54	26.56	3		
0	1.00E+00	0.00	0.00	4	0	6/11/2015
2	3.60E+01	1.56	3.58	4		
4	2.00E+02	2.30	5.30	4		
22	3.77E+02	2.58	5.93	4		
0	4.26E+05	5.63	12.96	5	0.25	5/20/2014
1	9.69E+03	3.99	9.18	5		
8	4.09E+02	2.61	6.01	5		
24	1.12E+10	10.05	23.14	5		
48	7.88E+10	10.90	25.09	5		
0	4.85E+05	5.69	13.09	6	1	5/20/2014
8	3.47E+02	2.54	5.85	6		
24	4.71E+09	9.67	22.27	6		
48	7.02E+10	10.85	24.97	6		
0	6.33E+05	5.80	13.36	7	2	5/29/2014
24	1.79E+06	6.25	14.40	7		
48	7.33E+10	10.87	25.02	7		
0	7.29E+05	5.86	13.50	8	4	5/29/2014
24	2.08E+06	6.32	14.55	8		
48	2.53E+09	9.40	21.65	8		
0	5.87E+05	5.77	13.28	9	0.0625	6/10/2014
1	1.10E+05	5.04	11.61	9		
2	4.91E+04	4.69	10.80	9		
0	6.26E+05	5.80	13.35	10	2	6/10/2014
8	3.88E+02	2.59	5.96	10		
24	4.70E+08	8.67	19.97	10		

Table F.15: Isolate 24 Polymyxin B Monotherapy Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
48	1.10E+11	11.04	25.42	10		
0	5.66E+05	5.75	13.25	11	4	6/10/2014
8	1.23E+02	2.09	4.81	11		
24	7.23E+06	6.86	15.79	11		
48	6.87E+10	10.84	24.95	11		
0	6.72E+05	5.83	13.42	12	0.25	6/10/2014
1	4.69E+04	4.67	10.76	12		
2	2.04E+02	2.31	5.32	12		
8	1.19E+03	3.08	7.08	12		
24	9.95E+09	10.00	23.02	12		
48	4.51E+10	10.65	24.53	12		
0	5.81E+05	5.76	13.27	13	1	6/10/2014
1	4.01E+03	3.60	8.30	13		
8	7.77E+02	2.89	6.66	13		
24	2.06E+10	10.31	23.75	13		
48	6.99E+10	10.84	24.97	13		
0	3.37E+05	5.53	12.73	14	0.0625	6/18/2014
1	2.78E+04	4.44	10.23	14		
2	1.19E+03	3.08	7.08	14		
0	3.67E+05	5.56	12.81	15	0.125	6/18/2014
4	1.23E+02	2.09	4.81	15		
8	1.83E+04	4.26	9.81	15		
24	4.96E+10	10.70	24.63	15		
48	1.42E+11	11.15	25.68	15		
0	7.51E+05	5.88	13.53	16	0.125	7/9/2014
1	3.70E+03	3.57	8.22	16		
2	9.40E+02	2.97	6.85	16		
4	5.33E+03	3.73	8.58	16		
8	2.22E+05	5.35	12.31	16		
24	8.15E+10	10.91	25.12	16		
48	1.39E+11	11.14	25.66	16		
0	5.37E+05	5.73	13.19	17	2	8/23/2014
8	9.40E+02	2.97	6.85	17		
24	4.95E+07	7.69	17.72	17		
48	1.02E+10	10.01	23.05	17		
0	5.04E+05	5.70	13.13	18	4	8/23/2014
8	1.23E+02	2.09	4.81	18		
24	6.66E+06	6.82	15.71	18		
48	2.12E+10	10.33	23.78	18		

Table F.16: Isolate 44 Growth Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	1.63E+06	6.21	14.31	1	0	1/26/2014
2	9.31E+07	7.97	18.35	1		
6	7.23E+08	8.86	20.40	1		
22	1.04E+11	11.02	25.37	1		
46	8.31E+11	11.92	27.45	1		
0	2.48E+06	6.39	14.72	2	0	2/15/2014
2	1.80E+09	9.26	21.31	2		
6	5.13E+10	10.71	24.66	2		
22	1.04E+11	11.02	25.37	2		
46	1.19E+12	12.08	27.81	2		
0	4.29E+06	6.63	15.27	3	0	3/30/2014
2	4.37E+09	9.64	22.20	3		
6	3.05E+10	10.48	24.14	3		
22	3.11E+11	11.49	26.46	3		
46	2.01E+11	11.30	26.02	3		
0	4.90E+06	6.69	15.40	4	0	6/11/2015
1	1.82E+07	7.26	16.72	4		
2	4.37E+08	8.64	19.89	4		
4	4.79E+10	10.68	24.59	4		
22	5.75E+11	11.76	27.08	4		
0	6.92E+04	4.84	11.14	5	0	6/11/2015
1	2.57E+05	5.41	12.46	5		
2	7.41E+06	6.87	15.82	5		
4	4.27E+08	8.63	19.87	5		
22	4.47E+08	8.65	19.92	5		
0	2.51E+01	1.40	3.22	6	0	6/11/2015
1	8.51E+01	1.93	4.44	6		
2	4.07E+02	2.61	6.01	6		
4	7.59E+02	2.88	6.63	6		
22	1.07E+04	4.03	9.28	6		

Table F.17: Isolate 44 Meropenem Initial Killing Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	1.63E+06	6.21	14.31	1	0	1/26/2014
2	9.31E+07	7.97	18.35	1		
6	7.23E+08	8.86	20.40	1		
22	1.04E+11	11.02	25.37	1		
46	8.31E+11	11.92	27.45	1		
0	2.48E+06	6.39	14.72	2	0	2/15/2014
2	1.80E+09	9.26	21.31	2		
6	5.13E+10	10.71	24.66	2		
22	1.04E+11	11.02	25.37	2		
46	1.19E+12	12.08	27.81	2		
0	4.29E+06	6.63	15.27	3	0	3/30/2014
2	4.37E+09	9.64	22.20	3		
6	3.05E+10	10.48	24.14	3		
22	3.11E+11	11.49	26.46	3		
46	2.01E+11	11.30	26.02	3		
0	4.90E+06	6.69	15.40	4	0	6/11/2015
1	1.82E+07	7.26	16.72	4		
2	4.37E+08	8.64	19.89	4		
4	4.79E+10	10.68	24.59	4		
22	5.75E+11	11.76	27.08	4		
0	6.80E+05	5.83	13.43	5	4	1/26/2014
1	1.97E+06	6.29	14.49	5		
3	5.56E+07	7.75	17.83	5		
0	6.97E+05	5.84	13.45	6	16	1/26/2014
1	1.73E+06	6.24	14.36	6		
0	9.32E+05	5.97	13.74	7	64	1/26/2014
1	6.86E+05	5.84	13.44	7		
3	1.88E+05	5.27	12.14	7		
0	1.34E+06	6.13	14.11	8	4	5/14/2014
1	4.97E+06	6.70	15.42	8		
3	3.66E+09	9.56	22.02	8		
0	1.38E+06	6.14	14.14	9	64	5/14/2014
1	3.05E+05	5.48	12.63	9		
0	7.32E+05	5.86	13.50	10	16	5/20/2014
1	1.87E+06	6.27	14.44	10		
3	2.14E+07	7.33	16.88	10		
1	1.86E+05	5.27	12.13	11	64	2/13/2017
0	8.30E+05	5.92	13.63	12	128	2/13/2017
2	2.64E+04	4.42	10.18	12		

Table F.18: Isolate 44 Polymyxin B Initial Killing Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	1.63E+06	6.21	14.31	1	0	1/26/2014
2	9.31E+07	7.97	18.35	1		
6	7.23E+08	8.86	20.40	1		
22	1.04E+11	11.02	25.37	1		
46	8.31E+11	11.92	27.45	1		
0	2.48E+06	6.39	14.72	2	0	2/15/2014
2	1.80E+09	9.26	21.31	2		
6	5.13E+10	10.71	24.66	2		
22	1.04E+11	11.02	25.37	2		
46	1.19E+12	12.08	27.81	2		
0	4.29E+06	6.63	15.27	3	0	3/30/2014
2	4.37E+09	9.64	22.20	3		
6	3.05E+10	10.48	24.14	3		
22	3.11E+11	11.49	26.46	3		
46	2.01E+11	11.30	26.02	3		
0	4.90E+06	6.69	15.40	4	0	6/11/2015
1	1.82E+07	7.26	16.72	4		
2	4.37E+08	8.64	19.89	4		
4	4.79E+10	10.68	24.59	4		
22	5.75E+11	11.76	27.08	4		
0	6.03E+05	5.78	13.31	5	0.0625	6/10/2014
1	6.92E+04	4.84	11.14	5		
0	5.25E+05	5.72	13.17	6	0.0625	6/18/2014
1	7.08E+02	2.85	6.56	6		
0	5.37E+05	5.73	13.19	7	0.125	6/18/2014
1	1.62E+02	2.21	5.09	7		
0	7.08E+05	5.85	13.47	8	0.125	7/9/2014
1	2.00E+03	3.30	7.60	8		
0	2.69E+05	5.43	12.50	9	0.25	3/30/2014
0	8.51E+05	5.93	13.65	10	0.25	4/19/2014
1	5.75E+02	2.76	6.36	10		
0	8.91E+05	5.95	13.70	11	0.25	5/14/2014
1	1.74E+03	3.24	7.46	11		
0	4.47E+05	5.65	13.01	12	1	3/30/2014
0	8.13E+05	5.91	13.61	13	1	4/19/2014
0	9.77E+05	5.99	13.79	14	1	5/14/2014
1	7.08E+02	2.85	6.56	14		
0	6.46E+05	5.81	13.38	15	2	5/29/2014
0	6.17E+05	5.79	13.33	16	2	6/10/2014
0	4.79E+05	5.68	13.08	17	2	8/23/2014

Table F.18: Isolate 44 Polymyxin B Initial Killing Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	5.62E+05	5.75	13.24	18	4	5/20/2014
0	6.46E+05	5.81	13.38	19	4	5/29/2014
0	5.89E+05	5.77	13.29	20	4	6/10/2014
0	7.76E+05	5.89	13.56	21	4	7/9/2014
1	2.88E+02	2.46	5.66	21		
0	1.10E+06	6.04	13.91	22	4	8/23/2014
0	9.55E+05	5.98	13.77	23	4	2/13/2017
1	1.95E+02	2.29	5.27	23		
0	1.02E+06	6.01	13.84	24	8	2/13/2017

Table F.19: Isolate 44 Meropenem Monotherapy Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	1.63E+06	6.21	14.31	1	0	1/26/2014
2	9.31E+07	7.97	18.35	1		
22	1.04E+11	11.02	25.37	1		
46	8.31E+11	11.92	27.45	1		
0	2.48E+06	6.39	14.72	2	0	2/15/2014
2	1.80E+09	9.26	21.31	2		
6	5.13E+10	10.71	24.66	2		
22	1.04E+11	11.02	25.37	2		
46	1.19E+12	12.08	27.81	2		
0	4.29E+06	6.63	15.27	3	0	3/30/2014
2	4.37E+09	9.64	22.20	3		
6	3.05E+10	10.48	24.14	3		
22	3.11E+11	11.49	26.46	3		
46	2.01E+11	11.30	26.02	3		
0	4.90E+06	6.69	15.40	4	0	6/11/2015
1	1.82E+07	7.26	16.72	4		
2	4.37E+08	8.64	19.89	4		
4	4.79E+10	10.68	24.59	4		
22	5.75E+11	11.76	27.08	4		
0	2.58E+05	5.41	12.46	5	0	6/11/2015
1	7.36E+06	6.87	15.81	5		
0	6.80E+05	5.83	13.43	6	4	1/26/2014
1	1.97E+06	6.29	14.49	6		
23	2.71E+10	10.43	24.02	6		
47	1.89E+10	10.28	23.66	6		
0	6.97E+05	5.84	13.45	7	16	1/26/2014
1	1.73E+06	6.24	14.36	7		
23	1.90E+10	10.28	23.67	7		
47	1.22E+10	10.09	23.22	7		
0	9.32E+05	5.97	13.74	8	64	1/26/2014
1	6.86E+05	5.84	13.44	8		
3	1.88E+05	5.27	12.14	8		
7	3.63E+05	5.56	12.80	8		
47	2.06E+10	10.31	23.75	8		
0	1.34E+06	6.13	14.11	9	4	5/14/2014
1	4.97E+06	6.70	15.42	9		
3	3.66E+09	9.56	22.02	9		
7	9.15E+10	10.96	25.24	9		
23	1.21E+11	11.08	25.52	9		
47	2.26E+10	10.35	23.84	9		

Table F.19: Isolate 44 Meropenem Monotherapy Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	1.38E+06	6.14	14.14	10	64	5/14/2014
1	3.05E+05	5.48	12.63	10		
3	4.01E+05	5.60	12.90	10		
7	6.40E+05	5.81	13.37	10		
23	5.77E+10	10.76	24.78	10		
47	1.01E+10	10.00	23.04	10		
0	7.32E+05	5.86	13.50	11	16	5/20/2014
1	1.87E+06	6.27	14.44	11		
3	2.14E+07	7.33	16.88	11		
7	4.04E+10	10.61	24.42	11		
23	5.93E+10	10.77	24.81	11		
47	1.69E+10	10.23	23.55	11		
0	8.75E+05	5.94	13.68	12	64	2/13/2017
2	1.86E+05	5.27	12.13	12		
4	1.27E+05	5.10	11.75	12		
6	1.25E+05	5.10	11.74	12		
8	2.06E+05	5.31	12.24	12		
24	9.93E+10	11.00	25.32	12	128	2/13/2017
0	8.30E+05	5.92	13.63	13		
2	2.64E+04	4.42	10.18	13		
4	6.48E+03	3.81	8.78	13		
6	3.92E+03	3.59	8.27	13		
24	1.69E+10	10.23	23.55	13		

Table F.20: Isolate 44 Polymyxin B Monotherapy Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	1.63E+06	6.21	14.31	1	0	1/26/2014
2	9.31E+07	7.97	18.35	1		
6	7.23E+08	8.86	20.40	1		
22	1.04E+11	11.02	25.37	1		
46	8.31E+11	11.92	27.45	1		
0	2.48E+06	6.39	14.72	2	0	2/15/2014
2	1.80E+09	9.26	21.31	2		
6	5.13E+10	10.71	24.66	2		
22	1.04E+11	11.02	25.37	2		
46	1.19E+12	12.08	27.81	2		
0	4.28E+06	6.63	15.27	3	0	3/30/2014
2	4.37E+09	9.64	22.20	3		
6	3.05E+10	10.48	24.14	3		
22	3.11E+11	11.49	26.46	3		
46	2.00E+11	11.30	26.02	3		
0	4.93E+06	6.69	15.41	4	0	6/11/2015
1	1.81E+07	7.26	16.71	4		
2	4.32E+08	8.64	19.88	4		
4	4.76E+10	10.68	24.59	4		
22	5.72E+11	11.76	27.07	4		
0	2.50E+01	1.40	3.22	5	0	6/11/2015
1	8.60E+01	1.93	4.45	5		
2	4.09E+02	2.61	6.01	5		
4	7.56E+02	2.88	6.63	5		
0	6.04E+05	5.78	13.31	6	0.0625	6/10/2014
1	6.95E+04	4.84	11.15	6		
0	5.29E+05	5.72	13.18	7	0.0625	6/18/2014
1	7.15E+02	2.85	6.57	7		
0	5.42E+05	5.73	13.20	8	0.125	6/18/2014
1	1.64E+02	2.21	5.10	8		
2	2.86E+02	2.46	5.66	8		
4	1.76E+03	3.25	7.47	8		
8	6.73E+04	4.83	11.12	8		
24	1.61E+11	11.21	25.80	8		
48	4.92E+10	10.69	24.62	8		
0	7.16E+05	5.85	13.48	9	0.125	7/9/2014
1	2.00E+03	3.30	7.60	9		
4	2.82E+03	3.45	7.94	9		
8	5.52E+04	4.74	10.92	9		
24	7.37E+10	10.87	25.02	9		

Table F.20: Isolate 44 Polymyxin B Monotherapy Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
48	1.41E+11	11.15	25.67	9		
0	2.68E+05	5.43	12.50	10	0.25	3/30/2014
8	1.00E+03	3.00	6.91	10		
24	4.30E+10	10.63	24.48	10		
48	2.43E+10	10.39	23.91	10		
0	8.53E+05	5.93	13.66	11	0.25	4/19/2014
1	5.72E+02	2.76	6.35	11		
4	1.23E+02	2.09	4.81	11		
8	9.81E+02	2.99	6.89	11		
24	1.05E+10	10.02	23.07	11		
48	9.97E+11	12.00	27.63	11		
0	8.93E+05	5.95	13.70	12	0.25	5/14/2014
1	1.74E+03	3.24	7.46	12		
4	1.64E+02	2.21	5.10	12		
8	2.76E+03	3.44	7.92	12		
24	1.76E+11	11.25	25.89	12		
48	3.44E+10	10.54	24.26	12		
0	4.48E+05	5.65	13.01	13	1	3/30/2014
8	5.11E+02	2.71	6.24	13		
24	8.29E+09	9.92	22.84	13		
48	8.94E+10	10.95	25.22	13		
0	8.13E+05	5.91	13.61	14	1	4/19/2014
4	2.25E+02	2.35	5.42	14		
8	7.15E+02	2.85	6.57	14		
24	8.23E+09	9.92	22.83	14		
48	3.09E+11	11.49	26.46	14		
0	9.82E+05	5.99	13.80	15	1	5/14/2014
1	7.15E+02	2.85	6.57	15		
4	1.84E+02	2.26	5.21	15		
8	2.55E+03	3.41	7.84	15		
24	1.11E+11	11.05	25.43	15		
48	1.67E+10	10.22	23.54	15		
0	6.47E+05	5.81	13.38	16	2	5/29/2014
8	1.64E+02	2.21	5.10	16		
24	2.27E+11	11.36	26.15	16		
48	3.74E+10	10.57	24.34	16		
0	6.18E+05	5.79	13.33	17	2	6/10/2014
2	2.04E+02	2.31	5.32	17		
24	1.21E+06	6.08	14.01	17		
48	1.27E+10	10.10	23.26	17		

Table F.20: Isolate 44 Polymyxin B Monotherapy Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	4.75E+05	5.68	13.07	18	2	8/23/2014
24	5.38E+04	4.73	10.89	18		
48	2.98E+09	9.47	21.82	18		
0	5.56E+05	5.75	13.23	19	4	5/20/2014
0	6.53E+05	5.81	13.39	20	4	5/29/2014
8	5.72E+02	2.76	6.35	20		
24	2.30E+10	10.36	23.86	20		
48	3.77E+10	10.58	24.35	20		
0	5.87E+05	5.77	13.28	21	4	6/10/2014
24	6.52E+05	5.81	13.39	21		
48	4.00E+10	10.60	24.41	21		
0	7.75E+05	5.89	13.56	22	4	7/9/2014
1	2.86E+02	2.46	5.66	22		
8	1.02E+02	2.01	4.62	22		
24	2.13E+07	7.33	16.87	22		
48	3.17E+07	7.50	17.27	22		
0	1.10E+06	6.04	13.91	23	4	8/23/2014
24	2.68E+07	7.43	17.10	23		
48	5.87E+10	10.77	24.80	23		
0	9.48E+05	5.98	13.76	24	4	2/13/2017
1	1.95E+02	2.29	5.27	24		
1	7.10E+01	1.85	4.26	25	4	2/13/2017
2	8.20E+01	1.91	4.41	25		
4	2.10E+02	2.32	5.35	25		
8	1.12E+03	3.05	7.02	25		
8	6.54E+02	2.82	6.48	25		
8	9.81E+02	2.99	6.89	25		
24	1.31E+10	10.12	23.30	25		
24	7.44E+10	10.87	25.03	25		

Table F.21: Meropenem Composite Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	2.63E+06	6.42	14.78	1	0	1/26/2014
2	5.07E+08	8.71	20.04	1		
22	4.43E+10	10.65	24.51	1		
46	4.43E+11	11.65	26.82	1		
0	4.44E+06	6.65	15.31	2	0	2/15/2014
2	1.36E+10	10.13	23.33	2		
6	3.42E+10	10.53	24.26	2		
22	4.12E+11	11.61	26.74	2		
46	3.93E+12	12.59	29.00	2		
0	1.13E+07	7.05	16.24	3	0	3/30/2014
2	3.25E+10	10.51	24.20	3		
6	1.06E+11	11.03	25.39	3		
22	5.43E+11	11.73	27.02	3		
46	9.31E+10	10.97	25.26	3		
0	1.14E+07	7.06	16.25	4	0	6/11/2015
1	2.85E+08	8.45	19.47	4		
3	5.11E+10	10.71	24.66	4		
21	5.86E+11	11.77	27.10	4		
0	3.71E+02	2.57	5.92	5	0	6/11/2015
1	5.09E+03	3.71	8.54	5		
3	2.98E+05	5.47	12.60	5		
0	8.06E+05	5.91	13.60	6	4	1/26/2014
1	5.94E+05	5.77	13.29	6		
2	2.26E+04	4.35	10.03	6		
4	1.21E+03	3.08	7.10	6		
8	5.74E+03	3.76	8.65	6		
24	1.06E+09	9.03	20.78	6		
48	1.71E+10	10.23	23.56	6		
0	7.48E+05	5.87	13.53	7	16	1/26/2014
1	8.31E+04	4.92	11.33	7		
2	2.19E+03	3.34	7.69	7		
4	4.29E+02	2.63	6.06	7		
0	6.54E+05	5.82	13.39	8	64	1/26/2014
1	3.39E+04	4.53	10.43	8		
2	3.47E+02	2.54	5.85	8		
0	1.04E+06	6.02	13.85	9	4	7/9/2014
1	5.86E+05	5.77	13.28	9		
2	8.73E+03	3.94	9.07	9		

Table F.21: Meropenem Composite Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
4	9.61E+02	2.98	6.87	9		
8	5.72E+04	4.76	10.95	9		
24	8.39E+10	10.92	25.15	9		
48	5.76E+10	10.76	24.78	9		
0	4.85E+05	5.69	13.09	10	16	7/9/2014
1	5.25E+04	4.72	10.87	10		
4	1.43E+02	2.16	4.96	10		
0	7.54E+05	5.88	13.53	11	64	7/9/2014
1	2.98E+04	4.47	10.30	11		
2	2.25E+02	2.35	5.42	11		
0	7.59E+05	5.88	13.54	12	8	2/13/2017
2	2.08E+03	3.32	7.64	12		
4	7.77E+02	2.89	6.66	12		
6	3.47E+02	2.54	5.85	12		
24	2.04E+02	2.31	5.32	12		
0	1.14E+06	6.06	13.95	13	16	2/13/2017
2	1.27E+03	3.10	7.15	13		
4	2.25E+02	2.35	5.42	13		
6	2.04E+02	2.31	5.32	13		
0	3.14E+06	6.50	14.96	14	0	5/29/2014
2	4.21E+09	9.62	22.16	14		
6	1.11E+11	11.05	25.43	14		
22	5.23E+10	10.72	24.68	14		
46	9.93E+10	11.00	25.32	14		
0	2.51E+06	6.40	14.74	15	0	8/23/2014
2	5.15E+09	9.71	22.36	15		
6	1.06E+11	11.03	25.39	15		
22	6.69E+10	10.83	24.93	15		
46	2.45E+09	9.39	21.62	15		
0	6.23E+06	6.79	15.64	16	0	6/11/2015
1	1.35E+08	8.13	18.72	16		
3	3.21E+10	10.51	24.19	16		
21	1.43E+11	11.16	25.69	16		
0	7.93E+02	2.90	6.68	17	0	6/11/2015
1	8.75E+03	3.94	9.08	17		
3	9.69E+04	4.99	11.48	17		
0	1.70E+06	6.23	14.35	18	4	5/14/2014
1	4.70E+04	4.67	10.76	18		

Table F.21: Meropenem Composite Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
3	1.68E+03	3.23	7.43	18		
7	1.37E+05	5.14	11.83	18		
23	1.19E+11	11.08	25.50	18		
47	7.98E+10	10.90	25.10	18		
0	1.26E+06	6.10	14.05	19	16	5/14/2014
1	2.43E+04	4.39	10.10	19		
3	2.02E+03	3.31	7.61	19		
7	4.09E+04	4.61	10.62	19		
23	9.27E+10	10.97	25.25	19		
47	4.13E+09	9.62	22.14	19		
0	9.34E+05	5.97	13.75	20	64	5/14/2014
1	1.43E+04	4.16	9.57	20		
2	2.86E+02	2.46	5.66	20		
0	6.11E+05	5.79	13.32	21	4	5/20/2014
1	3.78E+03	3.58	8.24	21		
3	2.86E+02	2.46	5.66	21		
7	5.23E+04	4.72	10.86	21		
23	1.03E+11	11.01	25.36	21		
47	1.01E+11	11.00	25.34	21		
0	3.52E+05	5.55	12.77	22	16	5/20/2014
1	6.70E+03	3.83	8.81	22		
3	1.02E+02	2.01	4.62	22		
7	2.08E+03	3.32	7.64	22		
23	6.68E+10	10.82	24.92	22		
47	5.12E+10	10.71	24.66	22		
0	5.28E+05	5.72	13.18	23	64	5/20/2014
1	4.19E+03	3.62	8.34	23		
0	7.82E+05	5.89	13.57	24	16	2/13/2017
2	1.22E+04	4.09	9.41	24		
4	1.84E+02	2.26	5.21	24		
6	1.64E+02	2.21	5.10	25		
8	5.00E+03	3.70	8.52	25		
24	2.86E+10	10.46	24.08	25		
4	6.00E+00	0.78	1.79	25	16	2/13/2017
6	1.54E+02	2.19	5.04	25		
8	4.94E+03	3.69	8.51	25	16	2/13/2017
24	3.19E+10	10.50	24.19	25		
0	8.00E+05	5.90	13.59	26	32	2/13/2017

Table F.21: Meropenem Composite Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
2	1.23E+03	3.09	7.11	26		
4	1.23E+02	2.09	4.81	26		
6	1.43E+02	2.16	4.96	26		
0	1.77E+06	6.25	14.39	27	0	5/29/2014
2	6.82E+07	7.83	18.04	27		
6	1.45E+10	10.16	23.40	27		
22	2.82E+10	10.45	24.06	27		
46	2.58E+10	10.41	23.97	27		
0	2.00E+06	6.30	14.51	28	0	8/23/2014
2	8.08E+07	7.91	18.21	28		
6	2.24E+10	10.35	23.83	28		
22	2.60E+10	10.41	23.98	28		
46	3.99E+10	10.60	24.41	28		
0	2.46E+06	6.39	14.72	29	0	6/11/2015
1	8.59E+06	6.93	15.97	29		
2	2.48E+08	8.39	19.33	29		
4	5.24E+10	10.72	24.68	29		
22	3.44E+11	11.54	26.56	29		
0	5.70E+01	1.76	4.04	30	0	6/11/2015
1	5.08E+02	2.71	6.23	30		
2	1.01E+03	3.00	6.92	30		
4	4.98E+03	3.70	8.51	30		
22	3.34E+04	4.52	10.42	30		
0	4.79E+05	5.68	13.08	31	4	5/20/2014
2	1.71E+04	4.23	9.75	31		
6	2.07E+06	6.32	14.54	31		
22	3.95E+10	10.60	24.40	31		
46	5.48E+10	10.74	24.73	31		
0	5.68E+05	5.75	13.25	32	16	5/20/2014
1	1.17E+05	5.07	11.67	32		
3	4.03E+03	3.61	8.30	32		
7	9.81E+04	4.99	11.49	32		
23	4.13E+10	10.62	24.44	32		
47	1.92E+10	10.28	23.68	32		
0	4.36E+05	5.64	12.99	33	64	5/20/2014
1	9.40E+04	4.97	11.45	33		
2	1.05E+04	4.02	9.26	33		
4	2.17E+03	3.34	7.68	33		

Table F.21: Meropenem Composite Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
8	1.96E+03	3.29	7.58	33		
24	2.36E+11	11.37	26.19	33		
48	8.95E+10	10.95	25.22	33		
0	4.84E+05	5.68	13.09	34	4	6/10/2014
2	1.08E+04	4.03	9.29	34		
6	1.37E+03	3.14	7.22	34		
22	2.79E+10	10.45	24.05	34		
46	1.18E+11	11.07	25.49	34		
0	6.34E+05	5.80	13.36	35	16	6/10/2014
1	1.17E+05	5.07	11.67	35		
3	2.15E+03	3.33	7.67	35		
7	6.18E+03	3.79	8.73	35		
23	3.10E+10	10.49	24.16	35		
47	1.11E+11	11.05	25.43	35		
0	5.40E+05	5.73	13.20	36	64	6/10/2014
1	3.19E+05	5.50	12.67	36		
2	8.73E+03	3.94	9.07	36		
4	8.79E+02	2.94	6.78	36		
8	1.29E+03	3.11	7.16	36		
24	8.83E+10	10.95	25.20	36		
48	9.27E+10	10.97	25.25	36		
0	5.29E+05	5.72	13.18	37	64	2/13/2017
2	1.28E+04	4.11	9.46	37		
4	3.68E+02	2.57	5.91	37		
6	2.66E+02	2.42	5.58	37		
8	2.45E+02	2.39	5.50	37		
24	1.58E+10	10.20	23.48	37		
0	5.31E+05	5.73	13.18	38	128	2/13/2017
2	3.43E+03	3.54	8.14	38		
4	2.45E+02	2.39	5.50	38		
6	1.23E+02	2.09	4.81	38		
8	4.09E+01	1.61	3.71	39	64	2/13/2017
24	1.50E+10	10.18	23.43	39		
6	3.00E+00	0.48	1.10	39	64	2/13/2017
8	2.70E+01	1.43	3.30	39		
0	1.63E+06	6.21	14.30	40	0	1/26/2014
2	9.33E+07	7.97	18.35	40		
22	1.05E+11	11.02	25.37	40		

Table F.21: Meropenem Composite Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
46	8.32E+11	11.92	27.45	40		
0	2.48E+06	6.39	14.72	41	0	2/15/2014
2	1.82E+09	9.26	21.32	41		
6	5.13E+10	10.71	24.66	41		
22	1.05E+11	11.02	25.37	41		
46	1.20E+12	12.08	27.82	41		
0	4.29E+06	6.63	15.27	42	0	3/30/2014
2	4.37E+09	9.64	22.20	42		
6	3.02E+10	10.48	24.13	42		
22	3.09E+11	11.49	26.46	42		
46	2.00E+11	11.30	26.02	42		
0	4.93E+06	6.69	15.41	43	0	6/11/2015
1	1.82E+07	7.26	16.72	43		
2	4.37E+08	8.64	19.89	43		
4	4.79E+10	10.68	24.59	43		
22	5.75E+11	11.76	27.08	43		
0	2.58E+05	5.41	12.46	44		6/11/2015
1	7.36E+06	6.87	15.81	44		
0	6.80E+05	5.83	13.43	45	4	1/26/2014
1	1.97E+06	6.29	14.49	45		
23	2.71E+10	10.43	24.02	45		
47	1.89E+10	10.28	23.66	45		
0	6.97E+05	5.84	13.45	46	16	1/26/2014
1	1.73E+06	6.24	14.36	46		
23	1.90E+10	10.28	23.67	46		
47	1.22E+10	10.09	23.22	46		
0	9.32E+05	5.97	13.74	47	64	1/26/2014
1	6.86E+05	5.84	13.44	47		
3	1.88E+05	5.27	12.14	47		
7	3.63E+05	5.56	12.80	47		
47	2.06E+10	10.31	23.75	47		
0	1.34E+06	6.13	14.11	48	4	5/14/2014
1	4.97E+06	6.70	15.42	48		
3	3.66E+09	9.56	22.02	48		
7	9.15E+10	10.96	25.24	48		
23	1.21E+11	11.08	25.52	48		
47	2.26E+10	10.35	23.84	48		
0	1.38E+06	6.14	14.14	49	64	5/14/2014

Table F.21: Meropenem Composite Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
1	3.05E+05	5.48	12.63	49		
3	4.01E+05	5.60	12.90	49		
7	6.40E+05	5.81	13.37	49		
23	5.77E+10	10.76	24.78	49		
47	1.01E+10	10.00	23.04	49		
0	7.32E+05	5.86	13.50	50	16	5/20/2014
1	1.87E+06	6.27	14.44	50		
3	2.14E+07	7.33	16.88	50		
7	4.04E+10	10.61	24.42	50		
23	5.93E+10	10.77	24.81	50		
47	1.69E+10	10.23	23.55	50		
0	8.75E+05	5.94	13.68	51	64	2/13/2017
2	1.86E+05	5.27	12.13	51		
4	1.27E+05	5.10	11.75	51		
6	1.25E+05	5.10	11.74	51		
8	2.06E+05	5.31	12.24	51		
24	9.93E+10	11.00	25.32	51		
0	8.30E+05	5.92	13.63	52	128	2/13/2017
2	2.64E+04	4.42	10.18	52		
4	6.48E+03	3.81	8.78	52		
6	3.92E+03	3.59	8.27	52		
24	1.69E+10	10.23	23.55	52		

Table F.22: Polymyxin B Composite Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	2.63E+06	6.42	14.78	1	0	1/26/2014
22	4.43E+10	10.65	24.51	1		
46	4.43E+11	11.65	26.82	1		
0	4.44E+06	6.65	15.31	2	0	2/15/2014
2	1.36E+10	10.13	23.33	2		
6	3.42E+10	10.53	24.26	2		
22	4.12E+11	11.61	26.74	2		
46	3.93E+12	12.59	29.00	2		
0	1.13E+07	7.05	16.24	3	0	3/30/2014
2	3.25E+10	10.51	24.20	3		
6	1.06E+11	11.03	25.39	3		
22	5.43E+11	11.73	27.02	3		
46	9.31E+10	10.97	25.26	3		
0	1.14E+07	7.06	16.25	4	0	6/11/2015
1	2.85E+08	8.45	19.47	4		
3	5.11E+10	10.71	24.66	4		
21	5.86E+11	11.77	27.10	4		
0	1.42E+02	2.15	4.96	5	0	6/11/2015
1	1.08E+03	3.03	6.98	5		
3	2.02E+03	3.31	7.61	5		
21	1.74E+04	4.24	9.76	5		
0	7.07E+05	5.85	13.47	6	0.25	1/26/2014
1	2.52E+04	4.40	10.13	6		
2	6.74E+02	2.83	6.51	6		
4	1.92E+03	3.28	7.56	6		
48	4.71E+10	10.67	24.58	6		
0	6.54E+05	5.82	13.39	7	1	1/26/2014
1	7.07E+03	3.85	8.86	7		
2	2.86E+02	2.46	5.66	7		
4	4.91E+02	2.69	6.20	7		
48	1.49E+10	10.17	23.42	7		
0	7.23E+05	5.86	13.49	8	0.25	3/30/2015
1	3.27E+02	2.51	5.79	8		
2	1.43E+02	2.16	4.96	8		
4	5.93E+02	2.77	6.39	8		
8	4.16E+04	4.62	10.64	8		
24	7.16E+10	10.85	24.99	8		
48	7.93E+09	9.90	22.79	8		

Table F.22: Polymyxin B Composite Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	5.84E+05	5.77	13.28	9	1	3/30/2015
4	2.66E+02	2.42	5.58	9		
8	1.56E+04	4.19	9.66	9		
24	1.67E+11	11.22	25.84	9		
48	1.19E+10	10.08	23.20	9		
0	8.00E+05	5.90	13.59	10	2	5/29/2014
1	6.13E+02	2.79	6.42	10		
2	1.02E+02	2.01	4.62	10		
4	8.99E+02	2.95	6.80	10		
8	7.16E+04	4.85	11.18	10		
24	1.29E+11	11.11	25.58	10		
48	5.73E+10	10.76	24.77	10		
0	7.79E+05	5.89	13.57	11	4	5/29/2014
1	1.64E+02	2.21	5.10	11		
4	3.07E+02	2.49	5.73	11		
8	3.09E+04	4.49	10.34	11		
24	5.31E+10	10.73	24.70	11		
48	1.67E+11	11.22	25.84	11		
0	6.92E+05	5.84	13.45	12	2	6/10/2014
1	1.00E+03	3.00	6.91	12		
2	2.45E+02	2.39	5.50	12		
4	1.84E+02	2.26	5.21	12		
8	2.25E+04	4.35	10.02	12		
24	6.13E+10	10.79	24.84	12		
48	1.57E+11	11.20	25.78	12		
0	6.85E+05	5.84	13.44	13	4	6/10/2014
1	7.97E+02	2.90	6.68	13		
4	1.02E+02	2.01	4.62	13		
8	8.36E+03	3.92	9.03	13		
24	9.78E+10	10.99	25.31	13		
48	1.96E+11	11.29	26.00	13		
0	5.01E+05	5.70	13.12	14	0.06	6/18/2014
1	6.26E+04	4.80	11.04	14		6/10/2014
2	6.12E+03	3.79	8.72	14		
4	5.87E+03	3.77	8.68	14		
8	1.81E+06	6.26	14.41	14		
24	9.88E+10	10.99	25.32	14		
48	1.56E+11	11.19	25.77	14		

Table F.22: Polymyxin B Composite Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
0	5.15E+05	5.71	13.15	15	0.125	6/18/2014
1	2.15E+04	4.33	9.98	15		
2	6.54E+02	2.82	6.48	15		
4	3.88E+02	2.59	5.96	15		
8	4.46E+05	5.65	13.01	15		
24	6.48E+10	10.81	24.89	15		
48	7.37E+10	10.87	25.02	15		
0	7.87E+05	5.90	13.58	16	0.06	7/9/2014
1	1.36E+05	5.13	11.82	16		
2	6.44E+04	4.81	11.07	16		
4	3.79E+04	4.58	10.54	16		
8	1.19E+08	8.08	18.59	16		
24	2.19E+11	11.34	26.11	16		
48	8.18E+10	10.91	25.13	16		
0	9.30E+05	5.97	13.74	17	0.125	7/9/2014
1	4.70E+04	4.67	10.76	17		
2	4.24E+03	3.63	8.35	17		
4	2.76E+03	3.44	7.92	17		
8	1.18E+06	6.07	13.98	17		
24	8.44E+10	10.93	25.16	17		
48	9.84E+10	10.99	25.31	17		
0	8.95E+05	5.95	13.70	18	0.25	8/23/2014
1	3.47E+03	3.54	8.15	18		
2	6.34E+02	2.80	6.45	18		
4	2.92E+03	3.47	7.98	18		
8	4.26E+04	4.63	10.66	18		
24	7.88E+10	10.90	25.09	18		
48	1.71E+10	10.23	23.56	18		
0	8.43E+05	5.93	13.64	19	1	8/23/2014
1	2.21E+03	3.34	7.70	19		
2	1.64E+02	2.21	5.10	19		
4	4.37E+03	3.64	8.38	19		
8	2.00E+04	4.30	9.90	19		
24	8.59E+10	10.93	25.18	19		
48	1.78E+10	10.25	23.60	19		
0	3.14E+06	6.50	14.96	20	0	5/29/2014
2	4.21E+09	9.62	22.16	20		
6	1.11E+11	11.05	25.43	20		

Table F.22: Polymyxin B Composite Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
22	5.23E+10	10.72	24.68	20		
46	9.93E+10	11.00	25.32	20		
0	2.51E+06	6.40	14.74	21	0	8/23/2014
2	5.15E+09	9.71	22.36	21		
6	1.06E+11	11.03	25.39	21		
22	6.69E+10	10.83	24.93	21		
46	2.45E+09	9.39	21.62	21		
0	6.23E+06	6.79	15.64	22	0	6/11/2015
1	1.35E+08	8.13	18.72	22		
3	3.21E+10	10.51	24.19	22		
21	1.43E+11	11.16	25.69	22		
0	2.54E+02	2.40	5.54	23	0	6/11/2015
1	1.58E+03	3.20	7.37	23		
21	9.18E+03	3.96	9.12	23		
0	1.34E+06	6.13	14.11	24	0.25	5/14/2014
1	1.78E+03	3.25	7.48	24		
8	3.94E+03	3.60	8.28	24		
24	2.21E+11	11.34	26.12	24		
48	2.65E+10	10.42	24.00	24		
0	1.43E+06	6.16	14.17	25	1	5/14/2014
8	2.25E+02	2.35	5.42	25		
24	5.95E+10	10.77	24.81	25		
48	1.04E+11	11.02	25.37	25		
0	5.01E+05	5.70	13.12	26	0.25	5/20/2014
8	2.23E+03	3.35	7.71	26		
24	1.35E+11	11.13	25.63	26		
48	9.15E+10	10.96	25.24	26		
0	5.53E+05	5.74	13.22	27	1	5/20/2014
8	8.18E+02	2.91	6.71	27		
24	1.08E+11	11.03	25.41	27		
48	1.23E+11	11.09	25.54	27		
0	6.68E+05	5.82	13.41	28	2	5/29/2014
8	2.41E+03	3.38	7.79	28		
24	6.37E+10	10.80	24.88	28		
48	1.21E+11	11.08	25.52	28		
0	6.60E+05	5.82	13.40	29	4	5/29/2014
8	2.86E+02	2.46	5.66	29		
24	2.11E+10	10.32	23.77	29		

Table F.22: Polymyxin B Composite Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
48	1.13E+11	11.05	25.45	29		
0	5.27E+05	5.72	13.17	30	0.06	6/10/2014
1	9.81E+04	4.99	11.49	30		
0	5.64E+05	5.75	13.24	31	2	6/10/2014
8	1.37E+03	3.14	7.22	31		
24	1.29E+11	11.11	25.58	31		
48	8.03E+10	10.90	25.11	31		
0	5.00E+05	5.70	13.12	32	4	6/10/2014
8	5.31E+02	2.73	6.27	32		
24	2.38E+10	10.38	23.89	32		
48	1.52E+11	11.18	25.75	32		
0	4.56E+05	5.66	13.03	33	0.06	6/18/2014
1	4.48E+03	3.65	8.41	33		
0	4.93E+05	5.69	13.11	34	0.125	6/18/2014
1	3.07E+02	2.49	5.73	34		
4	2.25E+02	2.35	5.42	34		
8	3.00E+05	5.48	12.61	34		
24	1.29E+11	11.11	25.58	34		
48	6.66E+10	10.82	24.92	34		
0	6.91E+05	5.84	13.45	35	0.125	7/9/2014
1	1.64E+02	2.21	5.10	35		
2	1.84E+02	2.26	5.21	35		
4	4.50E+02	2.65	6.11	35		
8	8.58E+04	4.93	11.36	35		
24	1.26E+11	11.10	25.56	35		
48	8.75E+10	10.94	25.19	35		
0	1.77E+06	6.25	14.39	36	0	5/29/2014
2	6.82E+07	7.83	18.04	36		
6	1.45E+10	10.16	23.40	36		
22	2.82E+10	10.45	24.06	36		
46	2.58E+10	10.41	23.97	36		
0	2.00E+06	6.30	14.51	37	0	8/23/2014
2	8.08E+07	7.91	18.21	37		
6	2.24E+10	10.35	23.83	37		
22	2.60E+10	10.41	23.98	37		
46	3.99E+10	10.60	24.41	37		
0	2.46E+06	6.39	14.72	38	0	6/11/2015
1	8.59E+06	6.93	15.97	38		

Table F.22: Polymyxin B Composite Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
2	2.48E+08	8.39	19.33	38		
4	5.24E+10	10.72	24.68	38		
22	3.44E+11	11.54	26.56	38		
0	1.00E+00	0.00	0.00	39	0	6/11/2015
2	3.60E+01	1.56	3.58	39		
4	2.00E+02	2.30	5.30	39		
22	3.77E+02	2.58	5.93	39		
0	4.26E+05	5.63	12.96	40	0.25	5/20/2014
1	9.69E+03	3.99	9.18	40		
8	4.09E+02	2.61	6.01	40		
24	1.12E+10	10.05	23.14	40		
48	7.88E+10	10.90	25.09	40		
0	4.85E+05	5.69	13.09	41	1	5/20/2014
8	3.47E+02	2.54	5.85	41		
24	4.71E+09	9.67	22.27	41		
48	7.02E+10	10.85	24.97	41		
0	6.33E+05	5.80	13.36	42	2	5/29/2014
24	1.79E+06	6.25	14.40	42		
48	7.33E+10	10.87	25.02	42		
0	7.29E+05	5.86	13.50	43	4	5/29/2014
24	2.08E+06	6.32	14.55	43		
48	2.53E+09	9.40	21.65	43		
0	5.87E+05	5.77	13.28	44	0.0625	6/10/2014
1	1.10E+05	5.04	11.61	44		
2	4.91E+04	4.69	10.80	44		
0	6.26E+05	5.80	13.35	45	2	6/10/2014
8	3.88E+02	2.59	5.96	45		
24	4.70E+08	8.67	19.97	45		
48	1.10E+11	11.04	25.42	45		
0	5.66E+05	5.75	13.25	46	4	6/10/2014
8	1.23E+02	2.09	4.81	46		
24	7.23E+06	6.86	15.79	46		
48	6.87E+10	10.84	24.95	46		
0	6.72E+05	5.83	13.42	47	0.25	6/10/2014
1	4.69E+04	4.67	10.76	47		
2	2.04E+02	2.31	5.32	47		
8	1.19E+03	3.08	7.08	47		
24	9.95E+09	10.00	23.02	47		

Table F.22: Polymyxin B Composite Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
48	4.51E+10	10.65	24.53	47		
0	5.81E+05	5.76	13.27	48	1	6/10/2014
1	4.01E+03	3.60	8.30	48		
8	7.77E+02	2.89	6.66	48		
24	2.06E+10	10.31	23.75	48		
48	6.99E+10	10.84	24.97	48		
0	3.37E+05	5.53	12.73	49	0.0625	6/18/2014
1	2.78E+04	4.44	10.23	49		
2	1.19E+03	3.08	7.08	49		
0	3.67E+05	5.56	12.81	50	0.125	6/18/2014
4	1.23E+02	2.09	4.81	50		
8	1.83E+04	4.26	9.81	50		
24	4.96E+10	10.70	24.63	50		
48	1.42E+11	11.15	25.68	50		
0	7.51E+05	5.88	13.53	51	0.125	7/9/2014
1	3.70E+03	3.57	8.22	51		
2	9.40E+02	2.97	6.85	51		
4	5.33E+03	3.73	8.58	51		
8	2.22E+05	5.35	12.31	51		
24	8.15E+10	10.91	25.12	51		
48	1.39E+11	11.14	25.66	51		
0	5.37E+05	5.73	13.19	52	2	8/23/2014
8	9.40E+02	2.97	6.85	52		
24	4.95E+07	7.69	17.72	52		
48	1.02E+10	10.01	23.05	52		
0	5.04E+05	5.70	13.13	53	4	8/23/2014
8	1.23E+02	2.09	4.81	53		
24	6.66E+06	6.82	15.71	53		
48	2.12E+10	10.33	23.78	53		
0	1.63E+06	6.21	14.31	54	0	1/26/2014
2	9.31E+07	7.97	18.35	54		
6	7.23E+08	8.86	20.40	54		
22	1.04E+11	11.02	25.37	54		
46	8.31E+11	11.92	27.45	54		
0	2.48E+06	6.39	14.72	55	0	2/15/2014
2	1.80E+09	9.26	21.31	55		
6	5.13E+10	10.71	24.66	55		
22	1.04E+11	11.02	25.37	55		

Table F.22: Polymyxin B Composite Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
46	1.19E+12	12.08	27.81	55		
0	4.28E+06	6.63	15.27	56	0	3/30/2014
2	4.37E+09	9.64	22.20	56		
6	3.05E+10	10.48	24.14	56		
22	3.11E+11	11.49	26.46	56		
46	2.00E+11	11.30	26.02	56		
0	4.93E+06	6.69	15.41	57	0	6/11/2015
1	1.81E+07	7.26	16.71	57		
2	4.32E+08	8.64	19.88	57		
4	4.76E+10	10.68	24.59	57		
22	5.72E+11	11.76	27.07	57		
0	2.50E+01	1.40	3.22	58	0	6/11/2015
1	8.60E+01	1.93	4.45	58		
2	4.09E+02	2.61	6.01	58		
4	7.56E+02	2.88	6.63	58		
0	6.04E+05	5.78	13.31	59	0.0625	6/10/2014
1	6.95E+04	4.84	11.15	59		
0	5.29E+05	5.72	13.18	60	0.0625	6/18/2014
1	7.15E+02	2.85	6.57	60		
0	5.42E+05	5.73	13.20	61	0.125	6/18/2014
1	1.64E+02	2.21	5.10	61		
2	2.86E+02	2.46	5.66	61		
4	1.76E+03	3.25	7.47	61		
8	6.73E+04	4.83	11.12	61		
24	1.61E+11	11.21	25.80	61		
48	4.92E+10	10.69	24.62	61		
0	7.16E+05	5.85	13.48	62	0.125	7/9/2014
1	2.00E+03	3.30	7.60	62		
4	2.82E+03	3.45	7.94	62		
8	5.52E+04	4.74	10.92	62		
24	7.37E+10	10.87	25.02	62		
48	1.41E+11	11.15	25.67	62		
0	2.68E+05	5.43	12.50	63	0.25	3/30/2014
8	1.00E+03	3.00	6.91	63		
24	4.30E+10	10.63	24.48	63		
48	2.43E+10	10.39	23.91	63		
0	8.53E+05	5.93	13.66	64	0.25	4/19/2014
1	5.72E+02	2.76	6.35	64		

Table F.22: Polymyxin B Composite Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
4	1.23E+02	2.09	4.81	64		
8	9.81E+02	2.99	6.89	64		
24	1.05E+10	10.02	23.07	64		
48	9.97E+11	12.00	27.63	64		
0	8.93E+05	5.95	13.70	65	0.25	5/14/2014
1	1.74E+03	3.24	7.46	65		
4	1.64E+02	2.21	5.10	65		
8	2.76E+03	3.44	7.92	65		
24	1.76E+11	11.25	25.89	65		
48	3.44E+10	10.54	24.26	65		
0	4.48E+05	5.65	13.01	66	1	3/30/2014
8	5.11E+02	2.71	6.24	66		
24	8.29E+09	9.92	22.84	66		
48	8.94E+10	10.95	25.22	66		
0	8.13E+05	5.91	13.61	67	1	4/19/2014
4	2.25E+02	2.35	5.42	67		
8	7.15E+02	2.85	6.57	67		
24	8.23E+09	9.92	22.83	67		
48	3.09E+11	11.49	26.46	67		
0	9.82E+05	5.99	13.80	68	1	5/14/2014
1	7.15E+02	2.85	6.57	68		
4	1.84E+02	2.26	5.21	68		
8	2.55E+03	3.41	7.84	68		
24	1.11E+11	11.05	25.43	68		
48	1.67E+10	10.22	23.54	68		
0	6.47E+05	5.81	13.38	69	2	5/29/2014
8	1.64E+02	2.21	5.10	69		
24	2.27E+11	11.36	26.15	69		
48	3.74E+10	10.57	24.34	69		
0	6.18E+05	5.79	13.33	70	2	6/10/2014
2	2.04E+02	2.31	5.32	70		
24	1.21E+06	6.08	14.01	70		
48	1.27E+10	10.10	23.26	70		
0	4.75E+05	5.68	13.07	71	2	8/23/2014
24	5.38E+04	4.73	10.89	71		
48	2.98E+09	9.47	21.82	71		
0	5.56E+05	5.75	13.23	72	4	5/20/2014
0	6.53E+05	5.81	13.39	73	4	5/29/2014

Table F.22: Polymyxin B Composite Model Input Data

Adjusted Time	Viable Count	Log	Ln	Function	Drug Concentration	Date
8	5.72E+02	2.76	6.35	73		
24	2.30E+10	10.36	23.86	73		
48	3.77E+10	10.58	24.35	73		
0	5.87E+05	5.77	13.28	74	4	6/10/2014
24	6.52E+05	5.81	13.39	74		
48	4.00E+10	10.60	24.41	74		
0	7.75E+05	5.89	13.56	75	4	7/9/2014
1	2.86E+02	2.46	5.66	75		
8	1.02E+02	2.01	4.62	75		
24	2.13E+07	7.33	16.87	75		
48	3.17E+07	7.50	17.27	75		
0	1.10E+06	6.04	13.91	76	4	8/23/2014
24	2.68E+07	7.43	17.10	76		
48	5.87E+10	10.77	24.80	76		
0	9.48E+05	5.98	13.76	77	4	2/13/2017
1	1.95E+02	2.29	5.27	77		
1	7.10E+01	1.85	4.26	78	4	2/13/2017
2	8.20E+01	1.91	4.41	78		
4	2.10E+02	2.32	5.35	78		
8	1.12E+03	3.05	7.02	78		
8	6.54E+02	2.82	6.48	78		
8	9.81E+02	2.99	6.89	78		
24	1.31E+10	10.12	23.30	78		
24	7.44E+10	10.87	25.03	78		

Appendix G:

Win Nonlin® Code for Mathematical Models

Tables G.1-G.22 contain the Win Nonlin® code for each model.

Table G.1: Isolate 34 Growth Model Code

```
MODEL
remark   Three-Population Simple Net Effect Growth Model
remark   Klebsiella pneumoniae 34
remark   Growth Control Experiments with total and resistant subpopulations
remark   6 Functions, 4 Parameters
remark
remark   *****************************************************
remark   Developer:      Brandon Kulengowski
remark   Model Date:     8-7-2018
remark   Model Version:          1.0
remark   *****************************************************
remark
remark   parameters
remark   ----------
remark  gRLS
remark
remark  gRHS
remark
remark  gRLR
remark
remark  Nmax

remark - model-specific commands
COMMANDS
NFUN 6
NDER 6
NPAR 4
PNAMES 'gRLS' 'gRHS' 'gRLR' 'Nmax'
remark -initial estimates
remark -gRLS and Nmax initial estimates from pooled model, gRHS and gRLR initial estimates
from subpopulation analysis
END

remark - differential equations starting values
START
remark - RLS experiments
Z(1)=2.63*10**6
Z(2)=4.44*10**6
Z(3)=1.13*10**7
Z(4)=1.14*10**7
remark - RHS experiment
Z(5)=3.71*10**2
remark - RLR experiment
Z(6)=1.42*10**2
END

remark - differential equations
DIFF
```

Table G.1: Isolate 34 Growth Model Code

```
remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth
1/26/14
DZ(1)=(gRLS*(1-(Z(1)+Z(5)+Z(6))/Nmax))*Z(1)

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth
2/15/14
DZ(2)=(gRLS*(1-(Z(2)+Z(5)+Z(6))/Nmax))*Z(2)

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth
3/30/14
DZ(3)=(gRLS*(1-(Z(3)+Z(5)+Z(6))/Nmax))*Z(3)

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth
6/11/15
DZ(4)=(gRLS*(1-(Z(4)+Z(5)+Z(6))/Nmax))*Z(4)

remark  high-level meropenem-resistant and polymyxin B-susceptible subpopulation growth
6/11/15
DZ(5)=(gRHS*(1-(Z(4)+Z(5)+Z(6))/Nmax))*Z(5)

remark  low-level meropenem-resistant and polymyxin B-resistant subpopulation growth 6/11/15
DZ(6)=(gRLR*(1-(Z(4)+Z(5)+Z(6))/Nmax))*Z(6)

END

remark - algebraic functions
FUNC 1
F=log10(Z(1))
END
FUNC 2
F=log10(Z(2))
END
FUNC 3
F=log10(Z(3))
END
FUNC 4
F=log10(Z(4))
END
FUNC 5
F=log10(Z(5))
END
FUNC 6
F=log10(Z(6))
END

remark - end of model
EOM
```

Table G.2: Isolate 34 Meropenem Initial Killing Model Code

```
MODEL
remark   Logistic Meropenem Initial Killing Model
remark   Klebsiella pneumoniae 34
remark   Growth Control Experiments with MEM initial killing
remark   12 Functions, 12 differential equations, 4 Parameters
remark
remark   ****************************************************
remark   Developer:       Brandon Kulengowski
remark   Model Date:      9-19-2018
remark   Model Version:           1.0
remark   ****************************************************
remark
remark   parameters
remark   ----------
remark  gRLS
remark
remark  Nmax
remark
remark  kS
remark
remark  EC50S

remark - model-specific commands
COMMANDS
NFUN 12
NDER 12
NPAR 4
PNAMES 'gRLS' 'Nmax' 'kS' 'EC50S'
NSEC 1
SNAM 'SC'
END

remark - differential equations starting values
START
remark - Growth Control Experiments
Z(1)=2.63*10**6
Z(2)=4.44*10**6
Z(3)=1.13*10**7
Z(4)=1.14*10**7

remark - MEM Experiments
Z(5)=8.06*10**5
Z(6)=7.48*10**5
Z(7)=6.54*10**5
Z(8)=1.04*10**6
Z(9)=4.85*10**5
Z(10)=7.54*10**5
Z(11)=7.59*10**5
Z(12)=1.14*10**6
END
```

Table G.2: Isolate 34 Meropenem Initial Killing Model Code

```
remark - differential equations
DIFF

remark  low-level meropenem-resistant and high-level meropenem-resistant subpopulation growth 1/26/14
DZ(1)=(gRLS*(1-(Z(1))/Nmax))*Z(1)

remark  low-level meropenem-resistant and high-level meropenem-resistant subpopulation growth 2/15/14
DZ(2)=(gRLS*(1-(Z(2))/Nmax))*Z(2)

remark  low-level meropenem-resistant and high-level meropenem-resistant subpopulation growth 3/30/14
DZ(3)=(gRLS*(1-(Z(3))/Nmax))*Z(3)

remark  low-level meropenem-resistant and high-level meropenem-resistant subpopulation growth 6/11/15
DZ(4)=(gRLS*(1-(Z(4))/Nmax))*Z(4)

remark  MEM 4 monotherapy 1/26/14
DZ(5)=(gRLS*(1-(Z(5))/Nmax)-(kS*(4/(4+EC50S))))*Z(5)

remark  MEM 16 monotherapy 1/26/14
DZ(6)=(gRLS*(1-(Z(6))/Nmax)-(kS*(16/(16+EC50S))))*Z(6)

remark  MEM 64 monotherapy 1/26/14
DZ(7)=(gRLS*(1-(Z(7))/Nmax)-(kS*(64/(64+EC50S))))*Z(7)

remark  MEM 4 monotherapy 7/9/14
DZ(8)=(gRLS*(1-(Z(8))/Nmax)-(kS*(4/(4+EC50S))))*Z(8)

remark  MEM 16 monotherapy 7/9/14
DZ(9)=(gRLS*(1-(Z(9))/Nmax)-(kS*(16/(16+EC50S))))*Z(9)

remark  MEM 64 monotherapy 7/9/14
DZ(10)=(gRLS*(1-(Z(10))/Nmax)-(kS*(64/(64+EC50S))))*Z(10)

remark  MEM 8 monotherapy 2/13/17
DZ(11)=(gRLS*(1-(Z(11))/Nmax)-(kS*(8/(8+EC50S))))*Z(11)

remark  MEM 16 monotherapy 2/13/17
DZ(12)=(gRLS*(1-(Z(12))/Nmax)-(kS*(16/(16+EC50S))))*Z(12)

END

remark - algebraic functions
FUNC 1
F=log10(Z(1))
END
```

Table G.2: Isolate 34 Meropenem Initial Killing Model Code

```
FUNC 2
F=log10(Z(2))
END
FUNC 3
F=log10(Z(3))
END
FUNC 4
F=log10(Z(4))
END
FUNC 5
F=log10(Z(5))
END
FUNC 6
F=log10(Z(6))
END
FUNC 7
F=log10(Z(7))
END
FUNC 8
F=log10(Z(8))
END
FUNC 9
F=log10(Z(9))
END
FUNC 10
F=log10(Z(10))
END
FUNC 11
F=log10(Z(11))
END
FUNC 12
F=log10(Z(12))
END

SECONDARY
S(1)=(kS-gRLS)/gRLS*EC50S
END

remark - end of model
EOM
```

Table G.3: Isolate 34 Polymyxin B Initial Killing Model Code

```
MODEL
remark   Logistic Polymyxin B Initial Killing Model
remark   Klebsiella pneumoniae 34
remark   Growth Control Experiments with PMB initial killing
remark   17 Functions, 17 differential equations, 4 Parameters
remark
remark   ****************************************************
remark   Developer:       Brandon Kulengowski
remark   Model Date:      9-16-2018
remark   Model Version:           1.0
remark   ****************************************************
remark
remark   parameters
remark   ----------
remark  gRLS
remark
remark  Nmax
remark
remark  kS
remark
remark  EC50S

remark - model-specific commands
COMMANDS
NFUN 17
NDER 17
NPAR 4
PNAMES 'gRLS' 'Nmax' 'kS' 'EC50S'
NSEC 1
SNAM 'SCS'
END

remark - differential equations starting values
START
remark - S experiments
Z(1)=2.63*10**6
Z(2)=4.44*10**6
Z(3)=1.13*10**7
Z(4)=1.14*10**7

remark - PMB monotherapy experiments
Z(5)=7.07*10**5
Z(6)=6.54*10**5
Z(7)=7.23*10**5
Z(8)=8.00*10**5
Z(9)=7.79*10**5
Z(10)=6.92*10**5
Z(11)=6.85*10**5
Z(12)=5.01*10**5
Z(13)=5.15*10**5
```

Table G.3: Isolate 34 Polymyxin B Initial Killing Model Code

```
Z(14)=7.87*10**5
Z(15)=9.30*10**5
Z(16)=8.95*10**5
Z(17)=8.43*10**5

END

remark - differential equations
DIFF

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth 1/26/14
DZ(1)=(gRLS*(1-(Z(1))/Nmax))*Z(1)

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth 2/15/14
DZ(2)=(gRLS*(1-(Z(2))/Nmax))*Z(2)

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth 3/30/14
DZ(3)=(gRLS*(1-(Z(3))/Nmax))*Z(3)

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth 6/11/15
DZ(4)=(gRLS*(1-(Z(4))/Nmax))*Z(4)

remark  PMB 0.25 monotherapy 1/26/14
DZ(5)=(gRLS*(1-(Z(5))/Nmax)-(kS*(0.25/(0.25+EC50S))))*Z(5)

remark  PMB 1 monotherapy 1/26/14
DZ(6)=(gRLS*(1-(Z(6))/Nmax)-(kS*(1/(1+EC50S))))*Z(6)

remark  PMB 0.25 monotherapy 3/30/15
DZ(7)=(gRLS*(1-(Z(7))/Nmax)-(kS*(0.25/(0.25+EC50S))))*Z(7)

remark  PMB 2 monotherapy 5/29/14
DZ(8)=(gRLS*(1-(Z(8))/Nmax)-(kS*(2/(2+EC50S))))*Z(8)

remark  PMB 4 monotherapy 5/29/14
DZ(9)=(gRLS*(1-(Z(9))/Nmax)-(kS*(4/(4+EC50S))))*Z(9)

remark  PMB 2 monotherapy 6/10/14
DZ(10)=(gRLS*(1-(Z(10))/Nmax)-(kS*(2/(2+EC50S))))*Z(10)

remark  PMB 4 monotherapy 6/10/14
DZ(11)=(gRLS*(1-(Z(11))/Nmax)-(kS*(4/(4+EC50S))))*Z(11)

remark  PMB 0.0625 monotherapy 6/18/14
DZ(12)=(gRLS*(1-(Z(12))/Nmax)-(kS*(0.0625/(0.0625+EC50S))))*Z(12)
```

Table G.3: Isolate 34 Polymyxin B Initial Killing Model Code

```
remark  PMB 0.125 monotherapy 6/18/14
DZ(13)=(gRLS*(1-(Z(13))/Nmax)-(kS*(0.125/(0.125+EC50S))))*Z(13)

remark  PMB 0.0625 monotherapy 7/9/14
DZ(14)=(gRLS*(1-(Z(14))/Nmax)-(kS*(0.0625/(0.0625+EC50S))))*Z(14)

remark  PMB 0.125 monotherapy 7/9/14
DZ(15)=(gRLS*(1-(Z(15))/Nmax)-(kS*(0.125/(0.125+EC50S))))*Z(15)

remark  PMB 0.25 monotherapy 8/23/14
DZ(16)=(gRLS*(1-(Z(16))/Nmax)-(kS*(0.25/(0.25+EC50S))))*Z(16)

remark  PMB 1 monotherapy 8/23/14
DZ(17)=(gRLS*(1-(Z(17))/Nmax)-(kS*(1/(1+EC50S))))*Z(17)

END

remark - algebraic functions
FUNC 1
F=log10(Z(1))
END
FUNC 2
F=log10(Z(2))
END
FUNC 3
F=log10(Z(3))
END
FUNC 4
F=log10(Z(4))
END
FUNC 5
F=log10(Z(5))
END
FUNC 6
F=log10(Z(6))
END
FUNC 7
F=log10(Z(7))
END
FUNC 8
F=log10(Z(8))
END
FUNC 9
F=log10(Z(9))
END
FUNC 10
F=log10(Z(10))
END
FUNC 11
F=log10(Z(11))
```

Table G.3: Isolate 34 Polymyxin B Initial Killing Model Code

```
END
FUNC 12
F=log10(Z(12))
END
FUNC 13
F=log10(Z(13))
END
FUNC 14
F=log10(Z(14))
END
FUNC 15
F=log10(Z(15))
END
FUNC 16
F=log10(Z(16))
END
FUNC 17
F=log10(Z(17))
END

SECO
SCS=(kS-gRLS)/gRLS*EC50S
END

remark - end of model
EOM
```

Table G.4: Isolate 34 Meropenem Monotherapy Model Code

```
MODEL
remark   Two-Population Simple Meropenem Net Effect Model
remark   Klebsiella pneumoniae 34
remark   Meropenem-low-resistant and high-resistant subpopulations
remark   13 Functions, 24 Differential Equations, 6 Parameters
remark
remark   ******************************************************
remark   Developer:       Brandon Kulengowski
remark   Model Date:      9-17-2018
remark   Model Version:           1.0
remark   ******************************************************
remark
remark   parameters
remark   ------------
remark  gS
remark
remark  gR
remark
remark  Nmax
remark
remark  kS
remark
remark  EC50S
remark
remark  kR

remark - model-specific commands
COMMANDS
NFUN 13
NDER 24
NPAR 6
PNAMES 'gS' 'gR' 'Nmax' 'kS' 'EC50S' 'kR'
NSEC 4
SNAMES 'SCS' 'SCR' 'dS' 'dR'
SIZE 6
END

TEMP
R0=64.2
END

remark - differential equations starting values
START
Z(1)=2.63*10**6
Z(2)=R0
Z(3)=4.44*10**6
Z(4)=R0
Z(5)=1.13*10**7
Z(6)=R0
Z(23)=1.14*10**7
```

Table G.4: Isolate 34 Meropenem Monotherapy Model Code

```
Z(24)=3.71*10**2
Z(7)=8.06*10**5
Z(8)=R0
Z(9)=7.48*10**5
Z(10)=R0
Z(11)=6.54*10**5
Z(12)=R0
Z(13)=1.04*10**6
Z(14)=R0
Z(15)=4.85*10**5
Z(16)=R0
Z(17)=7.54*10**5
Z(18)=R0
Z(19)=7.59*10**5
Z(20)=R0
Z(21)=1.14*10**6
Z(22)=R0
END

remark - differential equations
DIFF

remark  growth control experiment 1/26/14
DZ(1)=(gS*(1-(Z(1)+Z(2))/Nmax))*Z(1)
DZ(2)=(gR*(1-(Z(1)+Z(2))/Nmax))*Z(2)

remark  growth control experiment 2/15/14
DZ(3)=(gS*(1-(Z(3)+Z(4))/Nmax))*Z(3)
DZ(4)=(gR*(1-(Z(3)+Z(4))/Nmax))*Z(4)

remark  growth control experiment 3/30/14
DZ(5)=(gS*(1-(Z(5)+Z(6))/Nmax))*Z(5)
DZ(6)=(gR*(1-(Z(5)+Z(6))/Nmax))*Z(6)

remark  growth control experiment MEM low-resistance subpopulation 6/11/15
DZ(23)=(gS*(1-(Z(23)+Z(24))/Nmax))*Z(23)

remark    growth control experiment MEM high-resistance subpopulation 6/11/15
DZ(24)=(gR*(1-(Z(23)+Z(24))/Nmax))*Z(24)

remark  meropenem 4 mg/L time-kill experiment 1/26/14
DZ(7)=(gS*(1-(Z(7)+Z(8))/Nmax)-kS*(4/(4+EC50S)))*Z(7)
DZ(8)=(gR*(1-(Z(7)+Z(8))/Nmax)-kR*4)*Z(8)

remark  meropenem 16 mg/L time-kill experiment 1/26/14
DZ(9)=(gS*(1-(Z(9)+Z(10))/Nmax)-kS*(16/(16+EC50S)))*Z(9)
DZ(10)=(gR*(1-(Z(9)+Z(10))/Nmax)-kR*16)*Z(10)

remark  meropenem 64 mg/L time-kill experiment 1/26/14
DZ(11)=(gS*(1-(Z(11)+Z(12))/Nmax)-kS*(64/(64+EC50S)))*Z(11)
```

Table G.4: Isolate 34 Meropenem Monotherapy Model Code

```
DZ(12)=(gR*(1-(Z(11)+Z(12))/Nmax)-kR*64)*Z(12)

remark  meropenem 4 mg/L time-kill experiment 5/14/14
DZ(13)=(gS*(1-(Z(13)+Z(14))/Nmax)-kS*(4/(4+EC50S)))*Z(13)
DZ(14)=(gR*(1-(Z(13)+Z(14))/Nmax)-kR*4)*Z(14)

remark  meropenem 16 mg/L time-kill experiment 5/14/14
DZ(15)=(gS*(1-(Z(15)+Z(16))/Nmax)-kS*(16/(16+EC50S)))*Z(15)
DZ(16)=(gR*(1-(Z(15)+Z(16))/Nmax)-kR*16)*Z(16)

remark  meropenem 64 mg/L time-kill experiment 5/20/14
DZ(17)=(gS*(1-(Z(17)+Z(18))/Nmax)-kS*(64/(64+EC50S)))*Z(17)
DZ(18)=(gR*(1-(Z(17)+Z(18))/Nmax)-kR*64)*Z(18)

remark  meropenem 8 mg/L time-kill experiment 2/13/14
DZ(19)=(gS*(1-(Z(19)+Z(20))/Nmax)-kS*(8/(8+EC50S)))*Z(19)
DZ(20)=(gR*(1-(Z(19)+Z(20))/Nmax)-kR*8)*Z(20)

remark  meropenem 16 mg/L time-kill experiment 2/13/14
DZ(21)=(gS*(1-(Z(21)+Z(22))/Nmax)-kS*(16/(16+EC50S)))*Z(21)
DZ(22)=(gR*(1-(Z(21)+Z(22))/Nmax)-kR*16)*Z(22)

END

remark - algebraic functions
FUNC 1
F=log10(Z(1)+Z(2))
END
FUNC 2
F=log10(Z(3)+Z(4))
END
FUNC 3
F=log10(Z(5)+Z(6))
END
FUNC 4
F=log10(Z(23)+Z(24))
END
FUNC 5
F=log10(Z(24))
END
FUNC 6
F=log10(Z(7)+Z(8))
END
FUNC 7
F=log10(Z(9)+Z(10))
END
FUNC 8
F=log10(Z(11)+Z(12))
END
FUNC 9
```

Table G.4: Isolate 34 Meropenem Monotherapy Model Code

```
F=log10(Z(13)+Z(14))
END
FUNC 10
F=log10(Z(15)+Z(16))
END
FUNC 11
F=log10(Z(17)+Z(18))
END
FUNC 12
F=log10(Z(19)+Z(20)+1)
END
FUNC 13
F=log10(Z(21)+Z(22))
END


remark - secondary parameters
SECO
SCS=(gS/(kS-gS))*EC50S
SCR=gR/kR
dS=0.6931/gS*60
dR=0.6931/gR*60
END

remark - end of model
EOM
```

Table G.5: Isolate 34 Polymyxin B Monotherapy Model Code

```
MODEL
remark   Two-Population Simple Polymyxin B Net Effect Model
remark   Klebsiella pneumoniae 34
remark   Polymyxin B-susceptible and -resistant subpopulations
remark   19 Functions, 36 Differential Equations, 6 Parameters
remark
remark   ******************************************************
remark   Developer:      Brandon Kulengowski
remark   Model Date:     9-17-2018
remark   Model Version:          1.0
remark   ******************************************************
remark
remark   parameters
remark   ----------
remark  gS
remark
remark  gR
remark
remark  Nmax
remark
remark  kS
remark
remark  EC50S
remark
remark  kR

remark - model-specific commands
COMMANDS
NFUN 19
NDER 36
NPAR 6
PNAMES 'gS' 'gR' 'Nmax' 'kS' 'EC50S' 'kR'
NSEC 4
SNAMES 'SCS' 'SCR' 'dS' 'dR'
SIZE 6
END

TEMP
R0=19
END

remark - differential equations starting values
START
Z(1)=2.63*10**6
Z(2)=R0
Z(3)=2.44*10**6
Z(4)=R0
Z(5)=1.13*10**7
Z(6)=R0
Z(7)=1.14*10**7
```

Table G.5: Isolate 34 Polymyxin B Monotherapy Model Code

```
Z(8)=1.42*10**2
Z(9)=7.07*10**5
Z(10)=R0
Z(11)=6.54*10**5
Z(12)=R0
Z(13)=7.23*10**5
Z(14)=R0
Z(15)=5.84*10**5
Z(16)=R0
Z(17)=8.00*10**5
Z(18)=R0
Z(19)=7.79*10**5
Z(20)=R0
Z(21)=6.92*10**5
Z(22)=R0
Z(23)=6.85*10**5
Z(24)=R0
Z(25)=5.01*10**5
Z(26)=R0
Z(27)=5.15*10**5
Z(28)=R0
Z(29)=7.87*10**5
Z(30)=R0
Z(31)=9.30*10**5
Z(32)=R0
Z(33)=8.95*10**5
Z(34)=R0
Z(35)=8.43*10**5
Z(36)=R0
END

remark - differential equations
DIFF

remark  growth control experiment 1/26/14
DZ(1)=(gS*(1-(Z(1)+Z(2))/Nmax))*Z(1)
DZ(2)=(gR*(1-(Z(1)+Z(2))/Nmax))*Z(2)

remark  growth control experiment 2/15/14
DZ(3)=(gS*(1-(Z(3)+Z(4))/Nmax))*Z(3)
DZ(4)=(gR*(1-(Z(3)+Z(4))/Nmax))*Z(4)

remark  growth control experiment 3/30/14
DZ(5)=(gS*(1-(Z(5)+Z(6))/Nmax))*Z(5)
DZ(6)=(gR*(1-(Z(5)+Z(6))/Nmax))*Z(6)

remark  growth control experiment PMB susceptible subpopulation 6/11/15
DZ(7)=(gS*(1-(Z(7)+Z(8))/Nmax))*Z(7)

remark  growth control experiment PMB resistant subpopulation 6/11/15
```

Table G.5: Isolate 34 Polymyxin B Monotherapy Model Code

```
DZ(8)=(gR*(1-(Z(7)+Z(8))/Nmax))*Z(8)

remark  polymyxin B 0.25 mg/L time-kill experiment 1/26/14
DZ(9)=(gS*(1-(Z(9)+Z(10))/Nmax)-kS*(0.25/(0.25+EC50S)))*Z(9)
DZ(10)=(gR*(1-(Z(9)+Z(10))/Nmax)-kR*0.25)*Z(10)

remark  polymyxin B 1 mg/L time-kill experiment 1/26/14
DZ(11)=(gS*(1-(Z(11)+Z(12))/Nmax)-kS*(1/(1+EC50S)))*Z(11)
DZ(12)=(gR*(1-(Z(11)+Z(12))/Nmax)-kR*1)*Z(12)

remark  polymyxin B 0.25 mg/L time-kill experiment 3/30/15
DZ(13)=(gS*(1-(Z(13)+Z(14))/Nmax)-kS*(0.25/(0.25+EC50S)))*Z(13)
DZ(14)=(gR*(1-(Z(13)+Z(14))/Nmax)-kR*0.25)*Z(14)

remark  polymyxin B 1 mg/L time-kill experiment 3/30/15
DZ(15)=(gS*(1-(Z(15)+Z(16))/Nmax)-kS*(1/(1+EC50S)))*Z(15)
DZ(16)=(gR*(1-(Z(15)+Z(16))/Nmax)-kR*1)*Z(16)

remark  polymyxin B 2 mg/L time-kill experiment 5/29/14
DZ(17)=(gS*(1-(Z(17)+Z(18))/Nmax)-kS*(2/(2+EC50S)))*Z(17)
DZ(18)=(gR*(1-(Z(17)+Z(18))/Nmax)-kR*2)*Z(18)

remark  polymyxin B 4 mg/L time-kill experiment 5/29/14
DZ(19)=(gS*(1-(Z(19)+Z(20))/Nmax)-kS*(4/(4+EC50S)))*Z(19)
DZ(20)=(gR*(1-(Z(19)+Z(20))/Nmax)-kR*4)*Z(20)

remark  polymyxin B 2 mg/L time-kill experiment 6/10/14
DZ(21)=(gS*(1-(Z(21)+Z(22))/Nmax)-kS*(2/(2+EC50S)))*Z(21)
DZ(22)=(gR*(1-(Z(21)+Z(22))/Nmax)-kR*2)*Z(22)

remark  polymyxin B 4 mg/L time-kill experiment 6/10/14
DZ(23)=(gS*(1-(Z(23)+Z(24))/Nmax)-kS*(4/(4+EC50S)))*Z(23)
DZ(24)=(gR*(1-(Z(23)+Z(24))/Nmax)-kR*4)*Z(24)

remark  polymyxin B 0.0625 mg/L time-kill experiment 6/18/14
DZ(25)=(gS*(1-(Z(25)+Z(26))/Nmax)-kS*(0.0625/(0.0625+EC50S)))*Z(25)
DZ(26)=(gR*(1-(Z(25)+Z(26))/Nmax)-kR*0.0625)*Z(26)

remark  polymyxin B 0.125 mg/L time-kill experiment 6/18/14
DZ(27)=(gS*(1-(Z(27)+Z(28))/Nmax)-kS*(0.125/(0.125+EC50S)))*Z(27)
DZ(28)=(gR*(1-(Z(27)+Z(28))/Nmax)-kR*0.125)*Z(28)

remark  polymyxin B 0.0625 mg/L time-kill experiment 7/9/14
DZ(29)=(gS*(1-(Z(29)+Z(30))/Nmax)-kS*(0.0625/(0.0625+EC50S)))*Z(29)
DZ(30)=(gR*(1-(Z(29)+Z(30))/Nmax)-kR*0.0625)*Z(30)

remark  polymyxin B 0.125 mg/L time-kill experiment 7/9/14
DZ(31)=(gS*(1-(Z(31)+Z(32))/Nmax)-kS*(0.125/(0.125+EC50S)))*Z(31)
DZ(32)=(gR*(1-(Z(31)+Z(32))/Nmax)-kR*0.125)*Z(32)
```

Table G.5: Isolate 34 Polymyxin B Monotherapy Model Code

```
remark  polymyxin B 0.25 mg/L time-kill experiment 8/23/17
DZ(33)=(gS*(1-(Z(33)+Z(34))/Nmax)-kS*(0.25/(0.25+EC50S)))*Z(33)
DZ(34)=(gR*(1-(Z(33)+Z(34))/Nmax)-kR*0.25)*Z(34)

remark  polymyxin B 1 mg/L time-kill experiment 8/23/17
DZ(35)=(gS*(1-(Z(35)+Z(36))/Nmax)-kS*(1/(1+EC50S)))*Z(35)
DZ(36)=(gR*(1-(Z(35)+Z(36))/Nmax)-kR*1)*Z(36)

END

remark - algebraic functions
FUNC 1
F=log10(Z(1)+Z(2))
END
FUNC 2
F=log10(Z(3)+Z(4))
END
FUNC 3
F=log10(Z(5)+Z(6))
END
FUNC 4
F=log10(Z(7)+Z(8))
END
FUNC 5
F=log10(Z(8))
END
FUNC 6
F=log10(Z(9)+Z(10))
END
FUNC 7
F=log10(Z(11)+Z(12))
END
FUNC 8
F=log10(Z(13)+Z(14))
END
FUNC 9
F=log10(Z(15)+Z(16))
END
FUNC 10
F=log10(Z(17)+Z(18))
END
FUNC 11
F=log10(Z(19)+Z(20))
END
FUNC 12
F=log10(Z(21)+Z(22))
END
FUNC 13
F=log10(Z(23)+Z(24))
END
```

Table G.5: Isolate 34 Polymyxin B Monotherapy Model Code

```
FUNC 14
F=log10(Z(25)+Z(26))
END
FUNC 15
F=log10(Z(27)+Z(28))
END
FUNC 16
F=log10(Z(29)+Z(30)+1)
END
FUNC 17
F=log10(Z(31)+Z(32))
END
FUNC 18
F=log10(Z(33)+Z(34))
END
FUNC 19
F=log10(Z(35)+Z(36))
END

remark - secondary parameters
SECO
SCS=(gS/(kS-gS))*EC50S
SCR=gR/kR
dS=0.6931/gS*60
dR=0.6931/gR*60
END

remark - end of model
EOM
```

Table G.6: Isolate 22 Growth Model Code

```
MODEL
remark   Three-Population Simple Net Growth Model
remark   Klebsiella pneumoniae 22
remark   Growth Control Experiments with total and resistant subpopulations
remark   5 Functions, 5 differential equations 4 Parameters
remark
remark   ****************************************************
remark   Developer:      Brandon Kulengowski
remark   Model Date:     9-16-2018
remark   Model Version:          1.0
remark   ****************************************************
remark
remark   parameters
remark   ----------
remark  gRLS
remark
remark  gRHS
remark
remark  gRLR
remark
remark  Nmax

remark - model-specific commands
COMMANDS
NFUN 5
NDER 5
NPAR 4
PNAMES 'gRLS' 'gRHS' 'gRLR' 'Nmax'
remark -initial estimates
remark -gRLS and Nmax initial estimates from pooled model, gRHS and gRLR initial estimates
from subpopulation analysis
END

remark - differential equations starting values
START
remark - RLS experiments
Z(1)=3.14*10**6
Z(2)=2.51*10**6
Z(3)=6.23*10**6
remark - RHS experiment
Z(4)=7.93*10**2
remark - RLR experiment
Z(5)=2.54*10**2
END

remark - differential equations
DIFF

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth
5/29/14
```

Table G.6: Isolate 22 Growth Model Code

```
DZ(1)=(gRLS*(1-(Z(1)+Z(4)+Z(5))/Nmax))*Z(1)

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth
8/23/14
DZ(2)=(gRLS*(1-(Z(2)+Z(4)+Z(5))/Nmax))*Z(2)

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth
6/11/15
DZ(3)=(gRLS*(1-(Z(3)+Z(4)+Z(5))/Nmax))*Z(3)

remark  high-level meropenem-resistant and polymyxin B-susceptible subpopulation growth
6/11/15
DZ(4)=(gRHS*(1-(Z(3)+Z(4)+Z(5))/Nmax))*Z(4)

remark  low-level meropenem-resistant and polymyxin B-resistant subpopulation growth 6/11/15
DZ(5)=(gRLR*(1-(Z(3)+Z(4)+Z(5))/Nmax))*Z(5)

END

remark - algebraic functions
FUNC 1
F=log10(Z(1))
END
FUNC 2
F=log10(Z(2))
END
FUNC 3
F=log10(Z(3))
END
FUNC 4
F=log10(Z(4))
END
FUNC 5
F=log10(Z(5))
END

remark - end of model
EOM
```

Table G.7: Isolate 22 Meropenem Initial Killing Model Code

```
MODEL
remark   Logistic Meropenem Initial Killing Model
remark   Klebsiella pneumoniae 22
remark   Growth Control Experiments with MEM initial killing
remark   11 Functions, 4 Parameters
remark
remark   ****************************************************
remark   Developer:      Brandon Kulengowski
remark   Model Date:     9-19-2018
remark   Model Version:          1.0
remark   ****************************************************
remark
remark   parameters
remark   ----------
remark  gRLS
remark
remark  Nmax
remark
remark  kS
remark
remark  EC50S

remark - model-specific commands
COMMANDS
NFUN 11
NDER 11
NPAR 4
PNAMES 'gRLS' 'Nmax' 'kS' 'EC50S'
NSEC 1
SNAM 'SC'
END

remark - differential equations starting values
START
remark - RLS experiments
Z(1)=3.14*10**6
Z(2)=2.51*10**6
Z(3)=6.23*10**6
remark - MEM experiments
Z(4)=1.70*10**6
Z(5)=1.26*10**6
Z(6)=9.34*10**5
Z(7)=6.11*10**5
Z(8)=3.52*10**5
Z(9)=5.28*10**5
Z(10)=7.82*10**5
Z(11)=8.00*10**5

END
```

Table G.7: Isolate 22 Meropenem Initial Killing Model Code

```
remark - differential equations
DIFF

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth 5/29/14
DZ(1)=(gRLS*(1-(Z(1))/Nmax))*Z(1)

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth 8/23/14
DZ(2)=(gRLS*(1-(Z(2))/Nmax))*Z(2)

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth 6/11/15
DZ(3)=(gRLS*(1-(Z(3))/Nmax))*Z(3)

remark  MEM 4 monotherapy 5/14/14
DZ(4)=(gRLS*(1-(Z(4))/Nmax)-(kS*(4/(4+EC50S))))*Z(4)

remark  MEM 16 monotherapy 5/14/14
DZ(5)=(gRLS*(1-(Z(5))/Nmax)-(kS*(16/(16+EC50S))))*Z(5)

remark  MEM 64 monotherapy 5/14/14
DZ(6)=(gRLS*(1-(Z(6))/Nmax)-(kS*(64/(64+EC50S))))*Z(6)

remark  MEM 4 monotherapy 5/20/14
DZ(7)=(gRLS*(1-(Z(7))/Nmax)-(kS*(4/(4+EC50S))))*Z(7)

remark  MEM 16 monotherapy 5/20/14
DZ(8)=(gRLS*(1-(Z(8))/Nmax)-(kS*(16/(16+EC50S))))*Z(8)


remark  MEM 64 monotherapy 5/20/14
DZ(9)=(gRLS*(1-(Z(9))/Nmax)-(kS*(64/(64+EC50S))))*Z(9)


remark  MEM 16 monotherapy 2/13/17
DZ(10)=(gRLS*(1-(Z(10))/Nmax)-(kS*(16/(16+EC50S))))*Z(10)


remark  MEM 32 monotherapy 2/13/17
DZ(11)=(gRLS*(1-(Z(11))/Nmax)-(kS*(32/(32+EC50S))))*Z(11)
END

remark - algebraic functions
FUNC 1
F=log10(Z(1))
END
FUNC 2
F=log10(Z(2))
END
```

Table G.7: Isolate 22 Meropenem Initial Killing Model Code

```
FUNC 3
F=log10(Z(3))
END
FUNC 4
F=log10(Z(4))
END
FUNC 5
F=log10(Z(5))
END
FUNC 6
F=log10(Z(6))
END
FUNC 7
F=log10(Z(7))
END
FUNC 8
F=log10(Z(8))
END
FUNC 9
F=log10(Z(9))
END
FUNC 10
F=log10(Z(10))
END
FUNC 11
F=log10(Z(11))
END

SECONDARY
S(1)=(kS-gRLS)/gRLS*EC50S
END

remark - end of model
EOM
```

Table G.8: Isolate 22 Polymyxin B Initial Killing Model Code

```
MODEL
remark   Logistic Polymyxin B Initial Killing Model
remark   Klebsiella pneumoniae 22
remark   Growth Control Experiments with PMB initial killing
remark   8 Functions, 8 differential equations, 4 Parameters
remark
remark   ****************************************************
remark   Developer:       Brandon Kulengowski
remark   Model Date:      9-16-2018
remark   Model Version:           1.0
remark   ****************************************************
remark
remark   parameters
remark   ------------
remark  gRLS
remark
remark  Nmax
remark
remark  kS
remark
remark  EC50S

remark - model-specific commands
COMMANDS
NFUN 8
NDER 8
NPAR 4
PNAMES 'gRLS' 'Nmax' 'kS' 'EC50S'
NSEC 1
SNAM 'SC'
END

remark - differential equations starting values
START
remark - RLS experiments
Z(1)=3.14*10**6
Z(2)=2.51*10**6
Z(3)=6.23*10**6
remark - RLR experiment
Z(4)=1.34E+06
Z(5)=5.27E+05
Z(6)=4.56E+05
Z(7)=4.93E+05
Z(8)=6.91E+05
END

remark - differential equations
DIFF
```

Table G.8: Isolate 22 Polymyxin B Initial Killing Model Code

```
remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth 5/29/14
DZ(1)=(gRLS*(1-(Z(1))/Nmax))*Z(1)

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth 8/23/14
DZ(2)=(gRLS*(1-(Z(2))/Nmax))*Z(2)

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth 6/11/15
DZ(3)=(gRLS*(1-(Z(3))/Nmax))*Z(3)

remark  PMB 0.25 monotherapy 5/14/14
DZ(4)=(gRLS*(1-(Z(4))/Nmax)-(kS*(0.25/(0.25+EC50S))))*Z(4)

remark  PMB 0.0625 monotherapy 6/10/14
DZ(5)=(gRLS*(1-(Z(5))/Nmax)-(kS*(0.0625/(0.0625+EC50S))))*Z(5)

remark  PMB 0.0625 monotherapy 6/18/14
DZ(6)=(gRLS*(1-(Z(6))/Nmax)-(kS*(0.0625/(0.0625+EC50S))))*Z(6)

remark  PMB 0.125 monotherapy 6/18/14
DZ(7)=(gRLS*(1-(Z(7))/Nmax)-(kS*(0.125/(0.125+EC50S))))*Z(7)

remark  PMB 0.125 monotherapy 7/9/14
DZ(8)=(gRLS*(1-(Z(8))/Nmax)-(kS*(0.125/(0.125+EC50S))))*Z(8)
END

remark - algebraic functions
FUNC 1
F=log10(Z(1))
END
FUNC 2
F=log10(Z(2))
END
FUNC 3
F=log10(Z(3))
END
FUNC 4
F=log10(Z(4))
END
FUNC 5
F=log10(Z(5))
END
FUNC 6
F=log10(Z(6))
END
FUNC 7
F=log10(Z(7))
END
```

Table G.8: Isolate 22 Polymyxin B Initial Killing Model Code

```
FUNC 8
F=log10(Z(8))
END

SECONDARY
S(1)=(kS-gRLS)/gRLS*EC50S
END

remark - end of model
EOM
```

Table G.9: Isolate 22 Meropenem Monotherapy Model Code

```
MODEL
remark   Two-Population Simple Net Effect Model
remark   Klebsiella pneumoniae 22
remark   Meropenem-low-resistant and high-resistant subpopulations
remark   13 Functions, 22 Differential Equations, 6 Parameters
remark
remark   ******************************************************
remark   Developer:       Brandon Kulengowski
remark   Model Date:      9-17-2018
remark   Model Version:           1.0
remark   ******************************************************
remark
remark   parameters
remark   ------------
remark  gS
remark
remark  gR
remark
remark  Nmax
remark
remark  kS
remark
remark  EC50S
remark
remark  kR

remark - model-specific commands
COMMANDS
NFUN 13
NDER 22
NPAR 6
PNAMES 'gS' 'gR' 'Nmax' 'kS' 'EC50S' 'kR'
NSEC 4
SNAMES 'SCS' 'SCR' 'dS' 'dR'
SIZE 6
END

TEMP
R0=13
END

remark - differential equations starting values
START
Z(1)=3.14*10**6
Z(2)=R0
Z(3)=2.51*10**6
Z(4)=R0
Z(5)=6.23*10**6
Z(6)=7.93*10**2
Z(7)=1.70*10**6
```

Table G.9: Isolate 22 Meropenem Monotherapy Model Code

```
Z(8)=R0
Z(9)=1.26*10**6
Z(10)=R0
Z(11)=9.34*10**5
Z(12)=R0
Z(13)=6.11*10**5
Z(14)=R0
Z(15)=3.52*10**5
Z(16)=R0
Z(17)=5.28*10**5
Z(18)=R0
Z(19)=7.82*10**5
Z(20)=R0
Z(21)=8.00*10**5
Z(22)=R0
END

remark - differential equations
DIFF

remark  growth control experiment 5/29/14
DZ(1)=(gS*(1-(Z(1)+Z(2))/Nmax))*Z(1)
DZ(2)=(gR*(1-(Z(1)+Z(2))/Nmax))*Z(2)

remark  growth control experiment 8/23/14
DZ(3)=(gS*(1-(Z(3)+Z(4))/Nmax))*Z(3)
DZ(4)=(gR*(1-(Z(3)+Z(4))/Nmax))*Z(4)

remark  growth control experiment 6/11/15
DZ(5)=(gS*(1-(Z(5)+Z(6))/Nmax))*Z(5)

remark  growth control experiment MEM resistant subpopulation 6/11/15
DZ(6)=(gR*(1-(Z(5)+Z(6))/Nmax))*Z(6)

remark  meropenem 4 mg/L time-kill experiment 5/14/14
DZ(7)=(gS*(1-(Z(7)+Z(8))/Nmax)-kS*(4/(4+EC50S)))*Z(7)
DZ(8)=(gR*(1-(Z(7)+Z(8))/Nmax)-kR*4)*Z(8)

remark  meropenem 16 mg/L time-kill experiment 5/14/14
DZ(9)=(gS*(1-(Z(9)+Z(10))/Nmax)-kS*(16/(16+EC50S)))*Z(9)
DZ(10)=(gR*(1-(Z(9)+Z(10))/Nmax)-kR*16)*Z(10)

remark  meropenem 64 mg/L time-kill experiment 5/20/14
DZ(11)=(gS*(1-(Z(11)+Z(12))/Nmax)-kS*(64/(64+EC50S)))*Z(11)
DZ(12)=(gR*(1-(Z(11)+Z(12))/Nmax)-kR*64)*Z(12)

remark  meropenem 4 mg/L time-kill experiment 5/20/14
DZ(13)=(gS*(1-(Z(13)+Z(14))/Nmax)-kS*(4/(4+EC50S)))*Z(13)
DZ(14)=(gR*(1-(Z(13)+Z(14))/Nmax)-kR*4)*Z(14)
```

Table G.9: Isolate 22 Meropenem Monotherapy Model Code

```
remark  meropenem 16 mg/L time-kill experiment 5/29/14
DZ(15)=(gS*(1-(Z(15)+Z(16))/Nmax)-kS*(16/(16+EC50S)))*Z(15)
DZ(16)=(gR*(1-(Z(15)+Z(16))/Nmax)-kR*16)*Z(16)

remark  meropenem 64 mg/L time-kill experiment 5/29/14
DZ(17)=(gS*(1-(Z(17)+Z(18))/Nmax)-kS*(64/(64+EC50S)))*Z(17)
DZ(18)=(gR*(1-(Z(17)+Z(18))/Nmax)-kR*64)*Z(18)

remark  meropenem 16 mg/L time-kill experiment 2/13/14
DZ(19)=(gS*(1-(Z(19)+Z(20))/Nmax)-kS*(16/(16+EC50S)))*Z(19)

remark  meropenem 16 mg/L time-kill resistant subpopulation experiment 2/13/14
DZ(20)=(gR*(1-(Z(19)+Z(20))/Nmax)-kR*16)*Z(20)

remark  meropenem 32 mg/L time-kill experiment 2/13/14
DZ(21)=(gS*(1-(Z(21)+Z(22))/Nmax)-kS*(32/(32+EC50S)))*Z(21)

remark  meropenem 32 mg/L time-kill resistant subpopulation experiment 2/13/14
DZ(22)=(gR*(1-(Z(21)+Z(22))/Nmax)-kR*32)*Z(22)

END

remark - algebraic functions
FUNC 1
F=log10(Z(1)+Z(2))
END
FUNC 2
F=log10(Z(3)+Z(4))
END
FUNC 3
F=log10(Z(5)+Z(6))
END
FUNC 4
F=log10(Z(6))
END
FUNC 5
F=log10(Z(7)+Z(8))
END
FUNC 6
F=log10(Z(9)+Z(10))
END
FUNC 7
F=log10(Z(11)+Z(12))
END
FUNC 8
F=log10(Z(13)+Z(14))
END
FUNC 9
F=log10(Z(15)+Z(16))
END
```

Table G.9: Isolate 22 Meropenem Monotherapy Model Code

```
FUNC 10
F=log10(Z(17)+Z(18))
END
FUNC 11
F=log10(Z(19))
END
FUNC 12
F=log10(Z(20)+1)
END
FUNC 13
F=log10(Z(21)+Z(22))
END

remark - secondary parameters
SECO
SCS=(gS/(kS-gS))*EC50S
SCR=gR/kR
dS=0.6931/gS*60
dR=0.6931/gR*60
END

remark - end of model
EOM
```

Table G.10: Isolate 22 Polymyxin B Monotherapy Model Code

```
MODEL
remark   Two-Population Simple Net Effect Model
remark   Klebsiella pneumoniae 22
remark   Polymyxin B-susceptible and -resistant subpopulations
remark   16 Functions, 28 Differential Equations, 5 Parameters
remark
remark   ******************************************************
remark   Developer:       Brandon Kulengowski
remark   Model Date:      9-17-2018
remark   Model Version:           1.0
remark   ******************************************************
remark
remark   parameters
remark   ------------
remark  gS
remark
remark  gR
remark
remark  Nmax
remark
remark  kS
remark
remark  kR

remark - model-specific commands
COMMANDS
NFUN 16
NDER 28
NPAR 5
PNAMES 'gS' 'gR' 'Nmax' 'kS' 'kR'
NSEC 4
SNAMES 'SCS' 'SCR' 'dS' 'dR'
SIZE 6
END

TEMP
R0=0.63
END

remark - differential equations starting values
START
Z(1)=3.14*10**6
Z(2)=R0
Z(3)=2.51*10**6
Z(4)=R0
Z(5)=6.23*10**6
Z(6)=2.54*10**2
Z(7)=1.34*10**6
Z(8)=R0
Z(9)=1.43*10**6
```

Table G.10: Isolate 22 Polymyxin B Monotherapy Model Code

```
Z(10)=R0
Z(11)=5.01*10**5
Z(12)=R0
Z(13)=5.53*10**5
Z(14)=R0
Z(15)=6.68*10**5
Z(16)=R0
Z(17)=6.60*10**5
Z(18)=R0
Z(19)=5.27*10**5
Z(20)=5.64*10**5
Z(21)=R0
Z(22)=5.00*10**5
Z(23)=R0
Z(24)=4.56*10**5
Z(25)=4.93*10**5
Z(26)=R0
Z(27)=6.91*10**5
Z(28)=R0
END

remark - differential equations
DIFF

remark  growth control experiment 5/29/14
DZ(1)=(gS*(1-(Z(1)+Z(2))/Nmax))*Z(1)
DZ(2)=(gR*(1-(Z(1)+Z(2))/Nmax))*Z(2)

remark  growth control experiment 8/23/14
DZ(3)=(gS*(1-(Z(3)+Z(4))/Nmax))*Z(3)
DZ(4)=(gR*(1-(Z(3)+Z(4))/Nmax))*Z(4)

remark  growth control experiment 6/11/15
DZ(5)=(gS*(1-(Z(5)+Z(6))/Nmax))*Z(5)

remark  growth control experiment PMB resistant subpopulation 6/11/15
DZ(6)=(gR*(1-(Z(5)+Z(6))/Nmax))*Z(6)

remark  polymyxin B 0.25 mg/L time-kill experiment 5/14/14
DZ(7)=(gS*(1-(Z(7)+Z(8))/Nmax)-kS)*Z(7)
DZ(8)=(gR*(1-(Z(7)+Z(8))/Nmax)-kR*0.25)*Z(8)

remark  polymyxin B 1 mg/L time-kill experiment 5/14/14
DZ(9)=(gS*(1-(Z(9)+Z(10))/Nmax)-kS)*Z(9)
DZ(10)=(gR*(1-(Z(9)+Z(10))/Nmax)-kR*1)*Z(10)

remark  polymyxin B 0.25 mg/L time-kill experiment 5/20/14
DZ(11)=(gS*(1-(Z(11)+Z(12))/Nmax)-kS)*Z(11)
DZ(12)=(gR*(1-(Z(11)+Z(12))/Nmax)-kR*0.25)*Z(12)
```

Table G.10: Isolate 22 Polymyxin B Monotherapy Model Code

```
remark  polymyxin B 1 mg/L time-kill experiment 5/20/14
DZ(13)=(gS*(1-(Z(13)+Z(14))/Nmax)-kS)*Z(13)
DZ(14)=(gR*(1-(Z(13)+Z(14))/Nmax)-kR*1)*Z(14)

remark  polymyxin B 2 mg/L time-kill experiment 5/29/14
DZ(15)=(gS*(1-(Z(15)+Z(16))/Nmax)-kS)*Z(15)
DZ(16)=(gR*(1-(Z(15)+Z(16))/Nmax)-kR*2)*Z(16)

remark  polymyxin B 4 mg/L time-kill experiment 5/29/14
DZ(17)=(gS*(1-(Z(17)+Z(18))/Nmax)-kS)*Z(17)
DZ(18)=(gR*(1-(Z(17)+Z(18))/Nmax)-kR*4)*Z(18)

remark  polymyxin B 0.0625 mg/L time-kill experiment 6/10/14
DZ(19)=(gS*(1-(Z(19))/Nmax)-kS)*Z(19)

remark  polymyxin B 2 mg/L time-kill experiment 6/10/14
DZ(20)=(gS*(1-(Z(20)+Z(21))/Nmax)-kS)*Z(20)
DZ(21)=(gR*(1-(Z(20)+Z(21))/Nmax)-kR*2)*Z(21)

remark  polymyxin B 4 mg/L time-kill experiment 6/10/14
DZ(22)=(gS*(1-(Z(22)+Z(23))/Nmax)-kS)*Z(22)
DZ(23)=(gR*(1-(Z(22)+Z(23))/Nmax)-kR*4)*Z(23)

remark  polymyxin B 0.0625 mg/L time-kill experiment 6/18/14
DZ(24)=(gS*(1-Z(24)/Nmax)-kS)*Z(24)

remark  polymyxin B 0.125 mg/L time-kill experiment 6/18/14
DZ(25)=(gS*(1-(Z(25)+Z(26))/Nmax)-kS)*Z(25)
DZ(26)=(gR*(1-(Z(25)+Z(26))/Nmax)-kR*0.125)*Z(26)

remark  polymyxin B 0.125 mg/L time-kill experiment 7/9/14
DZ(27)=(gS*(1-(Z(27)+Z(28))/Nmax)-kS)*Z(27)
DZ(28)=(gR*(1-(Z(27)+Z(28))/Nmax)-kR*0.125)*Z(28)

END

remark - algebraic functions
FUNC 1
F=log10(Z(1)+Z(2))
END
FUNC 2
F=log10(Z(3)+Z(4))
END
FUNC 3
F=log10(Z(5)+Z(6))
END
FUNC 4
F=log10(Z(6))
END
FUNC 5
```

Table G.10: Isolate 22 Polymyxin B Monotherapy Model Code

```
F=log10(Z(7)+Z(8))
END
FUNC 6
F=log10(Z(9)+Z(10))
END
FUNC 7
F=log10(Z(11)+Z(12))
END
FUNC 8
F=log10(Z(13)+Z(14))
END
FUNC 9
F=log10(Z(15)+Z(16))
END
FUNC 10
F=log10(Z(17)+Z(18))
END
FUNC 11
F=log10(Z(19))
END
FUNC 12
F=log10(Z(20)+Z(21))
END
FUNC 13
F=log10(Z(22)+Z(23))
END
FUNC 14
F=log10(Z(24))
END
FUNC 15
F=log10(Z(25)+Z(26))
END
FUNC 16
F=log10(Z(27)+Z(28))
END

remark - secondary parameters
SECO
SCS=0
SCR=gR/kR
dS=0.6931/gS*60
dR=0.6931/gR*60
END

remark - end of model
EOM
```

Table G.11: Isolate 24 Growth Model Code

```
MODEL
remark   Three-Population Simple Net Growth Model
remark   Klebsiella pneumoniae 24
remark   Growth Control Experiments with total and resistant subpopulations
remark   5 Functions, 5 differential equations, 4 Parameters
remark
remark   ****************************************************
remark   Developer:      Brandon Kulengowski
remark   Model Date:     7-17-2018
remark   Model Version:          1.0
remark   ****************************************************
remark
remark   parameters
remark   ----------
remark  gRLS
remark
remark  gRHS
remark
remark  gRLR
remark
remark  Nmax

remark - model-specific commands
COMMANDS
NFUN 5
NDER 5
NPAR 4
PNAMES 'gRLS' 'gRHS' 'gRLR' 'Nmax'
remark -initial estimates
remark -gRLS and Nmax initial estimates from pooled model, gRHS and gRLR initial estimates
from subpopulation analysis
END

remark - differential equations starting values
START
remark - RLS experiments
Z(1)=1.77*10**6
Z(2)=2.00*10**6
Z(3)=2.46*10**6
remark - RHS experiment
Z(4)=5.7*10**1
remark - RLR experiment
Z(5)=7.0*10**0
END

remark - differential equations
DIFF

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth
5/29/14
```

Table G.11: Isolate 24 Growth Model Code

```
DZ(1)=(gRLS*(1-(Z(1)+Z(4)+Z(5))/Nmax))*Z(1)

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth
8/23/14
DZ(2)=(gRLS*(1-(Z(2)+Z(4)+Z(5))/Nmax))*Z(2)

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth
6/11/15
DZ(3)=(gRLS*(1-(Z(3)+Z(4)+Z(5))/Nmax))*Z(3)

remark  high-level meropenem-resistant and polymyxin B-susceptible subpopulation growth
6/11/15
DZ(4)=(gRHS*(1-(Z(3)+Z(4)+Z(5))/Nmax))*Z(4)

remark  low-level meropenem-resistant and polymyxin B-resistant subpopulation growth 6/11/15
DZ(5)=(gRLR*(1-(Z(3)+Z(4)+Z(5))/Nmax))*Z(5)

END

remark - algebraic functions
FUNC 1
F=log10(Z(1))
END
FUNC 2
F=log10(Z(2))
END
FUNC 3
F=log10(Z(3))
END
FUNC 4
F=log10(Z(4))
END
FUNC 5
F=log10(Z(5))
END

remark - end of model
EOM
```

Table G.12: Isolate 24 Meropenem Initial Killing Model Code

```
MODEL
remark   Logistic Meropenem Initial Killing Model
remark   Klebsiella pneumoniae 24
remark   Growth Control Experiments with MEM initial killing
remark   11 Functions, 11 differential equations, 4 Parameters
remark
remark   ****************************************************
remark   Developer:      Jeffrey J. Campion, Brandon Kulengowski
remark   Model Date:     9-19-2018
remark   Model Version:          1.0
remark   ****************************************************
remark
remark   parameters
remark   ----------
remark  gRLS
remark
remark  Nmax
remark
remark  kS
remark
remark  EC50S

remark - model-specific commands
COMMANDS
NFUN 11
NDER 11
NPAR 4
PNAMES 'gRLS' 'Nmax' 'kS' 'EC50S'
NSEC 1
SNAM 'SC'
END

remark - differential equations starting values
START
remark - Growth Control Experiments
Z(1)=1.77*10**6
Z(2)=2.00*10**6
Z(3)=2.46*10**6

remark - MEM Experiments
Z(4)=4.79*10**5
Z(5)=5.68*10**5
Z(6)=4.36*10**5
Z(7)=4.84*10**5
Z(8)=6.34*10**5
Z(9)=5.40*10**5
Z(10)=5.29*10**5
Z(11)=5.31*10**5
END
```

Table G.12: Isolate 24 Meropenem Initial Killing Model Code

```
remark - differential equations
DIFF

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth 5/29/14
DZ(1)=(gRLS*(1-(Z(1))/Nmax))*Z(1)

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth 8/23/14
DZ(2)=(gRLS*(1-(Z(2))/Nmax))*Z(2)

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth 6/11/15
DZ(3)=(gRLS*(1-(Z(3))/Nmax))*Z(3)

remark  MEM 4 monotherapy 5/20/14
DZ(4)=(gRLS*(1-(Z(4))/Nmax)-(kS*(4/(4+EC50S))))*Z(4)

remark  MEM 16 monotherapy 5/20/14
DZ(5)=(gRLS*(1-(Z(5))/Nmax)-(kS*(16/(16+EC50S))))*Z(5)

remark  MEM 64 monotherapy 5/20/14
DZ(6)=(gRLS*(1-(Z(6))/Nmax)-(kS*(64/(64+EC50S))))*Z(6)

remark  MEM 4 monotherapy 6/10/14
DZ(7)=(gRLS*(1-(Z(7))/Nmax)-(kS*(4/(4+EC50S))))*Z(7)

remark  MEM 16 monotherapy 6/10/14
DZ(8)=(gRLS*(1-(Z(8))/Nmax)-(kS*(16/(16+EC50S))))*Z(8)


remark  MEM 64 monotherapy 6/10/14
DZ(9)=(gRLS*(1-(Z(9))/Nmax)-(kS*(64/(64+EC50S))))*Z(9)


remark  MEM 64 monotherapy 2/13/17
DZ(10)=(gRLS*(1-(Z(10))/Nmax)-(kS*(64/(64+EC50S))))*Z(10)


remark  MEM 128 monotherapy 2/13/17
DZ(11)=(gRLS*(1-(Z(11))/Nmax)-(kS*(128/(128+EC50S))))*Z(11)
END

remark - algebraic functions
FUNC 1
F=log10(Z(1))
END
FUNC 2
F=log10(Z(2))
END
```

Table G.12: Isolate 24 Meropenem Initial Killing Model Code

```
FUNC 3
F=log10(Z(3))
END
FUNC 4
F=log10(Z(4))
END
FUNC 5
F=log10(Z(5))
END
FUNC 6
F=log10(Z(6))
END
FUNC 7
F=log10(Z(7))
END
FUNC 8
F=log10(Z(8))
END
FUNC 9
F=log10(Z(9))
END
FUNC 10
F=log10(Z(10))
END
FUNC 11
F=log10(Z(11))
END

SECONDARY
S(1)=(kS-gRLS)/gRLS*EC50S
END

remark - end of model
EOM
```

Table G.13: Isolate 24 Polymyxin B Initial Killing Model Code

```
MODEL
remark   Logistic Polymyxin B Initial Killing Model
remark   Klebsiella pneumoniae 24
remark   Growth Control Experiments with PMB initial killing
remark   9 Functions, 9 differential equations, 4 Parameters
remark
remark   ****************************************************
remark   Developer:       Brandon Kulengowski
remark   Model Date:      9-19-2018
remark   Model Version:           1.0
remark   ****************************************************
remark
remark   parameters
remark   ----------
remark  gRLS
remark
remark  Nmax
remark
remark  kS
remark
remark  EC50S

remark - model-specific commands
COMMANDS
NFUN 9
NDER 9
NPAR 4
PNAMES 'gRLS' 'Nmax' 'kS' 'EC50S'
NSEC 1
SNAM 'SC'
END

remark - differential equations starting values
START
remark - Growth Control Experiments
Z(1)=1.77*10**6
Z(2)=2.00*10**6
Z(3)=2.46*10**6

remark - PMB Experiments
Z(4)=4.26*10**5
Z(5)=5.87*10**5
Z(6)=6.72*10**5
Z(7)=5.81*10**5
Z(8)=3.37*10**5
Z(9)=7.51*10**5
END

remark - differential equations
DIFF
```

Table G.13: Isolate 24 Polymyxin B Initial Killing Model Code

```
remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth 5/29/14
DZ(1)=(gRLS*(1-(Z(1))/Nmax))*Z(1)

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth 8/23/14
DZ(2)=(gRLS*(1-(Z(2))/Nmax))*Z(2)

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth 6/11/15
DZ(3)=(gRLS*(1-(Z(3))/Nmax))*Z(3)

remark  PMB 0.25 monotherapy 5/20/14
DZ(4)=(gRLS*(1-(Z(4))/Nmax)-(kS*(0.25/(0.25+EC50S))))*Z(4)

remark  PMB 0.0625 monotherapy 6/10/14
DZ(5)=(gRLS*(1-(Z(5))/Nmax)-(kS*(0.0625/(0.0625+EC50S))))*Z(5)

remark  PMB 0.25 monotherapy 6/10/14
DZ(6)=(gRLS*(1-(Z(6))/Nmax)-(kS*(0.25/(0.25+EC50S))))*Z(6)

remark  PMB 1 monotherapy 6/10/14
DZ(7)=(gRLS*(1-(Z(7))/Nmax)-(kS*(1/(1+EC50S))))*Z(7)

remark  PMB 0.0625 monotherapy 6/18/14
DZ(8)=(gRLS*(1-(Z(8))/Nmax)-(kS*(0.0625/(0.0625+EC50S))))*Z(8)

remark  PMB 0.125 monotherapy 7/9/14
DZ(9)=(gRLS*(1-(Z(9))/Nmax)-(kS*(0.125/(0.125+EC50S))))*Z(9)

END

remark - algebraic functions
FUNC 1
F=log10(Z(1))
END
FUNC 2
F=log10(Z(2))
END
FUNC 3
F=log10(Z(3))
END
FUNC 4
F=log10(Z(4))
END
FUNC 5
F=log10(Z(5))
END
FUNC 6
```

Table G.13: Isolate 24 Polymyxin B Initial Killing Model Code

```
F=log10(Z(6))
END
FUNC 7
F=log10(Z(7))
END
FUNC 8
F=log10(Z(8))
END
FUNC 9
F=log10(Z(9))
END

SECONDARY
S(1)=(kS-gRLS)/gRLS*EC50S
END

remark - end of model
EOM
```

Table G.14: Isolate 24 Meropenem Monotherapy Model Code

```
MODEL
remark   Two-Population Simple Net Effect Model
remark   Klebsiella pneumoniae 24
remark   Meropenem-low-resistant and high-resistant subpopulations
remark   13 Functions, 23 Differential Equations, 5 Parameters
remark
remark   ****************************************************
remark   Developer:       Brandon Kulengowski
remark   Model Date:      9-17-2018
remark   Model Version:           1.0
remark   ****************************************************
remark
remark   parameters
remark   ------------
remark  gS
remark
remark  gR
remark
remark  Nmax
remark
remark  kS
remark
remark  kR

remark - model-specific commands
COMMANDS
NFUN 13
NDER 23
NPAR 5
PNAMES 'gS' 'gR' 'Nmax' 'kS' 'kR'
NSEC 4
SNAMES 'SCS' 'SCR' 'dS' 'dR'
SIZE 6
END

TEMP
R0=3.27
END

remark - differential equations starting values
START
Z(1)=1.77*10**6
Z(2)=R0
Z(3)=2.00*10**6
Z(4)=R0
Z(5)=2.446*10**6
Z(6)=5.7*10**1
Z(7)=4.79*10**5
Z(8)=R0
Z(9)=5.68*10**5
```

Table G.14: Isolate 24 Meropenem Monotherapy Model Code

```
Z(10)=R0
Z(11)=4.36*10**5
Z(12)=R0
Z(13)=4.84*10**5
Z(14)=R0
Z(15)=6.34*10**5
Z(16)=R0
Z(17)=5.40*10**5
Z(18)=R0
Z(19)=5.29*10**5
Z(20)=R0
Z(21)=5.31*10**5
Z(22)=R0
Z(23)=R0
END

remark - differential equations
DIFF

remark  growth control experiment 5/29/14
DZ(1)=(gS*(1-(Z(1)+Z(2))/Nmax))*Z(1)
DZ(2)=(gR*(1-(Z(1)+Z(2))/Nmax))*Z(2)

remark  growth control experiment 8/23/14
DZ(3)=(gS*(1-(Z(3)+Z(4))/Nmax))*Z(3)
DZ(4)=(gR*(1-(Z(3)+Z(4))/Nmax))*Z(4)

remark  growth control experiment 6/11/15
DZ(5)=(gS*(1-(Z(5)+Z(6))/Nmax))*Z(5)

remark  growth control experiment MEM resistant subpopulation 6/11/15
DZ(6)=(gR*(1-(Z(5)+Z(6))/Nmax))*Z(6)

remark  meropenem 4 mg/L time-kill experiment 5/20/14
DZ(7)=(gS*(1-(Z(7)+Z(8))/Nmax)-kS)*Z(7)
DZ(8)=(gR*(1-(Z(7)+Z(8))/Nmax)-kR*4)*Z(8)

remark  meropenem 16 mg/L time-kill experiment 5/20/14
DZ(9)=(gS*(1-(Z(9)+Z(10))/Nmax)-kS)*Z(9)
DZ(10)=(gR*(1-(Z(9)+Z(10))/Nmax)-kR*16)*Z(10)

remark  meropenem 64 mg/L time-kill experiment 5/20/14
DZ(11)=(gS*(1-(Z(11)+Z(12))/Nmax)-kS)*Z(11)
DZ(12)=(gR*(1-(Z(11)+Z(12))/Nmax)-kR*64)*Z(12)

remark  meropenem 4 mg/L time-kill experiment 6/10/14
DZ(13)=(gS*(1-(Z(13)+Z(14))/Nmax)-kS)*Z(13)
DZ(14)=(gR*(1-(Z(13)+Z(14))/Nmax)-kR*4)*Z(14)

remark  meropenem 16 mg/L time-kill experiment 6/10/14
```

Table G.14: Isolate 24 Meropenem Monotherapy Model Code

```
DZ(15)=(gS*(1-(Z(15)+Z(16))/Nmax)-kS)*Z(15)
DZ(16)=(gR*(1-(Z(15)+Z(16))/Nmax)-kR*16)*Z(16)

remark  meropenem 64 mg/L time-kill experiment 6/10/14
DZ(17)=(gS*(1-(Z(17)+Z(18))/Nmax)-kS)*Z(17)
DZ(18)=(gR*(1-(Z(17)+Z(18))/Nmax)-kR*64)*Z(18)

remark  meropenem 64 mg/L time-kill experiment 2/13/14
DZ(19)=(gS*(1-(Z(19)+Z(20))/Nmax)-kS)*Z(19)
DZ(20)=(gR*(1-(Z(19)+Z(20))/Nmax)-kR*64)*Z(20)

remark  meropenem 128 mg/L time-kill experiment 2/13/14
DZ(21)=(gS*(1-(Z(21)+Z(22))/Nmax)-kS)*Z(21)
DZ(22)=(gR*(1-(Z(21)+Z(22))/Nmax)-kR*128)*Z(22)

remark  meropenem 64 mg/L time-kill experiment 2/13/14 MEM resistant subpopulation MIC
>64 mg/L
DZ(23)=(gR*(1-(Z(19)+Z(23))/Nmax)-kR*64)*Z(23)

END

remark - algebraic functions
FUNC 1
F=log10(Z(1)+Z(2))
END
FUNC 2
F=log10(Z(3)+Z(4))
END
FUNC 3
F=log10(Z(5)+Z(6))
END
FUNC 4
F=log10(Z(6))
END
FUNC 5
F=log10(Z(7)+Z(8))
END
FUNC 6
F=log10(Z(9)+Z(10))
END
FUNC 7
F=log10(Z(11)+Z(12))
END
FUNC 8
F=log10(Z(13)+Z(14))
END
FUNC 9
F=log10(Z(15)+Z(16))
END
FUNC 10
```

Table G.14: Isolate 24 Meropenem Monotherapy Model Code

```
F=log10(Z(17)+Z(18)+1)
END
FUNC 11
F=log10(Z(19)+Z(20))
END
FUNC 12
F=log10(Z(21)+Z(22))
END
FUNC 13
F=log10(Z(23))
END

remark - secondary parameters
SECO
SCS=0
SCR=gR/kR
dS=0.6931/gS*60
dR=0.6931/gR*60
END

remark - end of model
EOM
```

Table G.15: Isolate 24 Polymyxin B Monotherapy Model Code

```
MODEL
remark   Two-Population Simple Net Effect Model
remark   Klebsiella pneumoniae 24
remark   Polymyxin B-susceptible and -resistant subpopulations
remark   18 Functions, 34 Differential Equations, 6 Parameters
remark
remark   ********************************************************
remark   Developer:       Brandon Kulengowski
remark   Model Date:      9-17-2018
remark   Model Version:           1.0
remark   ********************************************************
remark
remark   parameters
remark   ------------
remark  gS
remark
remark  gR
remark
remark  Nmax
remark
remark  kS
remark
remark  EC50S
remark
remark  kR

remark - model-specific commands
COMMANDS
NFUN 18
NDER 34
NPAR 6
PNAMES 'gS' 'gR' 'Nmax' 'kS' 'EC50S' 'kR'
NSEC 4
SNAMES 'SCS' 'SCR' 'dS' 'dR'
SIZE 6
END

TEMP
R0=1
END

remark - differential equations starting values
START
Z(1)=1.77*10**6
Z(2)=R0
Z(3)=2.00*10**6
Z(4)=R0
Z(5)=2.46*10**6
Z(6)=1.00*10**0
Z(7)=4.26*10**5
```

Table G.15: Isolate 24 Polymyxin B Monotherapy Model Code

```
Z(8)=R0
Z(9)=4.85*10**5
Z(10)=R0
Z(11)=6.33*10**5
Z(12)=R0
Z(13)=7.29*10**5
Z(14)=R0
Z(15)=5.87*10**5
Z(16)=R0
Z(17)=6.26*10**5
Z(18)=R0
Z(19)=5.66*10**5
Z(20)=R0
Z(21)=6.72*10**5
Z(22)=R0
Z(23)=5.81*10**5
Z(24)=R0
Z(25)=3.37*10**5
Z(26)=R0
Z(27)=3.67*10**5
Z(28)=R0
Z(29)=7.51*10**5
Z(30)=R0
Z(31)=5.37*10**5
Z(32)=R0
Z(33)=5.04*10**5
Z(34)=R0
END

remark - differential equations
DIFF

remark  growth control experiment 5/29/14
DZ(1)=(gS*(1-(Z(1)+Z(2))/Nmax))*Z(1)
DZ(2)=(gR*(1-(Z(1)+Z(2))/Nmax))*Z(2)

remark  growth control experiment 8/23/14
DZ(3)=(gS*(1-(Z(3)+Z(4))/Nmax))*Z(3)
DZ(4)=(gR*(1-(Z(3)+Z(4))/Nmax))*Z(4)

remark  growth control experiment 6/11/15
DZ(5)=(gS*(1-(Z(5)+Z(6))/Nmax))*Z(5)

remark  growth control experiment PMB resistant subpopulation 6/11/15
DZ(6)=(gR*(1-(Z(5)+Z(6))/Nmax))*Z(6)

remark  polymyxin B 0.25 mg/L time-kill experiment 5/20/14
DZ(7)=(gS*(1-(Z(7)+Z(8))/Nmax)-kS*(0.25/(0.25+EC50S)))*Z(7)
DZ(8)=(gR*(1-(Z(7)+Z(8))/Nmax)-kR*(0.25))*Z(8)
```

Table G.15: Isolate 24 Polymyxin B Monotherapy Model Code

```
remark  polymyxin B 1 mg/L time-kill experiment 5/20/14
DZ(9)=(gS*(1-(Z(9)+Z(10))/Nmax)-kS*(1/(1+EC50S)))*Z(9)
DZ(10)=(gR*(1-(Z(9)+Z(10))/Nmax)-kR*(1))*Z(10)

remark  polymyxin B 2 mg/L time-kill experiment 5/29/14
DZ(11)=(gS*(1-(Z(11)+Z(12))/Nmax)-kS*(2/(2+EC50S)))*Z(11)
DZ(12)=(gR*(1-(Z(11)+Z(12))/Nmax)-kR*(2))*Z(12)

remark  polymyxin B 4 mg/L time-kill experiment 5/29/14
DZ(13)=(gS*(1-(Z(13)+Z(14))/Nmax)-kS*(4/(4+EC50S)))*Z(13)
DZ(14)=(gR*(1-(Z(13)+Z(14))/Nmax)-kR*(4))*Z(14)

remark  polymyxin B 0.0625 mg/L time-kill experiment 6/10/14
DZ(15)=(gS*(1-(Z(15)+Z(16))/Nmax)-kS*(0.0625/(0.0625+EC50S)))*Z(15)
DZ(16)=(gR*(1-(Z(15)+Z(16))/Nmax)-kR*(0.0625))*Z(16)

remark  polymyxin B 2 mg/L time-kill experiment 6/10/14
DZ(17)=(gS*(1-(Z(17)+Z(18))/Nmax)-kS*(2/(2+EC50S)))*Z(17)
DZ(18)=(gR*(1-(Z(17)+Z(18))/Nmax)-kR*(2))*Z(18)

remark  polymyxin B 4 mg/L time-kill experiment 6/10/14
DZ(19)=(gS*(1-(Z(19)+Z(20))/Nmax)-kS*(4/(4+EC50S)))*Z(19)
DZ(20)=(gR*(1-(Z(19)+Z(20))/Nmax)-kR*(4))*Z(20)

remark  polymyxin B 0.25 mg/L time-kill experiment 6/10/14
DZ(21)=(gS*(1-(Z(21)+Z(22))/Nmax)-kS*(0.25/(0.25+EC50S)))*Z(21)
DZ(22)=(gR*(1-(Z(21)+Z(22))/Nmax)-kR*(0.25))*Z(22)

remark  polymyxin B 1 mg/L time-kill experiment 6/10/14
DZ(23)=(gS*(1-(Z(23)+Z(24))/Nmax)-kS*(1/(1+EC50S)))*Z(23)
DZ(24)=(gR*(1-(Z(23)+Z(24))/Nmax)-kR*(1))*Z(24)

remark  polymyxin B 0.0625 mg/L time-kill experiment 6/18/14
DZ(25)=(gS*(1-(Z(25)+Z(26))/Nmax)-kS*(0.0625/(0.0625+EC50S)))*Z(25)
DZ(26)=(gR*(1-(Z(15)+Z(26))/Nmax)-kR*(0.0625))*Z(26)

remark  polymyxin B 0.125 mg/L time-kill experiment 6/18/14
DZ(27)=(gS*(1-(Z(27)+Z(28))/Nmax)-kS*(0.125/(0.125+EC50S)))*Z(27)
DZ(28)=(gR*(1-(Z(27)+Z(28))/Nmax)-kR*(0.125))*Z(28)

remark  polymyxin B 0.125 mg/L time-kill experiment 7/9/14
DZ(29)=(gS*(1-(Z(29)+Z(30))/Nmax)-kS*(0.125/(0.125+EC50S)))*Z(29)
DZ(30)=(gR*(1-(Z(29)+Z(30))/Nmax)-kR*(0.125))*Z(30)

remark  polymyxin B 2 mg/L time-kill experiment 8/23/17
DZ(31)=(gS*(1-(Z(31)+Z(32))/Nmax)-kS*(2/(2+EC50S)))*Z(31)
DZ(32)=(gR*(1-(Z(31)+Z(32))/Nmax)-kR*(2))*Z(32)

remark  polymyxin B 4 mg/L time-kill experiment 8/23/17
DZ(33)=(gS*(1-(Z(33)+Z(34))/Nmax)-kS*(4/(4+EC50S)))*Z(33)
```

Table G.15: Isolate 24 Polymyxin B Monotherapy Model Code

```
DZ(34)=(gR*(1-(Z(33)+Z(34))/Nmax)-kR*(4))*Z(34)

END

remark - algebraic functions
FUNC 1
F=log10(Z(1)+Z(2))
END
FUNC 2
F=log10(Z(3)+Z(4))
END
FUNC 3
F=log10(Z(5)+Z(6))
END
FUNC 4
F=log10(Z(6))
END
FUNC 5
F=log10(Z(7)+Z(8))
END
FUNC 6
F=log10(Z(9)+Z(10))
END
FUNC 7
F=log10(Z(11)+Z(12))
END
FUNC 8
F=log10(Z(13)+Z(14))
END
FUNC 9
F=log10(Z(15)+Z(16))
END
FUNC 10
F=log10(Z(17)+Z(18))
END
FUNC 11
F=log10(Z(19)+Z(20))
END
FUNC 12
F=log10(Z(21)+Z(22))
END
FUNC 13
F=log10(Z(23)+Z(24))
END
FUNC 14
F=log10(Z(25)+Z(26))
END
FUNC 15
F=log10(Z(27)+Z(28))
END
```

Table G.15: Isolate 24 Polymyxin B Monotherapy Model Code

```
FUNC 16
F=log10(Z(29)+Z(30))
END
FUNC 17
F=log10(Z(31)+Z(32))
END
FUNC 18
F=log10(Z(33)+Z(34))
END

remark - secondary parameters
SECO
SCS=(gS/(kS-gS))*EC50S
SCR=gR/kR
dS=0.6931/gS*60
dR=0.6931/gR*60
END

remark - end of model
EOM
```

Table G.16: Isolate 44 Growth Model Code

```
MODEL
remark   Three-Population Simple Net Growth Model
remark   Klebsiella pneumoniae 44
remark   Growth Control Experiments with total and resistant subpopulations 6/11/15
remark   6 Functions, 4 Parameters
remark
remark   *****************************************************
remark   Developer:      Jeffrey J. Campion, Brandon Kulengowski
remark   Model Date:     7-17-2018
remark   Model Version:          1.0
remark   *****************************************************
remark
remark   parameters
remark   ----------
remark  gRLS
remark
remark  gRHS
remark
remark  gRLR
remark
remark  Nmax

remark - model-specific commands
COMMANDS
NFUN 6
NDER 6
NPAR 4
PNAMES 'gRLS' 'gRHS' 'gRLR' 'Nmax'
remark -initial estimates 2.6244093, 2.0018887, 1.1347168, 1.1476822E+11
remark -gRLS and Nmax initial estimates from pooled model, gRHS and gRLR initial estimates
from subpopulation model
END

remark - differential equations starting values
START
remark - RLS experiments
Z(1)=1.63*10**6
Z(2)=2.48*10**6
Z(3)=4.29*10**6
Z(4)=4.93*10**6
remark - RHS experiment
Z(5)=6.95*10**4
remark - RLR experiment
Z(6)=2.50*10**1
END

remark - differential equations
DIFF
```

Table G.16: Isolate 44 Growth Model Code

```
remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth
1/26/14
DZ(1)=(gRLS*(1-(Z(1)+Z(5)+Z(6))/Nmax))*Z(1)

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth
2/15/14
DZ(2)=(gRLS*(1-(Z(2)+Z(5)+Z(6))/Nmax))*Z(2)

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth
3/30/14
DZ(3)=(gRLS*(1-(Z(3)+Z(5)+Z(6))/Nmax))*Z(3)

remark  low-level meropenem-resistant and polymyxin B-susceptible subpopulation growth
6/11/15
DZ(4)=(gRLS*(1-(Z(4)+Z(5)+Z(6))/Nmax))*Z(4)

remark  high-level meropenem-resistant and polymyxin B-susceptible subpopulation growth
6/11/15
DZ(5)=(gRHS*(1-(Z(4)+Z(5)+Z(6))/Nmax))*Z(5)

remark  low-level meropenem-resistant and polymyxin B-resistant subpopulation growth 6/11/15
DZ(6)=(gRLR*(1-(Z(4)+Z(5)+Z(6))/Nmax))*Z(6)

END

remark - algebraic functions
FUNC 1
F=log10(Z(1))
END
FUNC 2
F=log10(Z(2))
END
FUNC 3
F=log10(Z(3))
END
FUNC 4
F=log10(Z(4))
END
FUNC 5
F=log10(Z(5))
END
FUNC 6
F=log10(Z(6))
END

remark - end of model
EOM
```

Table G.17: Isolate 44 Meropenem Initial Killing Model Code

```
MODEL
remark   Logistic Meropenem Initial Killing Model
remark   Klebsiella pneumoniae 44
remark   Growth Control Experiments with MEM initial killing
remark   12 Functions, 12 differential equations, 4 Parameters
remark
remark   ****************************************************
remark   Developer:      Jeffrey J. Campion, Brandon Kulengowski
remark   Model Date:     9-19-2018
remark   Model Version:          1.0
remark   ****************************************************
remark
remark   parameters
remark   ----------
remark  gRLS
remark
remark  Nmax
remark
remark  kS
remark
remark  EC50S

remark - model-specific commands
COMMANDS
NFUN 12
NDER 12
NPAR 4
PNAMES 'gRLS' 'Nmax' 'kS' 'EC50S'
NSEC 1
SNAM 'SC'
END

remark - differential equations starting values
START
remark - Growth Control Experiments
Z(1)=1.77*10**6
Z(2)=2.00*10**6
Z(3)=2.46*10**6
Z(4)=4.93*10**6

remark - MEM Experiments
Z(5)=6.80*10**5
Z(6)=6.97*10**5
Z(7)=9.32*10**5
Z(8)=1.34*10**6
Z(9)=1.38*10**6
Z(10)=7.32*10**5
Z(11)=1.86*10**5
Z(12)=8.30*10**5
END
```

Table G.17: Isolate 44 Meropenem Initial Killing Model Code

```
remark - differential equations
DIFF

remark  low-level meropenem-resistant and high-level meropenem-resistant subpopulation growth 1/26/14
DZ(1)=(gRLS*(1-(Z(1))/Nmax))*Z(1)

remark  low-level meropenem-resistant and high-level meropenem-resistant subpopulation growth 2/15/14
DZ(2)=(gRLS*(1-(Z(2))/Nmax))*Z(2)

remark  low-level meropenem-resistant and high-level meropenem-resistant subpopulation growth 3/30/14
DZ(3)=(gRLS*(1-(Z(3))/Nmax))*Z(3)

remark  low-level meropenem-resistant and high-level meropenem-resistant subpopulation growth 6/11/15
DZ(4)=(gRLS*(1-(Z(4))/Nmax))*Z(4)

remark  MEM 4 monotherapy 1/26/14
DZ(5)=(gRLS*(1-(Z(5))/Nmax)-(kS*(4/(4+EC50S))))*Z(5)

remark  MEM 16 monotherapy 1/26/14
DZ(6)=(gRLS*(1-(Z(6))/Nmax)-(kS*(16/(16+EC50S))))*Z(6)

remark  MEM 64 monotherapy 1/26/14
DZ(7)=(gRLS*(1-(Z(7))/Nmax)-(kS*(64/(64+EC50S))))*Z(7)

remark  MEM 4 monotherapy 5/14/14
DZ(8)=(gRLS*(1-(Z(8))/Nmax)-(kS*(4/(4+EC50S))))*Z(8)

remark  MEM 16 monotherapy 5/14/14
DZ(9)=(gRLS*(1-(Z(9))/Nmax)-(kS*(16/(16+EC50S))))*Z(9)

remark  MEM 64 monotherapy 5/20/14
DZ(10)=(gRLS*(1-(Z(10))/Nmax)-(kS*(64/(64+EC50S))))*Z(10)

remark  MEM 64 monotherapy 2/13/17
DZ(11)=(gRLS*(1-(Z(11))/Nmax)-(kS*(64/(64+EC50S))))*Z(11)

remark  MEM 128 monotherapy 2/13/17
DZ(12)=(gRLS*(1-(Z(12))/Nmax)-(kS*(128/(128+EC50S))))*Z(12)

END

remark - algebraic functions
FUNC 1
F=log10(Z(1))
END
```

Table G.17: Isolate 44 Meropenem Initial Killing Model Code

```
FUNC 2
F=log10(Z(2))
END
FUNC 3
F=log10(Z(3))
END
FUNC 4
F=log10(Z(4))
END
FUNC 5
F=log10(Z(5))
END
FUNC 6
F=log10(Z(6))
END
FUNC 7
F=log10(Z(7))
END
FUNC 8
F=log10(Z(8))
END
FUNC 9
F=log10(Z(9))
END
FUNC 10
F=log10(Z(10))
END
FUNC 11
F=log10(Z(11))
END
FUNC 12
F=log10(Z(12))
END

SECONDARY
S(1)=(kS-gRLS)/gRLS*EC50S
END

remark - end of model
EOM
```

Table G.18: Isolate 44 Polymyxin B Initial Killing Model Code

```
MODEL
remark   Logistic Polymyxin B Initial Killing Model
remark   Klebsiella pneumoniae 44
remark   Polymyxin B Monotherapy Initial Killing Phase
remark   24 Functions, 24 differential equations, 4 Parameters
remark
remark   ****************************************************
remark   Developer:      Jeffrey J. Campion, Brandon Kulengowski
remark   Model Date:     7-23-2018
remark   Model Version:          1.0
remark   ****************************************************
remark
remark   parameters
remark   ----------
remark  gRLS
remark
remark  Nmax
remark
remark  KmaxPMB
remark
remark  EC50PMB

remark - model-specific commands
COMMANDS
NFUN 24
NDER 24
NPAR 4
PNAMES 'gRLS' 'Nmax' 'KmaxPMB' 'EC50PMB'
remark -initial estimates 2.557385, 2.3627639E+11, 10.55, 0.041
NSEC 1
SNAM 'SC'
END

remark - differential equations starting values
START
remark ??? Growth experiments time-shifted T=0 and PMB monotherapy experiments count at
T=0
Z(1)=1.63E+06
Z(2)=2.48E+06
Z(3)=4.29E+06
Z(4)=4.93E+06
Z(5)=6.04E+05
Z(6)=5.29E+05
Z(7)=5.42E+05
Z(8)=7.16E+05
Z(9)=2.68E+05
Z(10)=8.53E+05
Z(11)=8.93E+05
Z(12)=4.48E+05
Z(13)=8.13E+05
```

Table G.18: Isolate 44 Polymyxin B Initial Killing Model Code

```
Z(14)=9.82E+05
Z(15)=6.47E+05
Z(16)=6.18E+05
Z(17)=4.75E+05
Z(18)=5.56E+05
Z(19)=6.53E+05
Z(20)=5.87E+05
Z(21)=7.75E+05
Z(22)=1.10E+06
Z(23)=9.48E+05
Z(24)=1.02E+06
END

remark - differential equations
DIFF
remark  growth control
DZ(1)=(gRLS*(1-(Z(1))/Nmax)-(KmaxPMB*(0/(0+EC50PMB))))*Z(1)

remark  growth control
DZ(2)=(gRLS*(1-(Z(2))/Nmax)-(KmaxPMB*(0/(0+EC50PMB))))*Z(2)

remark  growth control
DZ(3)=(gRLS*(1-(Z(3))/Nmax)-(KmaxPMB*(0/(0+EC50PMB))))*Z(3)

remark  growth control
DZ(4)=(gRLS*(1-(Z(4))/Nmax)-(KmaxPMB*(0/(0+EC50PMB))))*Z(4)

remark  PMB 0.0625 monotherapy 6/10/14
DZ(5)=(gRLS*(1-(Z(5))/Nmax)-(KmaxPMB*(0.0625/(0.0625+EC50PMB))))*Z(5)

remark  PMB 0.0625 monotherapy 6/18/14
DZ(6)=(gRLS*(1-(Z(6))/Nmax)-(KmaxPMB*(0.0625/(0.0625+EC50PMB))))*Z(6)

remark  PMB 0.125 monotherapy 6/18/14
DZ(7)=(gRLS*(1-(Z(7))/Nmax)-(KmaxPMB*(0.125/(0.125+EC50PMB))))*Z(7)

remark  PMB 0.125 monotherapy 7/9/14
DZ(8)=(gRLS*(1-(Z(8))/Nmax)-(KmaxPMB*(0.125/(0.125+EC50PMB))))*Z(8)

remark  PMB 0.25 monotherapy 3/30/14
DZ(9)=(gRLS*(1-(Z(9))/Nmax)-(KmaxPMB*(0.25/(0.25+EC50PMB))))*Z(9)

remark  PMB 0.25 monotherapy 4/19/14
DZ(10)=(gRLS*(1-(Z(10))/Nmax)-(KmaxPMB*(0.25/(0.25+EC50PMB))))*Z(10)

remark  PMB 0.25 monotherapy 5/14/14
DZ(11)=(gRLS*(1-(Z(11))/Nmax)-(KmaxPMB*(0.25/(0.25+EC50PMB))))*Z(11)

remark  PMB 1 monotherapy 3/30/14
DZ(12)=(gRLS*(1-(Z(12))/Nmax)-(KmaxPMB*(1/(1+EC50PMB))))*Z(12)
```

Table G.18: Isolate 44 Polymyxin B Initial Killing Model Code

```
remark  PMB 1 monotherapy 4/19/14
DZ(13)=(gRLS*(1-(Z(13))/Nmax)-(KmaxPMB*(1/(1+EC50PMB))))*Z(13)

remark  PMB 1 monotherapy 5/14/14
DZ(14)=(gRLS*(1-(Z(14))/Nmax)-(KmaxPMB*(1/(1+EC50PMB))))*Z(14)

remark  PMB 2 monotherapy 5/29/14
DZ(15)=(gRLS*(1-(Z(15))/Nmax)-(KmaxPMB*(2/(2+EC50PMB))))*Z(15)

remark  PMB 2 monotherapy 6/10/14
DZ(16)=(gRLS*(1-(Z(16))/Nmax)-(KmaxPMB*(2/(2+EC50PMB))))*Z(16)

remark  PMB 2 monotherapy 8/23/14
DZ(17)=(gRLS*(1-(Z(17))/Nmax)-(KmaxPMB*(2/(2+EC50PMB))))*Z(17)

remark  PMB 4 monotherapy 5/20/14
DZ(18)=(gRLS*(1-(Z(18))/Nmax)-(KmaxPMB*(4/(4+EC50PMB))))*Z(18)

remark  PMB 4 monotherapy 5/29/14
DZ(19)=(gRLS*(1-(Z(19))/Nmax)-(KmaxPMB*(4/(4+EC50PMB))))*Z(19)

remark  PMB 4 monotherapy 6/10/14
DZ(20)=(gRLS*(1-(Z(20))/Nmax)-(KmaxPMB*(4/(4+EC50PMB))))*Z(20)

remark  PMB 4 monotherapy 7/9/14
DZ(21)=(gRLS*(1-(Z(21))/Nmax)-(KmaxPMB*(4/(4+EC50PMB))))*Z(21)

remark  PMB 4 monotherapy 8/23/14
DZ(22)=(gRLS*(1-(Z(22))/Nmax)-(KmaxPMB*(4/(4+EC50PMB))))*Z(22)

remark  PMB 4 monotherapy 2/13/17
DZ(23)=(gRLS*(1-(Z(23))/Nmax)-(KmaxPMB*(4/(4+EC50PMB))))*Z(23)

remark  PMB 8 monotherapy 2/13/17
DZ(24)=(gRLS*(1-(Z(24))/Nmax)-(KmaxPMB*(8/(8+EC50PMB))))*Z(24)

END

SECONDARY
remark -Stationary concentration
S(1)=(KmaxPMB-gRLS)/gRLS*EC50PMB
END

remark - algebraic functions
FUNC 1
F=log10(Z(1))
END
FUNC 2
F=log10(Z(2))
```

Table G.18: Isolate 44 Polymyxin B Initial Killing Model Code

```
END
FUNC 3
F=log10(Z(3))
END
FUNC 4
F=log10(Z(4))
END
FUNC 5
F=log10(Z(5))
END
FUNC 6
F=log10(Z(6))
END
FUNC 7
F=log10(Z(7))
END
FUNC 8
F=log10(Z(8))
END
FUNC 9
F=log10(Z(9))
END
FUNC 10
F=log10(Z(10))
END
FUNC 11
F=log10(Z(11))
END
FUNC 12
F=log10(Z(12))
END
FUNC 13
F=log10(Z(13))
END
FUNC 14
F=log10(Z(14))
END
FUNC 15
F=log10(Z(15))
END
FUNC 16
F=log10(Z(16))
END
FUNC 17
F=log10(Z(17))
END
FUNC 18
F=log10(Z(18))
END
FUNC 19
```

Table G.18: Isolate 44 Polymyxin B Initial Killing Model Code

```
F=log10(Z(19))
END
FUNC 20
F=log10(Z(20))
END
FUNC 21
F=log10(Z(21))
END
FUNC 22
F=log10(Z(22))
END
FUNC 23
F=log10(Z(23))
END
FUNC 24
F=log10(Z(24))
END

remark - end of model
EOM
```

Table G.19: Isolate 44 Meropenem Monotherapy Model Code

```
MODEL
remark   Two-Population Meropenem Simple Net Effect Model
remark   Klebsiella pneumoniae 44
remark   Meropenem-low-resistant and high-resistant subpopulations
remark   13 Functions, 24 Differential Equations, 5 Parameters
remark
remark   ******************************************************
remark   Developer:      Jeffrey J. Campion, Brandon Kulengowski
remark   Model Date:     9-17-2018
remark   Model Version:          1.0
remark   ******************************************************
remark
remark   parameters
remark   ----------
remark  gS
remark
remark  gR
remark
remark  Nmax
remark
remark  kS
remark
remark  EC50S

remark - model-specific commands
COMMANDS
NFUN 13
NDER 24
NPAR 5
PNAMES 'gS' 'gR' 'Nmax' 'kS' 'EC50S'
NSEC 4
SNAMES 'SCS' 'SCR' 'dS' 'dR'
SIZE 6
END

TEMP
R0=0.52
END

remark - differential equations starting values
START
Z(1)=1.63*10**6
Z(2)=R0
Z(3)=2.48*10**6
Z(4)=R0
Z(5)=4.29*10**6
Z(6)=R0
Z(23)=4.93*10**6
Z(24)=2.58*10**5
Z(7)=6.80*10**5
```

Table G.19: Isolate 44 Meropenem Monotherapy Model Code

```
Z(8)=R0
Z(9)=6.97*10**5
Z(10)=R0
Z(11)=9.32*10**5
Z(12)=R0
Z(13)=1.34*10**6
Z(14)=R0
Z(15)=1.38*10**6
Z(16)=R0
Z(17)=7.32*10**5
Z(18)=R0
Z(19)=8.75*10**5
Z(20)=R0
Z(21)=8.30*10**5
Z(22)=R0
END

remark - differential equations
DIFF

remark  growth control experiment 1/26/14
DZ(1)=(gS*(1-(Z(1)+Z(2))/Nmax))*Z(1)
DZ(2)=(gR*(1-(Z(1)+Z(2))/Nmax))*Z(2)

remark  growth control experiment 2/15/14
DZ(3)=(gS*(1-(Z(3)+Z(4))/Nmax))*Z(3)
DZ(4)=(gR*(1-(Z(3)+Z(4))/Nmax))*Z(4)

remark  growth control experiment 3/30/14
DZ(5)=(gS*(1-(Z(5)+Z(6))/Nmax))*Z(5)
DZ(6)=(gR*(1-(Z(5)+Z(6))/Nmax))*Z(6)

remark  growth control experiment MEM low-resistance subpopulation 6/11/15
DZ(23)=(gS*(1-(Z(23)+Z(24))/Nmax))*Z(23)

remark    growth control experiment MEM high-resistance subpopulation 6/11/15
DZ(24)=(gR*(1-(Z(23)+Z(24))/Nmax))*Z(24)

remark  meropenem 4 mg/L time-kill experiment 1/26/14
DZ(7)=(gS*(1-(Z(7)+Z(8))/Nmax)-kS*(4/(4+EC50S)))*Z(7)
DZ(8)=(gR*(1-(Z(7)+Z(8))/Nmax))*Z(8)

remark  meropenem 16 mg/L time-kill experiment 1/26/14
DZ(9)=(gS*(1-(Z(9)+Z(10))/Nmax)-kS*(16/(16+EC50S)))*Z(9)
DZ(10)=(gR*(1-(Z(9)+Z(10))/Nmax))*Z(10)

remark  meropenem 64 mg/L time-kill experiment 1/26/14
DZ(11)=(gS*(1-(Z(11)+Z(12))/Nmax)-kS*(64/(64+EC50S)))*Z(11)
DZ(12)=(gR*(1-(Z(11)+Z(12))/Nmax))*Z(12)
```

Table G.19: Isolate 44 Meropenem Monotherapy Model Code

```
remark  meropenem 4 mg/L time-kill experiment 5/14/14
DZ(13)=(gS*(1-(Z(13)+Z(14))/Nmax)-kS*(4/(4+EC50S)))*Z(13)
DZ(14)=(gR*(1-(Z(13)+Z(14))/Nmax))*Z(14)

remark  meropenem 64 mg/L time-kill experiment 5/14/14
DZ(15)=(gS*(1-(Z(15)+Z(16))/Nmax)-kS*(64/(64+EC50S)))*Z(15)
DZ(16)=(gR*(1-(Z(15)+Z(16))/Nmax))*Z(16)

remark  meropenem 16 mg/L time-kill experiment 5/20/14
DZ(17)=(gS*(1-(Z(17)+Z(18))/Nmax)-kS*(16/(16+EC50S)))*Z(17)
DZ(18)=(gR*(1-(Z(17)+Z(18))/Nmax))*Z(18)

remark  meropenem 64 mg/L time-kill experiment 2/13/14
DZ(19)=(gS*(1-(Z(19)+Z(20))/Nmax)-kS*(64/(64+EC50S)))*Z(19)
DZ(20)=(gR*(1-(Z(19)+Z(20))/Nmax))*Z(20)

remark  meropenem 128 mg/L time-kill experiment 2/13/14
DZ(21)=(gS*(1-(Z(21)+Z(22))/Nmax)-kS*(128/(128+EC50S)))*Z(21)
DZ(22)=(gR*(1-(Z(21)+Z(22))/Nmax))*Z(22)

END

remark - algebraic functions
FUNC 1
F=log10(Z(1)+Z(2))
END
FUNC 2
F=log10(Z(3)+Z(4))
END
FUNC 3
F=log10(Z(5)+Z(6))
END
FUNC 4
F=log10(Z(23)+Z(24))
END
FUNC 5
F=log10(Z(24))
END
FUNC 6
F=log10(Z(7)+Z(8))
END
FUNC 7
F=log10(Z(9)+Z(10))
END
FUNC 8
F=log10(Z(11)+Z(12))
END
FUNC 9
F=log10(Z(13)+Z(14))
END
```

Table G.19: Isolate 44 Meropenem Monotherapy Model Code

```
FUNC 10
F=log10(Z(15)+Z(16))
END
FUNC 11
F=log10(Z(17)+Z(18))
END
FUNC 12
F=log10(Z(19)+Z(20))
END
FUNC 13
F=log10(Z(21)+Z(22))
END


remark - secondary parameters
SECO
SCS=(gS/(kS-gS))*EC50S
SCR=0
dS=0.6931/gS*60
dR=0.6931/gR*60
END

remark - end of model
EOM
```

Table G.20: Isolate 44 Polymyxin B Monotherapy Model Code

```
MODEL
remark   Two-Population Polymyxin B Simple Net Effect Model
remark   Klebsiella pneumoniae 44
remark   Polymyxin B-susceptible and -resistant subpopulations
remark   25 Functions, 43 Differential Equations, 5 Parameters
remark
remark   *****************************************************
remark   Developer:      Jeffrey J. Campion, Brandon Kulengowski
remark   Model Date:     9-6-2018
remark   Model Version:          1.0
remark   *****************************************************
remark
remark   parameters
remark   ----------
remark  gS
remark
remark  gR
remark
remark  Nmax
remark
remark  kS
remark
remark  kR
remark

remark - model-specific commands
COMMANDS
NFUN 25
NDER 43
NPAR 5
PNAMES 'gS' 'gR' 'Nmax' 'kS' 'kR'
NSEC 4
SNAMES 'SCS' 'SCR' 'dS' 'dR'
END

TEMP
R0=44
END

remark - differential equations starting values
START
Z(1)=1.63*10**6
Z(2)=R0
Z(3)=2.48*10**6
Z(4)=R0
Z(5)=4.29*10**6
Z(6)=R0
Z(7)=4.93*10**6
Z(8)=2.50*10**1
Z(9)=6.04*10**5
```

Table G.20: Isolate 44 Polymyxin B Monotherapy Model Code

```
Z(10)=5.29*10**5
Z(11)=5.42*10**5
Z(12)=R0
Z(13)=7.16*10**5
Z(14)=R0
Z(15)=2.68*10**5
Z(16)=R0
Z(17)=8.53*10**5
Z(18)=R0
Z(19)=8.93*10**5
Z(20)=R0
Z(21)=4.48*10**5
Z(22)=R0
Z(23)=8.13*10**5
Z(24)=R0
Z(25)=9.82*10**5
Z(26)=R0
Z(27)=6.47*10**5
Z(28)=R0
Z(29)=6.18*10**5
Z(30)=R0
Z(31)=4.75*10**5
Z(32)=R0
Z(33)=5.56*10**5
Z(34)=6.53*10**5
Z(35)=R0
Z(36)=5.87*10**5
Z(37)=R0
Z(38)=7.75*10**5
Z(39)=R0
Z(40)=1.10*10**6
Z(41)=R0
Z(42)=9.48*10**5
Z(43)=R0
END

remark - differential equations
DIFF

remark  growth control experiment 1/26/14
DZ(1)=(gS*(1-(Z(1)+Z(2))/Nmax))*Z(1)
DZ(2)=(gR*(1-(Z(1)+Z(2))/Nmax))*Z(2)

remark  growth control experiment 2/15/14
DZ(3)=(gS*(1-(Z(3)+Z(4))/Nmax))*Z(3)
DZ(4)=(gR*(1-(Z(3)+Z(4))/Nmax))*Z(4)

remark  growth control experiment 3/30/14
DZ(5)=(gS*(1-(Z(5)+Z(6))/Nmax))*Z(5)
DZ(6)=(gR*(1-(Z(5)+Z(6))/Nmax))*Z(6)
```

Table G.20: Isolate 44 Polymyxin B Monotherapy Model Code

```
remark  polymyxin B-susceptible subpopulation growth 6/11/15
DZ(7)=(gS*(1-(Z(7)+Z(8))/Nmax))*Z(7)

remark  polymyxin B-resistant subpopulation growth 6/11/15
DZ(8)=(gR*(1-(Z(7)+Z(8))/Nmax))*Z(8)

remark  polymyxin B 0.0625 mg/L time-kill experiment 6/10/14
DZ(9)=(gS*(1-(Z(9))/Nmax)-kS)*Z(9)

remark  polymyxin B 0.0625 mg/L time-kill experiment 6/18/14
DZ(10)=(gS*(1-(Z(10))/Nmax)-kS)*Z(10)

remark  polymyxin B 0.125 mg/L time-kill experiment 6/18/14
DZ(11)=(gS*(1-(Z(11)+Z(12))/Nmax)-kS)*Z(11)
DZ(12)=(gR*(1-(Z(11)+Z(12))/Nmax)-kR*0.125)*Z(12)

remark  polymyxin B 0.125 mg/L time-kill experiment 7/9/14
DZ(13)=(gS*(1-(Z(13)+Z(14))/Nmax)-kS)*Z(13)
DZ(14)=(gR*(1-(Z(13)+Z(14))/Nmax)-kR*0.125)*Z(14)

remark  polymyxin B 0.25 mg/L time-kill experiment 3/30/14
DZ(15)=(gS*(1-(Z(15)+Z(16))/Nmax)-kS)*Z(15)
DZ(16)=(gR*(1-(Z(15)+Z(16))/Nmax)-kR*0.25)*Z(16)

remark  polymyxin B 0.25 mg/L time-kill experiment 4/19/14
DZ(17)=(gS*(1-(Z(17)+Z(18))/Nmax)-kS)*Z(17)
DZ(18)=(gR*(1-(Z(17)+Z(18))/Nmax)-kR*0.25)*Z(18)

remark  polymyxin B 0.25 mg/L time-kill experiment 5/14/14
DZ(19)=(gS*(1-(Z(19)+Z(20))/Nmax)-kS)*Z(19)
DZ(20)=(gR*(1-(Z(19)+Z(20))/Nmax)-kR*0.25)*Z(20)

remark  polymyxin B 1 mg/L time-kill experiment 3/30/14
DZ(21)=(gS*(1-(Z(21)+Z(22))/Nmax)-kS)*Z(21)
DZ(22)=(gR*(1-(Z(21)+Z(22))/Nmax)-kR*1)*Z(22)

remark  polymyxin B 1 mg/L time-kill experiment 4/19/14
DZ(23)=(gS*(1-(Z(23)+Z(24))/Nmax)-kS)*Z(23)
DZ(24)=(gR*(1-(Z(23)+Z(24))/Nmax)-kR*1)*Z(24)

remark  polymyxin B 1 mg/L time-kill experiment 5/14/14
DZ(25)=(gS*(1-(Z(25)+Z(26))/Nmax)-kS)*Z(25)
DZ(26)=(gR*(1-(Z(25)+Z(26))/Nmax)-kR*1)*Z(26)

remark  polymyxin B 2 mg/L time-kill experiment 5/29/14
DZ(27)=(gS*(1-(Z(27)+Z(28))/Nmax)-kS)*Z(27)
DZ(28)=(gR*(1-(Z(27)+Z(28))/Nmax)-kR*2)*Z(28)

remark  polymyxin B 2 mg/L time-kill experiment 6/10/14
```

Table G.20: Isolate 44 Polymyxin B Monotherapy Model Code

```
DZ(29)=(gS*(1-(Z(29)+Z(30))/Nmax)-kS)*Z(29)
DZ(30)=(gR*(1-(Z(29)+Z(30))/Nmax)-kR*2)*Z(30)

remark  polymyxin B 2 mg/L time-kill experiment 8/23/14
DZ(31)=(gS*(1-(Z(31)+Z(32))/Nmax)-kS)*Z(31)
DZ(32)=(gR*(1-(Z(31)+Z(32))/Nmax)-kR*2)*Z(32)

remark  polymyxin B 4 mg/L time-kill experiment 5/20/14
DZ(33)=(gS*(1-(Z(33))/Nmax)-kS)*Z(33)

remark  polymyxin B 4 mg/L time-kill experiment 5/29/14
DZ(34)=(gS*(1-(Z(34)+Z(35))/Nmax)-kS)*Z(34)
DZ(35)=(gR*(1-(Z(34)+Z(35))/Nmax)-kR*4)*Z(35)

remark  polymyxin B 4 mg/L time-kill experiment 6/10/14
DZ(36)=(gS*(1-(Z(36)+Z(37))/Nmax)-kS)*Z(36)
DZ(37)=(gR*(1-(Z(36)+Z(37))/Nmax)-kR*4)*Z(37)

remark  polymyxin B 4 mg/L time-kill experiment 7/9/14
DZ(38)=(gS*(1-(Z(38)+Z(39))/Nmax)-kS)*Z(38)
DZ(39)=(gR*(1-(Z(38)+Z(39))/Nmax)-kR*4)*Z(39)

remark  polymyxin B 4 mg/L time-kill experiment 8/23/14
DZ(40)=(gS*(1-(Z(40)+Z(41))/Nmax)-kS)*Z(40)
DZ(41)=(gR*(1-(Z(40)+Z(41))/Nmax)-kR*4)*Z(41)

remark  polymyxin B-susceptible subpopulation for polymyxin B 4 mg/L time-kill experiment
2/13/17
DZ(42)=(gS*(1-(Z(42)+Z(43))/Nmax)-kS)*Z(42)

remark  polymyxin B-resistant subpopulation for polymyxin B 4 mg/L time-kill experiment
2/13/17
DZ(43)=(gR*(1-(Z(42)+Z(43))/Nmax)-kR*4)*Z(43)

END

remark - algebraic functions
FUNC 1
F=log10(Z(1)+Z(2))
END
FUNC 2
F=log10(Z(3)+Z(4))
END
FUNC 3
F=log10(Z(5)+Z(6))
END
FUNC 4
F=log10(Z(7))
END
FUNC 5
```

Table G.20: Isolate 44 Polymyxin B Monotherapy Model Code

```
F=log10(Z(8))
END
FUNC 6
F=log10(Z(9))
END
FUNC 7
F=log10(Z(10))
END
FUNC 8
F=log10(Z(11)+Z(12))
END
FUNC 9
F=log10(Z(13)+Z(14))
END
FUNC 10
F=log10(Z(15)+Z(16))
END
FUNC 11
F=log10(Z(17)+Z(18))
END
FUNC 12
F=log10(Z(19)+Z(20))
END
FUNC 13
F=log10(Z(21)+Z(22))
END
FUNC 14
F=log10(Z(23)+Z(24))
END
FUNC 15
F=log10(Z(25)+Z(26))
END
FUNC 16
F=log10(Z(27)+Z(28))
END
FUNC 17
F=log10(Z(29)+Z(30))
END
FUNC 18
F=log10(Z(31)+Z(32))
END
FUNC 19
F=log10(Z(33))
END
FUNC 20
F=log10(Z(34)+Z(35))
END
FUNC 21
F=log10(Z(36)+Z(37))
END
```

Table G.20: Isolate 44 Polymyxin B Monotherapy Model Code

```
FUNC 22
F=log10(Z(38)+Z(39))
END
FUNC 23
F=log10(Z(40)+Z(41))
END
FUNC 24
F=log10(Z(42))
END
FUNC 25
F=log10(Z(43))
END

remark - secondary parameters
SECO
SCS=0
SCR=gR/kR
dS=0.6931/gS*60
dR=0.6931/gR*60
END

remark - end of model
EOM
```

Table G.21: Meropenem Composite Model Code

```
MODEL
remark   Two-Population Meropenem Composite Simple Net Effect Model
remark   Klebsiella pneumoniae isolates 34, 22, 24, 44
remark   Meropenem low and high resistant subpopulations
remark   52 Functions, 93 Differential Equations, 6 Parameters
remark
remark   ******************************************************
remark   Developer:       Brandon Kulengowski
remark   Model Date:      9-22-2018
remark   Model Version:           1.0
remark   ******************************************************
remark
remark   parameters
remark   ------------
remark  gS
remark
remark  gR
remark
remark  Nmax
remark
remark  kS
remark
remark  EC50S
remark
remark  kR

remark - model-specific commands
COMMANDS
NFUN 52
NDER 93
NPAR 6
PNAMES 'gS' 'gR' 'Nmax' 'kS' 'EC50S' 'kR'
NSEC 4
SNAMES 'SCS' 'SCR' 'dS' 'dR'
SIZE 15
END

TEMP
remark - initial inocula
R034=64.2
R022=13
R024=3.27
R044=0.52

remark - static concentrations
c34=4
c22=16
c24=32
c44=128
```

Table G.21: Meropenem Composite Model Code

```
END

remark - differential equations starting values
START

remark - isolate 34 initial conditions

Z(1)=2.63*10**6
Z(2)=R034
Z(3)=4.44*10**6
Z(4)=R034
Z(5)=1.13*10**7
Z(6)=R034
Z(23)=1.14*10**7
Z(24)=3.71*10**2
Z(7)=8.06*10**5
Z(8)=R034
Z(9)=7.48*10**5
Z(10)=R034
Z(11)=6.54*10**5
Z(12)=R034
Z(13)=1.04*10**6
Z(14)=R034
Z(15)=4.85*10**5
Z(16)=R034
Z(17)=7.54*10**5
Z(18)=R034
Z(19)=7.59*10**5
Z(20)=R034
Z(21)=1.14*10**6
Z(22)=R034

remark - isolate 22 initial conditions

Z(25)=3.14*10**6
Z(26)=R022
Z(27)=2.51*10**6
Z(28)=R022
Z(29)=6.23*10**6
Z(30)=7.93*10**2
Z(31)=1.70*10**6
Z(32)=R022
Z(33)=1.26*10**6
Z(34)=R022
Z(35)=9.34*10**5
Z(36)=R022
Z(37)=6.11*10**5
Z(38)=R022
Z(39)=3.52*10**5
Z(40)=R022
```

Table G.21: Meropenem Composite Model Code

```
Z(41)=5.28*10**5
Z(42)=R022
Z(43)=7.82*10**5
Z(44)=R022
Z(45)=8.00*10**5
Z(46)=R022

remark - isolate 24 initial conditions

Z(47)=1.77*10**6
Z(48)=R024
Z(49)=2.00*10**6
Z(50)=R024
Z(51)=2.446*10**6
Z(52)=5.7*10**1
Z(53)=4.79*10**5
Z(54)=R024
Z(55)=5.68*10**5
Z(56)=R024
Z(57)=4.36*10**5
Z(58)=R024
Z(59)=4.84*10**5
Z(60)=R024
Z(61)=6.34*10**5
Z(62)=R024
Z(63)=5.40*10**5
Z(64)=R024
Z(65)=5.29*10**5
Z(66)=R024
Z(67)=5.31*10**5
Z(68)=R024
Z(69)=R024

remark - isolate 44 initial conditions

Z(70)=1.63*10**6
Z(71)=R044
Z(72)=2.48*10**6
Z(73)=R044
Z(74)=4.29*10**6
Z(75)=R044
Z(76)=4.93*10**6
Z(77)=2.58*10**5
Z(78)=6.80*10**5
Z(79)=R044
Z(80)=6.97*10**5
Z(81)=R044
Z(82)=9.32*10**5
Z(83)=R044
Z(84)=1.34*10**6
```

Table G.21: Meropenem Composite Model Code

```
Z(85)=R044
Z(86)=1.38*10**6
Z(87)=R044
Z(88)=7.32*10**5
Z(89)=R044
Z(90)=8.75*10**5
Z(91)=R044
Z(92)=8.30*10**5
Z(93)=R044

END

remark - differential equations
DIFF

remark - isolate 34 DEs
remark - All MEM doses are normalized to 4 mg/L

remark  growth control experiment 1/26/14
DZ(1)=(gS*(1-(Z(1)+Z(2))/Nmax))*Z(1)
DZ(2)=(gR*(1-(Z(1)+Z(2))/Nmax))*Z(2)

remark  growth control experiment 2/15/14
DZ(3)=(gS*(1-(Z(3)+Z(4))/Nmax))*Z(3)
DZ(4)=(gR*(1-(Z(3)+Z(4))/Nmax))*Z(4)

remark  growth control experiment 3/30/14
DZ(5)=(gS*(1-(Z(5)+Z(6))/Nmax))*Z(5)
DZ(6)=(gR*(1-(Z(5)+Z(6))/Nmax))*Z(6)

remark  growth control experiment MEM low-resistance subpopulation 6/11/15
DZ(23)=(gS*(1-(Z(23)+Z(24))/Nmax))*Z(23)

remark    growth control experiment MEM high-resistance subpopulation 6/11/15
DZ(24)=(gR*(1-(Z(23)+Z(24))/Nmax))*Z(24)

remark  meropenem 4 mg/L time-kill experiment 1/26/14
DZ(7)=(gS*(1-(Z(7)+Z(8))/Nmax)-kS*((4/c34)/((4/c34)+EC50S)))*Z(7)
DZ(8)=(gR*(1-(Z(7)+Z(8))/Nmax)-kR*((4/c34)))*Z(8)

remark  meropenem 16 mg/L time-kill experiment 1/26/14
DZ(9)=(gS*(1-(Z(9)+Z(10))/Nmax)-kS*((16/c34)/((16/c34)+EC50S)))*Z(9)
DZ(10)=(gR*(1-(Z(9)+Z(10))/Nmax)-kR*((16/c34)))*Z(10)

remark  meropenem 64 mg/L time-kill experiment 1/26/14
DZ(11)=(gS*(1-(Z(11)+Z(12))/Nmax)-kS*((64/c34)/((64/c34)+EC50S)))*Z(11)
DZ(12)=(gR*(1-(Z(11)+Z(12))/Nmax)-kR*((64/c34)))*Z(12)

remark  meropenem 4 mg/L time-kill experiment 5/14/14
DZ(13)=(gS*(1-(Z(13)+Z(14))/Nmax)-kS*((4/c34)/((4/C34)+EC50S)))*Z(13)
```

Table G.21: Meropenem Composite Model Code

```
DZ(14)=(gR*(1-(Z(13)+Z(14))/Nmax)-kR*((4/c34)))*Z(14)

remark  meropenem 16 mg/L time-kill experiment 5/14/14
DZ(15)=(gS*(1-(Z(15)+Z(16))/Nmax)-kS*((16/c34)/((16/C34)+EC50S)))*Z(15)
DZ(16)=(gR*(1-(Z(15)+Z(16))/Nmax)-kR*((16/c34)))*Z(16)

remark  meropenem 64 mg/L time-kill experiment 5/20/14
DZ(17)=(gS*(1-(Z(17)+Z(18))/Nmax)-kS*((64/c34)/((64/C34)+EC50S)))*Z(17)
DZ(18)=(gR*(1-(Z(17)+Z(18))/Nmax)-kR*((64/c34)))*Z(18)

remark  meropenem 8 mg/L time-kill experiment 2/13/14
DZ(19)=(gS*(1-(Z(19)+Z(20))/Nmax)-kS*((8/c34)/((8/C34)+EC50S)))*Z(19)
DZ(20)=(gR*(1-(Z(19)+Z(20))/Nmax)-kR*((8/c34)))*Z(20)

remark  meropenem 16 mg/L time-kill experiment 2/13/14
DZ(21)=(gS*(1-(Z(21)+Z(22))/Nmax)-kS*((16/c34)/((16/C34)+EC50S)))*Z(21)
DZ(22)=(gR*(1-(Z(21)+Z(22))/Nmax)-kR*((16/c34)))*Z(22)

remark - isolate 22 DEs
remark - All MEM doses are normalized to 16 mg/L

remark  growth control experiment 5/29/14
DZ(25)=(gS*(1-(Z(25)+Z(26))/Nmax))*Z(25)
DZ(26)=(gR*(1-(Z(25)+Z(26))/Nmax))*Z(26)

remark  growth control experiment 8/23/14
DZ(27)=(gS*(1-(Z(27)+Z(28))/Nmax))*Z(27)
DZ(28)=(gR*(1-(Z(27)+Z(28))/Nmax))*Z(28)

remark  growth control experiment 6/11/15
DZ(29)=(gS*(1-(Z(29)+Z(30))/Nmax))*Z(29)

remark  growth control experiment MEM resistant subpopulation 6/11/15
DZ(30)=(gR*(1-(Z(29)+Z(30))/Nmax))*Z(30)

remark  meropenem 4 mg/L time-kill experiment 5/14/14
DZ(31)=(gS*(1-(Z(31)+Z(32))/Nmax)-kS*((4/c22)/((4/c22)+EC50S)))*Z(31)
DZ(32)=(gR*(1-(Z(31)+Z(32))/Nmax)-kR*((4/c22)))*Z(32)

remark  meropenem 16 mg/L time-kill experiment 5/14/14
DZ(33)=(gS*(1-(Z(33)+Z(34))/Nmax)-kS*((16/c22)/((16/c22)+EC50S)))*Z(33)
DZ(34)=(gR*(1-(Z(33)+Z(34))/Nmax)-kR*((16/c22)))*Z(34)

remark  meropenem 64 mg/L time-kill experiment 5/20/14
DZ(35)=(gS*(1-(Z(35)+Z(36))/Nmax)-kS*((64/c22)/((64/c22)+EC50S)))*Z(35)
DZ(36)=(gR*(1-(Z(35)+Z(36))/Nmax)-kR*((64/c22)))*Z(36)

remark  meropenem 4 mg/L time-kill experiment 5/20/14
DZ(37)=(gS*(1-(Z(37)+Z(38))/Nmax)-kS*((4/c22)/((4/c22)+EC50S)))*Z(37)
DZ(38)=(gR*(1-(Z(37)+Z(38))/Nmax)-kR*((4/c22)))*Z(38)
```

Table G.21: Meropenem Composite Model Code

```
remark  meropenem 16 mg/L time-kill experiment 5/29/14
DZ(39)=(gS*(1-(Z(39)+Z(40))/Nmax)-kS*((16/c22)/((16/c22)+EC50S)))*Z(39)
DZ(40)=(gR*(1-(Z(39)+Z(40))/Nmax)-kR*((16/c22)))*Z(40)

remark  meropenem 64 mg/L time-kill experiment 5/29/14
DZ(41)=(gS*(1-(Z(41)+Z(42))/Nmax)-kS*((64/c22)/((64/c22)+EC50S)))*Z(41)
DZ(42)=(gR*(1-(Z(41)+Z(42))/Nmax)-kR*((64/c22)))*Z(42)

remark  meropenem 16 mg/L time-kill experiment 2/13/14
DZ(43)=(gS*(1-(Z(43)+Z(44))/Nmax)-kS*((16/c22)/((16/c22)+EC50S)))*Z(43)

remark  meropenem 16 mg/L time-kill resistant subpopulation experiment 2/13/14
DZ(44)=(gR*(1-(Z(43)+Z(44))/Nmax)-kR*((16/c22)))*Z(44)

remark  meropenem 32 mg/L time-kill experiment 2/13/14
DZ(45)=(gS*(1-(Z(45)+Z(46))/Nmax)-kS*((32/c22)/((32/c22)+EC50S)))*Z(45)

remark  meropenem 32 mg/L time-kill resistant subpopulation experiment 2/13/14
DZ(46)=(gR*(1-(Z(45)+Z(46))/Nmax)-kR*((32/c22)))*Z(46)

remark - isolate 24 DEs
remark - All MEM doses are normalized to 32 mg/L

remark  growth control experiment 5/29/14
DZ(47)=(gS*(1-(Z(47)+Z(48))/Nmax))*Z(47)
DZ(48)=(gR*(1-(Z(47)+Z(48))/Nmax))*Z(48)

remark  growth control experiment 8/23/14
DZ(49)=(gS*(1-(Z(49)+Z(50))/Nmax))*Z(49)
DZ(50)=(gR*(1-(Z(49)+Z(50))/Nmax))*Z(50)

remark  growth control experiment 6/11/15
DZ(51)=(gS*(1-(Z(51)+Z(52))/Nmax))*Z(51)

remark  growth control experiment MEM resistant subpopulation 6/11/15
DZ(52)=(gR*(1-(Z(51)+Z(52))/Nmax))*Z(52)

remark  meropenem 4 mg/L time-kill experiment 5/20/14
DZ(53)=(gS*(1-(Z(53)+Z(54))/Nmax)-kS*((4/c24)/((4/c24)+EC50S)))*Z(53)
DZ(54)=(gR*(1-(Z(53)+Z(54))/Nmax)-kR*((4/c24)))*Z(54)

remark  meropenem 16 mg/L time-kill experiment 5/20/14
DZ(55)=(gS*(1-(Z(55)+Z(56))/Nmax)-kS*((16/c24)/((16/c24)+EC50S)))*Z(55)
DZ(56)=(gR*(1-(Z(55)+Z(56))/Nmax)-kR*((16/c24)))*Z(56)

remark  meropenem 64 mg/L time-kill experiment 5/20/14
DZ(57)=(gS*(1-(Z(57)+Z(58))/Nmax)-kS*((64/c24)/((64/c24)+EC50S)))*Z(57)
DZ(58)=(gR*(1-(Z(57)+Z(58))/Nmax)-kR*((64/c24)))*Z(58)
```

Table G.21: Meropenem Composite Model Code

```
remark  meropenem 4 mg/L time-kill experiment 6/10/14
DZ(59)=(gS*(1-(Z(59)+Z(60))/Nmax)-kS*((4/c24)/((4/c24)+EC50S)))*Z(59)
DZ(60)=(gR*(1-(Z(59)+Z(60))/Nmax)-kR*((4/c24)))*Z(60)

remark  meropenem 16 mg/L time-kill experiment 6/10/14
DZ(61)=(gS*(1-(Z(61)+Z(62))/Nmax)-kS*((16/c24)/((16/c24)+EC50S)))*Z(61)
DZ(62)=(gR*(1-(Z(61)+Z(62))/Nmax)-kR*((16/c24)))*Z(62)

remark  meropenem 64 mg/L time-kill experiment 6/10/14
DZ(63)=(gS*(1-(Z(63)+Z(64))/Nmax)-kS*((64/c24)/((64/c24)+EC50S)))*Z(63)
DZ(64)=(gR*(1-(Z(63)+Z(64))/Nmax)-kR*((64/c24)))*Z(64)

remark  meropenem 64 mg/L time-kill experiment 2/13/14
DZ(65)=(gS*(1-(Z(65)+Z(66))/Nmax)-kS*((64/c24)/((64/c24)+EC50S)))*Z(65)
DZ(66)=(gR*(1-(Z(65)+Z(66))/Nmax)-kR*((64/c24)))*Z(66)

remark  meropenem 128 mg/L time-kill experiment 2/13/14
DZ(67)=(gS*(1-(Z(67)+Z(68))/Nmax)-kS*((128/c24)/((128/c24)+EC50S)))*Z(67)
DZ(68)=(gR*(1-(Z(67)+Z(68))/Nmax)-kR*((128/c24)))*Z(68)

remark  meropenem 64 mg/L time-kill experiment 2/13/14 MEM resistant subpopulation MIC
>64 mg/L
DZ(69)=(gR*(1-(Z(65)+Z(69))/Nmax)-kR*((64/c24)))*Z(69)

remark - isolate 44 DEs
remark - All MEM doses are normalized to 128 mg/L

remark  growth control experiment 1/26/14
DZ(70)=(gS*(1-(Z(70)+Z(71))/Nmax))*Z(70)
DZ(71)=(gR*(1-(Z(70)+Z(71))/Nmax))*Z(71)

remark  growth control experiment 2/15/14
DZ(72)=(gS*(1-(Z(72)+Z(73))/Nmax))*Z(72)
DZ(73)=(gR*(1-(Z(72)+Z(73))/Nmax))*Z(73)

remark  growth control experiment 3/30/14
DZ(74)=(gS*(1-(Z(74)+Z(75))/Nmax))*Z(74)
DZ(75)=(gR*(1-(Z(74)+Z(75))/Nmax))*Z(75)

remark  growth control experiment MEM low-resistance subpopulation 6/11/15
DZ(76)=(gS*(1-(Z(76)+Z(77))/Nmax))*Z(76)

remark    growth control experiment MEM high-resistance subpopulation 6/11/15
DZ(77)=(gR*(1-(Z(76)+Z(77))/Nmax))*Z(77)

remark  meropenem 4 mg/L time-kill experiment 1/26/14
DZ(78)=(gS*(1-(Z(78)+Z(79))/Nmax)-kS*((4/c44)/((4/c44)+EC50S)))*Z(78)
DZ(79)=(gR*(1-(Z(78)+Z(79))/Nmax)-kR*((4/c44)))*Z(79)

remark  meropenem 16 mg/L time-kill experiment 1/26/14
```

Table G.21: Meropenem Composite Model Code

```
DZ(80)=(gS*(1-(Z(80)+Z(81))/Nmax)-kS*((16/c44)/((16/c44)+EC50S)))*Z(80)
DZ(81)=(gR*(1-(Z(80)+Z(81))/Nmax)-kR*((16/c44)))*Z(81)

remark  meropenem 64 mg/L time-kill experiment 1/26/14
DZ(82)=(gS*(1-(Z(82)+Z(83))/Nmax)-kS*((64/c44)/((64/c44)+EC50S)))*Z(82)
DZ(83)=(gR*(1-(Z(82)+Z(83))/Nmax)-kR*((64/c44)))*Z(83)

remark  meropenem 4 mg/L time-kill experiment 5/14/14
DZ(84)=(gS*(1-(Z(84)+Z(85))/Nmax)-kS*((4/c44)/((4/c44)+EC50S)))*Z(84)
DZ(85)=(gR*(1-(Z(84)+Z(85))/Nmax)-kR*((4/c44)))*Z(85)

remark  meropenem 64 mg/L time-kill experiment 5/14/14
DZ(86)=(gS*(1-(Z(86)+Z(87))/Nmax)-kS*((64/c44)/((64/c44)+EC50S)))*Z(86)
DZ(87)=(gR*(1-(Z(86)+Z(87))/Nmax)-kR*((64/c44)))*Z(87)

remark  meropenem 16 mg/L time-kill experiment 5/20/14
DZ(88)=(gS*(1-(Z(88)+Z(89))/Nmax)-kS*((16/c44)/((16/c44)+EC50S)))*Z(88)
DZ(89)=(gR*(1-(Z(88)+Z(89))/Nmax)-kR*((16/c44)))*Z(89)

remark  meropenem 64 mg/L time-kill experiment 2/13/14
DZ(90)=(gS*(1-(Z(90)+Z(91))/Nmax)-kS*((64/c44)/((64/c44)+EC50S)))*Z(90)
DZ(91)=(gR*(1-(Z(90)+Z(91))/Nmax)-kR*((64/c44)))*Z(91)

remark  meropenem 128 mg/L time-kill experiment 2/13/14
DZ(92)=(gS*(1-(Z(92)+Z(93))/Nmax)-kS*((128/c44)/((128/c44)+EC50S)))*Z(92)
DZ(93)=(gR*(1-(Z(92)+Z(93))/Nmax)-kR*((128/c44)))*Z(93)

END

remark - algebraic functions

remark - isolate 34 functions

FUNC 1
F=log10(Z(1)+Z(2))
END
FUNC 2
F=log10(Z(3)+Z(4))
END
FUNC 3
F=log10(Z(5)+Z(6))
END
FUNC 4
F=log10(Z(23)+Z(24))
END
FUNC 5
F=log10(Z(24))
END
FUNC 6
F=log10(Z(7)+Z(8))
```

Table G.21: Meropenem Composite Model Code

```
END
FUNC 7
F=log10(Z(9)+Z(10))
END
FUNC 8
F=log10(Z(11)+Z(12))
END
FUNC 9
F=log10(Z(13)+Z(14))
END
FUNC 10
F=log10(Z(15)+Z(16))
END
FUNC 11
F=log10(Z(17)+Z(18))
END
FUNC 12
F=log10(Z(19)+Z(20)+1)
END
FUNC 13
F=log10(Z(21)+Z(22))
END

remark - isolate 22 functions

FUNC 14
F=log10(Z(25)+Z(26))
END
FUNC 15
F=log10(Z(27)+Z(28))
END
FUNC 16
F=log10(Z(29)+Z(30))
END
FUNC 17
F=log10(Z(30))
END
FUNC 18
F=log10(Z(31)+Z(32))
END
FUNC 19
F=log10(Z(33)+Z(34))
END
FUNC 20
F=log10(Z(35)+Z(36))
END
FUNC 21
F=log10(Z(37)+Z(38))
END
FUNC 22
```

Table G.21: Meropenem Composite Model Code

```
F=log10(Z(39)+Z(40))
END
FUNC 23
F=log10(Z(41)+Z(42))
END
FUNC 24
F=log10(Z(43))
END
FUNC 25
F=log10(Z(44)+1)
END
FUNC 26
F=log10(Z(45)+Z(46))
END

remark - isolate 24 functions

FUNC 27
F=log10(Z(47)+Z(48))
END
FUNC 28
F=log10(Z(49)+Z(50))
END
FUNC 29
F=log10(Z(51)+Z(52))
END
FUNC 30
F=log10(Z(52))
END
FUNC 31
F=log10(Z(53)+Z(54))
END
FUNC 32
F=log10(Z(55)+Z(56))
END
FUNC 33
F=log10(Z(57)+Z(58))
END
FUNC 34
F=log10(Z(59)+Z(60))
END
FUNC 35
F=log10(Z(61)+Z(62))
END
FUNC 36
F=log10(Z(63)+Z(64)+1)
END
FUNC 37
F=log10(Z(65)+Z(66))
END
```

Table G.21: Meropenem Composite Model Code

```
FUNC 38
F=log10(Z(67)+Z(68))
END
FUNC 39
F=log10(Z(69))
END

remark - isolate 44 functions

FUNC 40
F=log10(Z(70)+Z(71))
END
FUNC 41
F=log10(Z(72)+Z(73))
END
FUNC 42
F=log10(Z(74)+Z(75))
END
FUNC 43
F=log10(Z(76)+Z(77))
END
FUNC 44
F=log10(Z(77))
END
FUNC 45
F=log10(Z(78)+Z(79))
END
FUNC 46
F=log10(Z(80)+Z(81))
END
FUNC 47
F=log10(Z(82)+Z(83))
END
FUNC 48
F=log10(Z(84)+Z(85))
END
FUNC 49
F=log10(Z(86)+Z(87))
END
FUNC 50
F=log10(Z(88)+Z(89))
END
FUNC 51
F=log10(Z(90)+Z(91))
END
FUNC 52
F=log10(Z(92)+Z(93))
END

SECO
```

Table G.21: Meropenem Composite Model Code

```
SCS=gS/(kS-gS)*EC50S
SCR=gR/kR
dS=0.6931/gS*60
dR=0.6931/gR*60
END

remark - end of model
EOM
```

Table G.22: Polymyxin B Composite Model Code

```
MODEL
remark   Two-Population Polymyxin B Composite Simple Net Effect Model
remark   Klebsiella pneumoniae isolates 34, 22, 24, 44
remark   Polymyxin B-susceptible and -resistant subpopulations
remark   78 Functions, 141 Differential Equations, 6 Parameters
remark
remark   ******************************************************
remark   Developer:       Brandon Kulengowski
remark   Model Date:      9-22-2018
remark   Model Version:           1.0
remark   ******************************************************
remark
remark   parameters
remark   ------------
remark  gS
remark
remark  gR
remark
remark  Nmax
remark
remark  kS
remark
remark  EC50S
remark
remark  kR

remark - model-specific commands
COMMANDS
NFUN 78
NDER 141
NPAR 6
PNAMES 'gS' 'gR' 'Nmax' 'kS' 'EC50S' 'kR'
NSEC 4
SNAMES 'SCS' 'SCR' 'dS' 'dR'
SIZE 15
END

TEMP
R034=19
R022=0.63
R024=1
R044=44
END

remark - differential equations starting values
START

remark - isolate 34 initial conditions

Z(1)=2.63*10**6
```

Table G.22: Polymyxin B Composite Model Code

```
Z(2)=R034
Z(3)=2.44*10**6
Z(4)=R034
Z(5)=1.13*10**7
Z(6)=R034
Z(7)=1.14*10**7
Z(8)=1.42*10**2
Z(9)=7.07*10**5
Z(10)=R034
Z(11)=6.54*10**5
Z(12)=R034
Z(13)=7.23*10**5
Z(14)=R034
Z(15)=5.84*10**5
Z(16)=R034
Z(17)=8.00*10**5
Z(18)=R034
Z(19)=7.79*10**5
Z(20)=R034
Z(21)=6.92*10**5
Z(22)=R034
Z(23)=6.85*10**5
Z(24)=R034
Z(25)=5.01*10**5
Z(26)=R034
Z(27)=5.15*10**5
Z(28)=R034
Z(29)=7.87*10**5
Z(30)=R034
Z(31)=9.30*10**5
Z(32)=R034
Z(33)=8.95*10**5
Z(34)=R034
Z(35)=8.43*10**5
Z(36)=R034

remark - isolate 22 initial conditions

Z(37)=3.14*10**6
Z(38)=R022
Z(39)=2.51*10**6
Z(40)=R022
Z(41)=6.23*10**6
Z(42)=2.54*10**2
Z(43)=1.34*10**6
Z(44)=R022
Z(45)=1.43*10**6
Z(46)=R022
Z(47)=5.01*10**5
Z(48)=R022
```

Table G.22: Polymyxin B Composite Model Code

```
Z(49)=5.53*10**5
Z(50)=R022
Z(51)=6.68*10**5
Z(52)=R022
Z(53)=6.60*10**5
Z(54)=R022
Z(55)=5.27*10**5
Z(56)=5.64*10**5
Z(57)=R022
Z(58)=5.00*10**5
Z(59)=R022
Z(60)=4.56*10**5
Z(61)=4.93*10**5
Z(62)=R022
Z(63)=6.91*10**5
Z(64)=R022

remark - isolate 24 initial conditions

Z(65)=1.77*10**6
Z(66)=R024
Z(67)=2.00*10**6
Z(68)=R024
Z(69)=2.46*10**6
Z(70)=1.00*10**0
Z(71)=4.26*10**5
Z(72)=R024
Z(73)=4.85*10**5
Z(74)=R024
Z(75)=6.33*10**5
Z(76)=R024
Z(77)=7.29*10**5
Z(78)=R024
Z(79)=5.87*10**5
Z(80)=R024
Z(81)=6.26*10**5
Z(82)=R024
Z(83)=5.66*10**5
Z(84)=R024
Z(85)=6.72*10**5
Z(86)=R024
Z(87)=5.81*10**5
Z(88)=R024
Z(89)=3.37*10**5
Z(90)=R024
Z(91)=3.67*10**5
Z(92)=R024
Z(93)=7.51*10**5
Z(94)=R024
Z(95)=5.37*10**5
```

Table G.22: Polymyxin B Composite Model Code

```
Z(96)=R024
Z(97)=5.04*10**5
Z(98)=R024

remark - isolate 44 initial conditions

Z(99)=1.63*10**6
Z(100)=R044
Z(101)=2.48*10**6
Z(102)=R044
Z(103)=4.29*10**6
Z(104)=R044
Z(105)=4.93*10**6
Z(106)=2.50*10**1
Z(107)=6.04*10**5
Z(108)=5.29*10**5
Z(109)=5.42*10**5
Z(110)=R044
Z(111)=7.16*10**5
Z(112)=R044
Z(113)=2.68*10**5
Z(114)=R044
Z(115)=8.53*10**5
Z(116)=R044
Z(117)=8.93*10**5
Z(118)=R044
Z(119)=4.48*10**5
Z(120)=R044
Z(121)=8.13*10**5
Z(122)=R044
Z(123)=9.82*10**5
Z(124)=R044
Z(125)=6.47*10**5
Z(126)=R044
Z(127)=6.18*10**5
Z(128)=R044
Z(129)=4.75*10**5
Z(130)=R044
Z(131)=5.56*10**5
Z(132)=6.53*10**5
Z(133)=R044
Z(134)=5.87*10**5
Z(135)=R044
Z(136)=7.75*10**5
Z(137)=R044
Z(138)=1.10*10**6
Z(139)=R044
Z(140)=9.48*10**5
Z(141)=R044
```

Table G.22: Polymyxin B Composite Model Code

```
END

remark - differential equations
DIFF

remark - isolate 34 DEs

remark  growth control experiment 1/26/14
DZ(1)=(gS*(1-(Z(1)+Z(2))/Nmax))*Z(1)
DZ(2)=(gR*(1-(Z(1)+Z(2))/Nmax))*Z(2)

remark  growth control experiment 2/15/14
DZ(3)=(gS*(1-(Z(3)+Z(4))/Nmax))*Z(3)
DZ(4)=(gR*(1-(Z(3)+Z(4))/Nmax))*Z(4)

remark  growth control experiment 3/30/14
DZ(5)=(gS*(1-(Z(5)+Z(6))/Nmax))*Z(5)
DZ(6)=(gR*(1-(Z(5)+Z(6))/Nmax))*Z(6)

remark  growth control experiment PMB susceptible subpopulation 6/11/15
DZ(7)=(gS*(1-(Z(7)+Z(8))/Nmax))*Z(7)

remark  growth control experiment PMB resistant subpopulation 6/11/15
DZ(8)=(gR*(1-(Z(7)+Z(8))/Nmax))*Z(8)

remark  polymyxin B 0.25 mg/L time-kill experiment 1/26/14
DZ(9)=(gS*(1-(Z(9)+Z(10))/Nmax)-kS*(0.25/(0.25+EC50S)))*Z(9)
DZ(10)=(gR*(1-(Z(9)+Z(10))/Nmax)-kR*0.25)*Z(10)

remark  polymyxin B 1 mg/L time-kill experiment 1/26/14
DZ(11)=(gS*(1-(Z(11)+Z(12))/Nmax)-kS*(1/(1+EC50S)))*Z(11)
DZ(12)=(gR*(1-(Z(11)+Z(12))/Nmax)-kR*1)*Z(12)

remark  polymyxin B 0.25 mg/L time-kill experiment 3/30/15
DZ(13)=(gS*(1-(Z(13)+Z(14))/Nmax)-kS*(0.25/(0.25+EC50S)))*Z(13)
DZ(14)=(gR*(1-(Z(13)+Z(14))/Nmax)-kR*0.25)*Z(14)

remark  polymyxin B 1 mg/L time-kill experiment 3/30/15
DZ(15)=(gS*(1-(Z(15)+Z(16))/Nmax)-kS*(1/(1+EC50S)))*Z(15)
DZ(16)=(gR*(1-(Z(15)+Z(16))/Nmax)-kR*1)*Z(16)

remark  polymyxin B 2 mg/L time-kill experiment 5/29/14
DZ(17)=(gS*(1-(Z(17)+Z(18))/Nmax)-kS*(2/(2+EC50S)))*Z(17)
DZ(18)=(gR*(1-(Z(17)+Z(18))/Nmax)-kR*2)*Z(18)

remark  polymyxin B 4 mg/L time-kill experiment 5/29/14
DZ(19)=(gS*(1-(Z(19)+Z(20))/Nmax)-kS*(4/(4+EC50S)))*Z(19)
DZ(20)=(gR*(1-(Z(19)+Z(20))/Nmax)-kR*4)*Z(20)

remark  polymyxin B 2 mg/L time-kill experiment 6/10/14
```

Table G.22: Polymyxin B Composite Model Code

```
DZ(21)=(gS*(1-(Z(21)+Z(22))/Nmax)-kS*(2/(2+EC50S)))*Z(21)
DZ(22)=(gR*(1-(Z(21)+Z(22))/Nmax)-kR*2)*Z(22)

remark  polymyxin B 4 mg/L time-kill experiment 6/10/14
DZ(23)=(gS*(1-(Z(23)+Z(24))/Nmax)-kS*(4/(4+EC50S)))*Z(23)
DZ(24)=(gR*(1-(Z(23)+Z(24))/Nmax)-kR*4)*Z(24)

remark  polymyxin B 0.0625 mg/L time-kill experiment 6/18/14
DZ(25)=(gS*(1-(Z(25)+Z(26))/Nmax)-kS*(0.0625/(0.0625+EC50S)))*Z(25)
DZ(26)=(gR*(1-(Z(25)+Z(26))/Nmax)-kR*0.0625)*Z(26)

remark  polymyxin B 0.125 mg/L time-kill experiment 6/18/14
DZ(27)=(gS*(1-(Z(27)+Z(28))/Nmax)-kS*(0.125/(0.125+EC50S)))*Z(27)
DZ(28)=(gR*(1-(Z(27)+Z(28))/Nmax)-kR*0.125)*Z(28)

remark  polymyxin B 0.0625 mg/L time-kill experiment 7/9/14
DZ(29)=(gS*(1-(Z(29)+Z(30))/Nmax)-kS*(0.0625/(0.0625+EC50S)))*Z(29)
DZ(30)=(gR*(1-(Z(29)+Z(30))/Nmax)-kR*0.0625)*Z(30)

remark  polymyxin B 0.125 mg/L time-kill experiment 7/9/14
DZ(31)=(gS*(1-(Z(31)+Z(32))/Nmax)-kS*(0.125/(0.125+EC50S)))*Z(31)
DZ(32)=(gR*(1-(Z(31)+Z(32))/Nmax)-kR*0.125)*Z(32)

remark  polymyxin B 0.25 mg/L time-kill experiment 8/23/17
DZ(33)=(gS*(1-(Z(33)+Z(34))/Nmax)-kS*(0.25/(0.25+EC50S)))*Z(33)
DZ(34)=(gR*(1-(Z(33)+Z(34))/Nmax)-kR*0.25)*Z(34)

remark  polymyxin B 1 mg/L time-kill experiment 8/23/17
DZ(35)=(gS*(1-(Z(35)+Z(36))/Nmax)-kS*(1/(1+EC50S)))*Z(35)
DZ(36)=(gR*(1-(Z(35)+Z(36))/Nmax)-kR*1)*Z(36)

remark - isolate 22 DEs

remark  growth control experiment 5/29/14
DZ(37)=(gS*(1-(Z(37)+Z(38))/Nmax))*Z(37)
DZ(38)=(gR*(1-(Z(37)+Z(38))/Nmax))*Z(38)

remark  growth control experiment 8/23/14
DZ(39)=(gS*(1-(Z(39)+Z(40))/Nmax))*Z(39)
DZ(40)=(gR*(1-(Z(39)+Z(40))/Nmax))*Z(40)

remark  growth control experiment 6/11/15
DZ(41)=(gS*(1-(Z(41)+Z(42))/Nmax))*Z(41)

remark  growth control experiment PMB resistant subpopulation 6/11/15
DZ(42)=(gR*(1-(Z(41)+Z(42))/Nmax))*Z(42)

remark  polymyxin B 0.25 mg/L time-kill experiment 5/14/14
DZ(43)=(gS*(1-(Z(43)+Z(44))/Nmax)-kS*(0.25/(0.25+EC50S)))*Z(43)
DZ(44)=(gR*(1-(Z(43)+Z(44))/Nmax)-kR*0.25)*Z(44)
```

Table G.22: Polymyxin B Composite Model Code

```
remark  polymyxin B 1 mg/L time-kill experiment 5/14/14
DZ(45)=(gS*(1-(Z(45)+Z(46))/Nmax)-kS*(1/(1+EC50S)))*Z(45)
DZ(46)=(gR*(1-(Z(45)+Z(46))/Nmax)-kR*1)*Z(46)

remark  polymyxin B 0.25 mg/L time-kill experiment 5/20/14
DZ(47)=(gS*(1-(Z(47)+Z(48))/Nmax)-kS*(0.25/(0.25+EC50S)))*Z(47)
DZ(48)=(gR*(1-(Z(47)+Z(48))/Nmax)-kR*0.25)*Z(48)

remark  polymyxin B 1 mg/L time-kill experiment 5/20/14
DZ(49)=(gS*(1-(Z(49)+Z(50))/Nmax)-kS*(1/(1+EC50S)))*Z(49)
DZ(50)=(gR*(1-(Z(49)+Z(50))/Nmax)-kR*1)*Z(50)

remark  polymyxin B 2 mg/L time-kill experiment 5/29/14
DZ(51)=(gS*(1-(Z(51)+Z(52))/Nmax)-kS*(2/(2+EC50S)))*Z(51)
DZ(52)=(gR*(1-(Z(51)+Z(52))/Nmax)-kR*2)*Z(52)

remark  polymyxin B 4 mg/L time-kill experiment 5/29/14
DZ(53)=(gS*(1-(Z(53)+Z(54))/Nmax)-kS*(4/(4+EC50S)))*Z(53)
DZ(54)=(gR*(1-(Z(53)+Z(54))/Nmax)-kR*4)*Z(54)

remark  polymyxin B 0.0625 mg/L time-kill experiment 6/10/14
DZ(55)=(gS*(1-(Z(55))/Nmax)-kS*(0.0625/(0.0625+EC50S)))*Z(55)

remark  polymyxin B 2 mg/L time-kill experiment 6/10/14
DZ(56)=(gS*(1-(Z(56)+Z(57))/Nmax)-kS*(2/(2+EC50S)))*Z(56)
DZ(57)=(gR*(1-(Z(56)+Z(57))/Nmax)-kR*2)*Z(57)

remark  polymyxin B 4 mg/L time-kill experiment 6/10/14
DZ(58)=(gS*(1-(Z(58)+Z(59))/Nmax)-kS*(4/(4+EC50S)))*Z(58)
DZ(59)=(gR*(1-(Z(58)+Z(59))/Nmax)-kR*4)*Z(59)

remark  polymyxin B 0.0625 mg/L time-kill experiment 6/18/14
DZ(60)=(gS*(1-Z(60)/Nmax)-kS*(0.0625/(0.0625+EC50S)))*Z(60)

remark  polymyxin B 0.125 mg/L time-kill experiment 6/18/14
DZ(61)=(gS*(1-(Z(61)+Z(62))/Nmax)-kS*(0.125/(0.125+EC50S)))*Z(61)
DZ(62)=(gR*(1-(Z(61)+Z(62))/Nmax)-kR*0.125)*Z(62)

remark  polymyxin B 0.125 mg/L time-kill experiment 7/9/14
DZ(63)=(gS*(1-(Z(63)+Z(64))/Nmax)-kS*(0.125/(0.125+EC50S)))*Z(63)
DZ(64)=(gR*(1-(Z(63)+Z(64))/Nmax)-kR*0.125)*Z(64)

remark - isolate 24 DEs

remark  growth control experiment 5/29/14
DZ(65)=(gS*(1-(Z(65)+Z(66))/Nmax))*Z(65)
DZ(66)=(gR*(1-(Z(65)+Z(66))/Nmax))*Z(66)

remark  growth control experiment 8/23/14
```

Table G.22: Polymyxin B Composite Model Code

```
DZ(67)=(gS*(1-(Z(67)+Z(68))/Nmax))*Z(67)
DZ(68)=(gR*(1-(Z(67)+Z(68))/Nmax))*Z(68)

remark  growth control experiment 6/11/15
DZ(69)=(gS*(1-(Z(69)+Z(70))/Nmax))*Z(69)

remark  growth control experiment PMB resistant subpopulation 6/11/15
DZ(70)=(gR*(1-(Z(69)+Z(70))/Nmax))*Z(70)

remark  polymyxin B 0.25 mg/L time-kill experiment 5/20/14
DZ(71)=(gS*(1-(Z(71)+Z(72))/Nmax)-kS*(0.25/(0.25+EC50S)))*Z(71)
DZ(72)=(gR*(1-(Z(71)+Z(72))/Nmax)-kR*0.25)*Z(72)

remark  polymyxin B 1 mg/L time-kill experiment 5/20/14
DZ(73)=(gS*(1-(Z(73)+Z(74))/Nmax)-kS*(1/(1+EC50S)))*Z(73)
DZ(74)=(gR*(1-(Z(73)+Z(74))/Nmax)-kR*1)*Z(74)

remark  polymyxin B 2 mg/L time-kill experiment 5/29/14
DZ(75)=(gS*(1-(Z(75)+Z(76))/Nmax)-kS*(2/(2+EC50S)))*Z(75)
DZ(76)=(gR*(1-(Z(75)+Z(76))/Nmax)-kR*2)*Z(76)

remark  polymyxin B 4 mg/L time-kill experiment 5/29/14
DZ(77)=(gS*(1-(Z(77)+Z(78))/Nmax)-kS*(4/(4+EC50S)))*Z(77)
DZ(78)=(gR*(1-(Z(77)+Z(78))/Nmax)-kR*4)*Z(78)

remark  polymyxin B 0.0625 mg/L time-kill experiment 6/10/14
DZ(79)=(gS*(1-(Z(79)+Z(80))/Nmax)-kS*(0.0625/(0.0625+EC50S)))*Z(79)
DZ(80)=(gR*(1-(Z(79)+Z(80))/Nmax)-kR*0.0625)*Z(80)

remark  polymyxin B 2 mg/L time-kill experiment 6/10/14
DZ(81)=(gS*(1-(Z(81)+Z(82))/Nmax)-kS*(2/(2+EC50S)))*Z(81)
DZ(82)=(gR*(1-(Z(81)+Z(82))/Nmax)-kR*2)*Z(82)

remark  polymyxin B 4 mg/L time-kill experiment 6/10/14
DZ(83)=(gS*(1-(Z(83)+Z(84))/Nmax)-kS*(4/(4+EC50S)))*Z(83)
DZ(84)=(gR*(1-(Z(83)+Z(84))/Nmax)-kR*4)*Z(84)

remark  polymyxin B 0.25 mg/L time-kill experiment 6/10/14
DZ(85)=(gS*(1-(Z(85)+Z(86))/Nmax)-kS*(0.25/(0.25+EC50S)))*Z(85)
DZ(86)=(gR*(1-(Z(85)+Z(86))/Nmax)-kR*0.25)*Z(86)

remark  polymyxin B 1 mg/L time-kill experiment 6/10/14
DZ(87)=(gS*(1-(Z(87)+Z(88))/Nmax)-kS*(1/(1+EC50S)))*Z(87)
DZ(88)=(gR*(1-(Z(87)+Z(88))/Nmax)-kR*1)*Z(88)

remark  polymyxin B 0.0625 mg/L time-kill experiment 6/18/14
DZ(89)=(gS*(1-(Z(89)+Z(90))/Nmax)-kS*(0.0625/(0.0625+EC50S)))*Z(89)
DZ(90)=(gR*(1-(Z(79)+Z(90))/Nmax)-kR*0.0625)*Z(90)

remark  polymyxin B 0.125 mg/L time-kill experiment 6/18/14
```

Table G.22: Polymyxin B Composite Model Code

```
DZ(91)=(gS*(1-(Z(91)+Z(92))/Nmax)-kS*(0.125/(0.125+EC50S)))*Z(91)
DZ(92)=(gR*(1-(Z(91)+Z(92))/Nmax)-kR*0.125)*Z(92)

remark  polymyxin B 0.125 mg/L time-kill experiment 7/9/14
DZ(93)=(gS*(1-(Z(93)+Z(94))/Nmax)-kS*(0.125/(0.125+EC50S)))*Z(93)
DZ(94)=(gR*(1-(Z(93)+Z(94))/Nmax)-kR*0.125)*Z(94)

remark  polymyxin B 2 mg/L time-kill experiment 8/23/17
DZ(95)=(gS*(1-(Z(95)+Z(96))/Nmax)-kS*(2/(2+EC50S)))*Z(95)
DZ(96)=(gR*(1-(Z(95)+Z(96))/Nmax)-kR*2)*Z(96)

remark  polymyxin B 4 mg/L time-kill experiment 8/23/17
DZ(97)=(gS*(1-(Z(97)+Z(98))/Nmax)-kS*(4/(4+EC50S)))*Z(97)
DZ(98)=(gR*(1-(Z(97)+Z(98))/Nmax)-kR*4)*Z(98)

remark - isolate 44 DEs

remark  growth control experiment 1/26/14
DZ(99)=(gS*(1-(Z(99)+Z(100))/Nmax))*Z(99)
DZ(100)=(gR*(1-(Z(99)+Z(100))/Nmax))*Z(100)

remark  growth control experiment 2/15/14
DZ(101)=(gS*(1-(Z(101)+Z(102))/Nmax))*Z(101)
DZ(102)=(gR*(1-(Z(101)+Z(102))/Nmax))*Z(102)

remark  growth control experiment 3/30/14
DZ(103)=(gS*(1-(Z(103)+Z(104))/Nmax))*Z(103)
DZ(104)=(gR*(1-(Z(103)+Z(104))/Nmax))*Z(104)

remark  polymyxin B-susceptible subpopulation gRowth 6/11/15
DZ(105)=(gS*(1-(Z(105)+Z(106))/Nmax))*Z(105)

remark  polymyxin B-resistant subpopulation gRowth 6/11/15
DZ(106)=(gR*(1-(Z(105)+Z(106))/Nmax))*Z(106)

remark  polymyxin B 0.0625 mg/L time-kill experiment 6/10/14
DZ(107)=(gS*(1-(Z(107))/Nmax)-kS*(0.0625/(0.0625+EC50S)))*Z(107)

remark  polymyxin B 0.0625 mg/L time-kill experiment 6/18/14
DZ(108)=(gS*(1-(Z(108))/Nmax)-kS*(0.0625/(0.0625+EC50S)))*Z(108)

remark  polymyxin B 0.125 mg/L time-kill experiment 6/18/14
DZ(109)=(gS*(1-(Z(109)+Z(110))/Nmax)-kS*(0.125/(0.125+EC50S)))*Z(109)
DZ(110)=(gR*(1-(Z(109)+Z(110))/Nmax)-kR*0.125)*Z(110)

remark  polymyxin B 0.125 mg/L time-kill experiment 7/9/14
DZ(111)=(gS*(1-(Z(111)+Z(112))/Nmax)-kS*(0.125/(0.125+EC50S)))*Z(111)
DZ(112)=(gR*(1-(Z(111)+Z(112))/Nmax)-kR*0.125)*Z(112)

remark  polymyxin B 0.25 mg/L time-kill experiment 3/30/14
```

Table G.22: Polymyxin B Composite Model Code

```
DZ(113)=(gS*(1-(Z(113)+Z(114))/Nmax)-kS*(0.25/(0.25+EC50S)))*Z(113)
DZ(114)=(gR*(1-(Z(113)+Z(114))/Nmax)-kR*0.25)*Z(114)

remark  polymyxin B 0.25 mg/L time-kill experiment 4/19/14
DZ(115)=(gS*(1-(Z(115)+Z(116))/Nmax)-kS*(0.25/(0.25+EC50S)))*Z(115)
DZ(116)=(gR*(1-(Z(115)+Z(116))/Nmax)-kR*0.25)*Z(116)

remark  polymyxin B 0.25 mg/L time-kill experiment 5/14/14
DZ(117)=(gS*(1-(Z(117)+Z(118))/Nmax)-kS*(0.25/(0.25+EC50S)))*Z(117)
DZ(118)=(gR*(1-(Z(117)+Z(118))/Nmax)-kR*0.25)*Z(118)

remark  polymyxin B 1 mg/L time-kill experiment 3/30/14
DZ(119)=(gS*(1-(Z(119)+Z(120))/Nmax)-kS*(1/(1+EC50S)))*Z(119)
DZ(120)=(gR*(1-(Z(119)+Z(120))/Nmax)-kR*1)*Z(120)

remark  polymyxin B 1 mg/L time-kill experiment 4/19/14
DZ(121)=(gS*(1-(Z(121)+Z(122))/Nmax)-kS*(1/(1+EC50S)))*Z(121)
DZ(122)=(gR*(1-(Z(121)+Z(122))/Nmax)-kR*1)*Z(122)

remark  polymyxin B 1 mg/L time-kill experiment 5/14/14
DZ(123)=(gS*(1-(Z(123)+Z(124))/Nmax)-kS*(1/(1+EC50S)))*Z(123)
DZ(124)=(gR*(1-(Z(123)+Z(124))/Nmax)-kR*1)*Z(124)

remark  polymyxin B 2 mg/L time-kill experiment 5/29/14
DZ(125)=(gS*(1-(Z(125)+Z(126))/Nmax)-kS*(2/(2+EC50S)))*Z(125)
DZ(126)=(gR*(1-(Z(125)+Z(126))/Nmax)-kR*2)*Z(126)

remark  polymyxin B 2 mg/L time-kill experiment 6/10/14
DZ(127)=(gS*(1-(Z(127)+Z(128))/Nmax)-kS*(2/(2+EC50S)))*Z(127)
DZ(128)=(gR*(1-(Z(127)+Z(128))/Nmax)-kR*2)*Z(128)

remark  polymyxin B 2 mg/L time-kill experiment 8/23/14
DZ(129)=(gS*(1-(Z(129)+Z(130))/Nmax)-kS*(2/(2+EC50S)))*Z(129)
DZ(130)=(gR*(1-(Z(129)+Z(130))/Nmax)-kR*2)*Z(130)

remark  polymyxin B 4 mg/L time-kill experiment 5/20/14
DZ(131)=(gS*(1-(Z(131))/Nmax)-kS*(4/(4+EC50S)))*Z(131)

remark  polymyxin B 4 mg/L time-kill experiment 5/29/14
DZ(132)=(gS*(1-(Z(132)+Z(133))/Nmax)-kS*(4/(4+EC50S)))*Z(132)
DZ(133)=(gR*(1-(Z(132)+Z(133))/Nmax)-kR*4)*Z(133)

remark  polymyxin B 4 mg/L time-kill experiment 6/10/14
DZ(134)=(gS*(1-(Z(134)+Z(135))/Nmax)-kS*(4/(4+EC50S)))*Z(134)
DZ(135)=(gR*(1-(Z(134)+Z(135))/Nmax)-kR*4)*Z(135)

remark  polymyxin B 4 mg/L time-kill experiment 7/9/14
DZ(136)=(gS*(1-(Z(136)+Z(137))/Nmax)-kS*(4/(4+EC50S)))*Z(136)
DZ(137)=(gR*(1-(Z(136)+Z(137))/Nmax)-kR*4)*Z(137)
```

Table G.22: Polymyxin B Composite Model Code

```
remark  polymyxin B 4 mg/L time-kill experiment 8/23/14
DZ(138)=(gS*(1-(Z(138)+Z(139))/Nmax)-kS*(4/(4+EC50S)))*Z(138)
DZ(139)=(gR*(1-(Z(138)+Z(139))/Nmax)-kR*4)*Z(139)

remark  polymyxin B-susceptible subpopulation for polymyxin B 4 mg/L time-kill experiment
2/13/17
DZ(140)=(gS*(1-(Z(140)+Z(141))/Nmax)-kS*(4/(4+EC50S)))*Z(140)

remark  polymyxin B-resistant subpopulation for polymyxin B 4 mg/L time-kill experiment
2/13/17
DZ(141)=(gR*(1-(Z(140)+Z(141))/Nmax)-kR*4)*Z(141)

END

remark - algebraic functions

remark - isolate 34 functions

FUNC 1
F=log10(Z(1)+Z(2))
END
FUNC 2
F=log10(Z(3)+Z(4))
END
FUNC 3
F=log10(Z(5)+Z(6))
END
FUNC 4
F=log10(Z(7)+Z(8))
END
FUNC 5
F=log10(Z(8))
END
FUNC 6
F=log10(Z(9)+Z(10))
END
FUNC 7
F=log10(Z(11)+Z(12))
END
FUNC 8
F=log10(Z(13)+Z(14))
END
FUNC 9
F=log10(Z(15)+Z(16))
END
FUNC 10
F=log10(Z(17)+Z(18))
END
FUNC 11
F=log10(Z(19)+Z(20))
```

Table G.22: Polymyxin B Composite Model Code

```
END
FUNC 12
F=log10(Z(21)+Z(22))
END
FUNC 13
F=log10(Z(23)+Z(24))
END
FUNC 14
F=log10(Z(25)+Z(26))
END
FUNC 15
F=log10(Z(27)+Z(28))
END
FUNC 16
F=log10(Z(29)+Z(30)+1)
END
FUNC 17
F=log10(Z(31)+Z(32))
END
FUNC 18
F=log10(Z(33)+Z(34))
END
FUNC 19
F=log10(Z(35)+Z(36))
END

remark - isolate 22 functions

FUNC 20
F=log10(Z(37)+Z(38))
END
FUNC 21
F=log10(Z(39)+Z(40))
END
FUNC 22
F=log10(Z(41)+Z(42))
END
FUNC 23
F=log10(Z(42))
END
FUNC 24
F=log10(Z(43)+Z(44))
END
FUNC 25
F=log10(Z(45)+Z(46))
END
FUNC 26
F=log10(Z(47)+Z(48))
END
FUNC 27
```

Table G.22: Polymyxin B Composite Model Code

```
F=log10(Z(49)+Z(50))
END
FUNC 28
F=log10(Z(51)+Z(52))
END
FUNC 29
F=log10(Z(53)+Z(54))
END
FUNC 30
F=log10(Z(55))
END
FUNC 31
F=log10(Z(56)+Z(57))
END
FUNC 32
F=log10(Z(58)+Z(59))
END
FUNC 33
F=log10(Z(60))
END
FUNC 34
F=log10(Z(61)+Z(62))
END
FUNC 35
F=log10(Z(63)+Z(64))
END

remark - isolate 24 functions

FUNC 36
F=log10(Z(65)+Z(66))
END
FUNC 37
F=log10(Z(67)+Z(68))
END
FUNC 38
F=log10(Z(69)+Z(70))
END
FUNC 39
F=log10(Z(70))
END
FUNC 40
F=log10(Z(71)+Z(72))
END
FUNC 41
F=log10(Z(73)+Z(74))
END
FUNC 42
F=log10(Z(75)+Z(76))
END
```

Table G.22: Polymyxin B Composite Model Code

```
FUNC 43
F=log10(Z(77)+Z(78))
END
FUNC 44
F=log10(Z(79)+Z(80))
END
FUNC 45
F=log10(Z(81)+Z(82))
END
FUNC 46
F=log10(Z(83)+Z(84))
END
FUNC 47
F=log10(Z(85)+Z(86))
END
FUNC 48
F=log10(Z(87)+Z(88))
END
FUNC 49
F=log10(Z(89)+Z(90))
END
FUNC 50
F=log10(Z(91)+Z(92))
END
FUNC 51
F=log10(Z(93)+Z(94))
END
FUNC 52
F=log10(Z(95)+Z(96))
END
FUNC 53
F=log10(Z(97)+Z(98))
END

remark - isolate 44 functions

FUNC 54
F=log10(Z(99)+Z(100))
END
FUNC 55
F=log10(Z(101)+Z(102))
END
FUNC 56
F=log10(Z(103)+Z(104))
END
FUNC 57
F=log10(Z(105))
END
FUNC 58
F=log10(Z(106))
```

Table G.22: Polymyxin B Composite Model Code

```
END
FUNC 59
F=log10(Z(107))
END
FUNC 60
F=log10(Z(108))
END
FUNC 61
F=log10(Z(109)+Z(110))
END
FUNC 62
F=log10(Z(111)+Z(112))
END
FUNC 63
F=log10(Z(113)+Z(114))
END
FUNC 64
F=log10(Z(115)+Z(116))
END
FUNC 65
F=log10(Z(117)+Z(118))
END
FUNC 66
F=log10(Z(119)+Z(120))
END
FUNC 67
F=log10(Z(121)+Z(122))
END
FUNC 68
F=log10(Z(123)+Z(124))
END
FUNC 69
F=log10(Z(125)+Z(126))
END
FUNC 70
F=log10(Z(127)+Z(128))
END
FUNC 71
F=log10(Z(129)+Z(130))
END
FUNC 72
F=log10(Z(131))
END
FUNC 73
F=log10(Z(132)+Z(133))
END
FUNC 74
F=log10(Z(134)+Z(135))
END
FUNC 75
```

Table G.22: Polymyxin B Composite Model Code

```
F=log10(Z(136)+Z(137))
END
FUNC 76
F=log10(Z(138)+Z(139))
END
FUNC 77
F=log10(Z(140))
END
FUNC 78
F=log10(Z(141))
END

remark - secondary parameters
SECO
SCS=(gS/(kS-gS))*EC50S
SCR=gR/kR
dS=0.6931/gS*60
dR=0.6931/gR*60
END

remark - end of model
EOM
```

Appendix H:

Pharmacodynamic Mathematical Models

Data presented here lend support for the final models discussed in Pharmacodynamic Modeling. Model input data for all mathematical models can be found in Appendix F. Phoenix® 8.1 Win Nonlin® code utilized for each model can be found in Appendix G. Determination of initial parameter estimates for the growth control models are discussed in Determining Initial Pharmacodynamic Model Estimates for Growth Rate Constants and Maximum Population Count.

Pharmacodynamic Mathematical Model of Growth Experiments

The growth model (Equations 5, 6, and 7), initial parameter estimates, final parameter estimates, and fit of the growth control experiments are presented here. Growth model equation (5) reproduced here for easier reader reference:

$$\frac{dRLS}{dt} = \left(g_{RLS}\left[1 - \frac{RLS + RLR + RHS}{Nmax}\right]\right) RLS$$

Initial Pharmacodynamic Model of Isolate 34 Growth Control Experiments

Table H.1: Initial Parameter Estimates for 34 Growth Control Model

Parameter	gRLS	gRHS	gRLR	Nmax
Value	3.21	2.62	0.80	4.65E+11
Lower Bound	2.26	0.00	0.00	1.04E+11
Upper Bound	4.16	4.16	6.20	2.08E+12
Type	User-defined	User-defined	User-defined	User-defined
Source of estimate	Linear regression of pooled growth control data	Slope of single experiment meropenem highly resistant subpopulation data	Linear regression of single experiment polymyxin resistant subpopulation data	Average of pooled growth control data
Source of bounds	95% confidence interval of linear regression	Lower bound can't be <0. Upper bound likely not greater than gRLS upper bound	95% confidence interval of linear regression. Lower bound can't be <0	95% confidence interval of mean

Table H.2: Final Parameter Estimates for 34 Growth Control Model

Parameter	Estimate	SE	CV %	Lower univariate CI	Upper univariate CI
gRLS	3.43	0.28	8	2.84	4.01
gRHS	2.39	0.31	13	1.75	3.03
gRLR	1.37	0.28	20	0.79	1.94
Nmax	2.34E+11	8.25E+10	35	6.31E+10	4.04E+11

SE – Standard Error; CV – Coefficient of Variation; CI – 95% confidence interval; Units: g (h^{-1}); Nmax (CFU/mL)

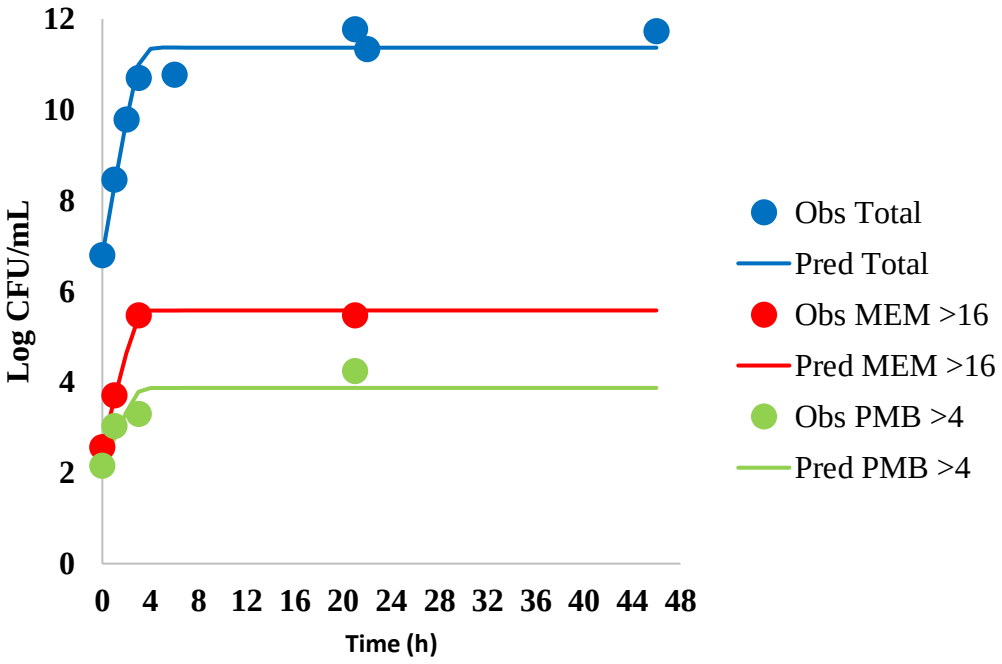

Figure H.1: Predicted and Observed Growth Control Experiments for Isolate 34. Observed data represent geometric mean data of at least 2 time-kill experiments. Blue data represent the sum of all three populations. Red data represent the subpopulation with a meropenem MIC > 16 mg/L. Green data represent the subpopulation with a polymyxin B MIC > 4 mg/L.

Initial Pharmacodynamic Model of Isolate 22 Growth Control Experiments

Table H.3: Initial Parameter Estimates for 22 Growth Control Model

Parameter	gRLS	gRHS	gRLR	Nmax
Value	3.23	2.4	1.83	6.22E+10
Lower Bound	2.72	0.00	0.00	1.41E+10
Upper Bound	3.74	3.74	3.74	1.10E+11
Type	User-defined	User-defined	User-defined	User-defined
Source of estimate	Linear regression of pooled growth control data	Slope of single experiment meropenem highly resistant subpopulation data	Slope of single experiment polymyxin resistant subpopulation data	Average of pooled growth control data
Source of bounds	95% confidence interval of linear regression	Lower bound can't be <0. Upper bound likely not greater than gRLS upper bound	Lower bound can't be <0. Upper bound likely not greater than gRLS upper bound	95% confidence interval of mean

Units: g (h^{-1}); Nmax (CFU/mL)

Table H.4: Final Parameter Estimates for 22 Growth Control Model

Parameter	Estimate	SE	CV %	Lower univariate CI	Upper univariate CI
gRLS	3.69	0.30	8	3.05	4.34
gRHS	2.06	0.36	18	1.30	2.83
gRLR	1.52	0.35	23	0.79	2.26
Nmax	5.14E+10	1.59E+10	31	1.78E+10	8.50E+10

SE – Standard Error; CV – Coefficient of Variation; CI – 95% confidence interval; Units: g (h^{-1}); Nmax (CFU/mL)

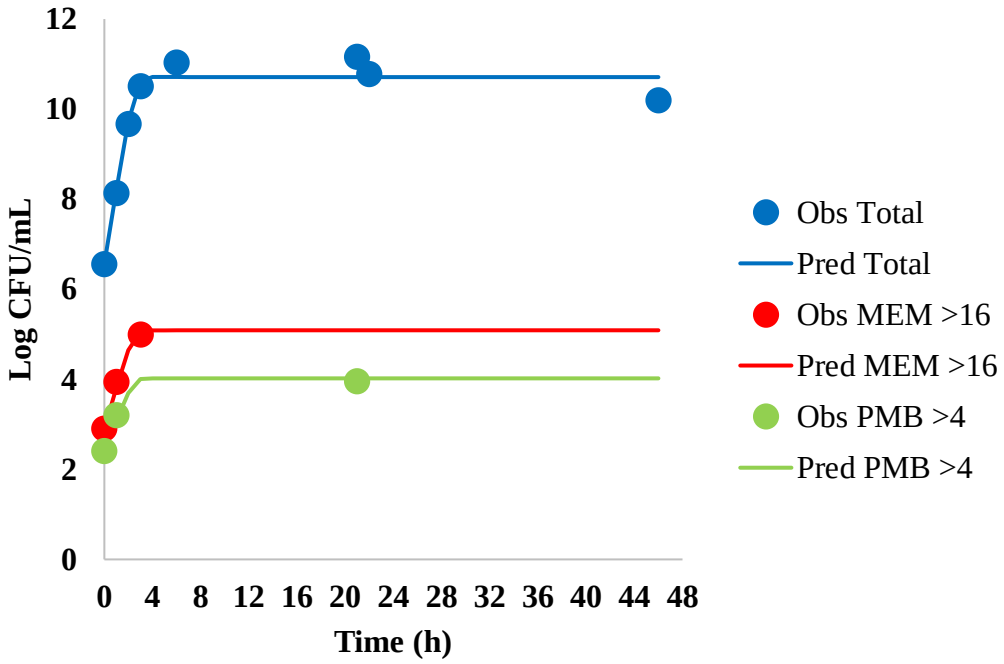

Figure H.2: Predicted and Observed Growth Control Experiments for Isolate 22. Observed data represent geometric mean data of at least 2 time-kill experiments. Blue data represent the sum of all three populations. Red data represent the subpopulation with a meropenem MIC > 16 mg/L. Green data represent the subpopulation with a polymyxin B MIC > 4 mg/L.

Initial Pharmacodynamic Model of Isolate 24 Growth Control Experiments

Table H.5: Initial Parameter Estimates for 24 Growth Control Model

Parameter	gRLS	gRHS	gRLR	Nmax
Value	1.68	1.04	0.86	3.58E+10
Lower Bound	0.00	0.14	0.00	1.09E+10
Upper Bound	3.47	1.95	3.47	1.18E+11
Type	User-defined	User-defined	User-defined	User-defined
Source of estimate	Linear regression of pooled growth control data	Linear regression of meropenem high resistant subpopulation data	Slope of single experiment polymyxin resistant subpopulation data	Average of pooled growth control data
Source of bounds	95% confidence interval of linear regression	95% confidence interval of linear regression	Lower bound can't be <0. Upper bound likely not greater than gRLS upper bound	95% confidence interval of mean

Units: g (h^{-1}); Nmax (CFU/mL)

Table H.6: Final Parameter Estimates for 24 Growth Control Model

Parameter	Estimate	SE	CV %	Lower univariate CI	Upper univariate CI
gRLS	1.71	0.15	9	1.40	2.03
gRHS	1.22	0.18	15	0.86	1.59
gRLR	0.70	0.17	24	0.35	1.05
Nmax	4.33E+10	2.21E+10	51	-1.94E+09	8.84E+10

SE – Standard Error; CV – Coefficient of Variation; CI – 95% confidence interval; Units: g (h^{-1}); Nmax (CFU/mL)

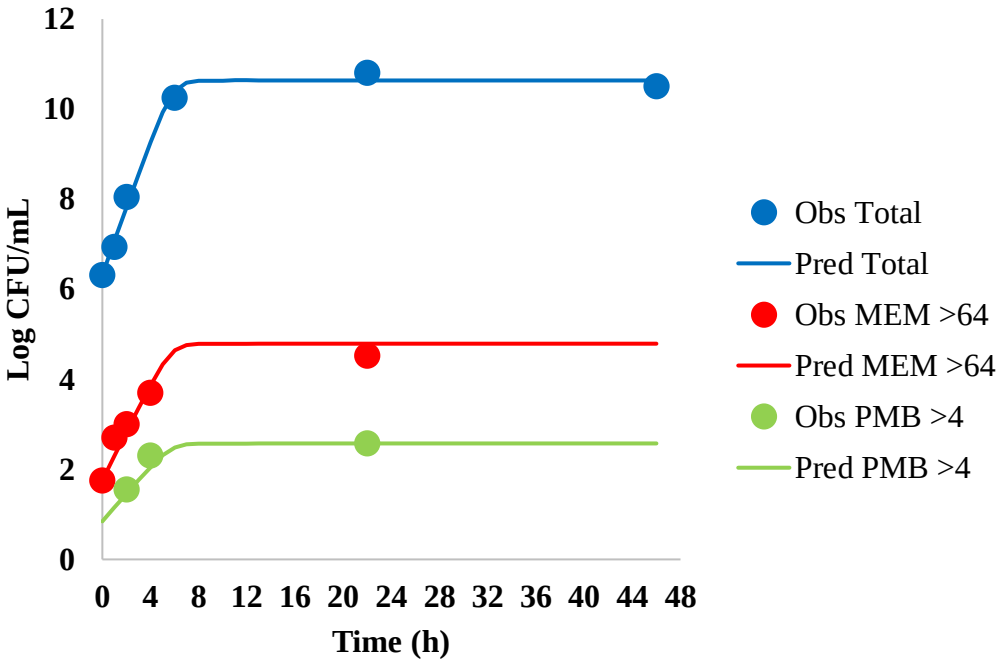

Figure H.3: Predicted and Observed Growth Control Experiments for Isolate 24. Observed data represent geometric mean data of at least 2 time-kill experiments. Blue data represent the sum of all three populations. Red data represent the subpopulation with a meropenem MIC > 16 mg/L. Green data represent the subpopulation with a polymyxin B MIC > 4 mg/L.

Initial Pharmacodynamic Model of Isolate 44 Growth Control Experiments

Table H.7: Initial Parameter Estimates for 44 Growth Control Model

Parameter	gRLS	gRHS	gRLR	Nmax
Value	2.38	2.33	1.40	5.72E+11
Lower Bound	1.56	0.00	0.21	1.04E+11
Upper Bound	3.19	9.81	2.59	1.19E+12
Type	User-defined	User-defined	User-defined	User-defined
Source of estimate	Linear regression of pooled growth control data	Linear regression of pooled growth control data	Linear regression of pooled growth control data	Average of pooled growth control data
Source of bounds	95% confidence interval of linear regression	95% confidence interval of linear regression. Lower bound can't be <0	95% confidence interval of linear regression	95% confidence interval of mean

Units: g (h^{-1}); Nmax (CFU/mL)

Table H.8: Final Parameter Estimates for 44 Growth Control Model

Parameter	Estimate	SE	CV %	Lower univariate CI	Upper univariate CI
gRLS	2.55	0.28	11	1.97	3.13
gRHS	2.27	0.30	13	1.65	2.88
gRLR	1.26	0.26	21	0.73	1.80
Nmax	1.20E+11	5.22E+10	44	1.29E+10	2.27E+11

SE – Standard Error; CV – Coefficient of Variation; CI – 95% confidence interval; Units: g (h^{-1}); Nmax (CFU/mL)

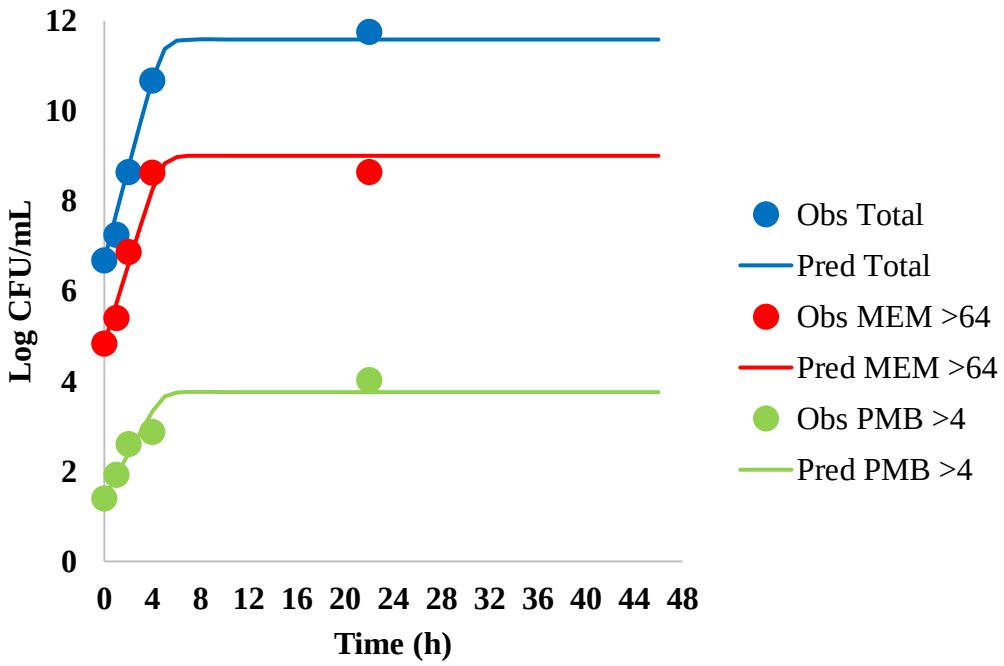

Figure H.4: Predicted and Observed Growth Control Experiments for Isolate 44. Observed data represent geometric mean data of at least 2 time-kill experiments. Blue data represent the sum of all three populations. Red data represent the subpopulation with a meropenem MIC > 16 mg/L. Green data represent the subpopulation with a polymyxin B MIC > 4 mg/L.

Pharmacodynamic Mathematical Model of the More Susceptible Population to Meropenem Monotherapy

Model input data for all mathematical models can be found in Appendix F. Phoenix® 8.1 Win Nonlin® code utilized for each model can be found in Appendix G. Determination of initial parameter estimates for the meropenem low resistance models are discussed in Determining Initial Pharmacodynamic Model Estimates for Susceptible Population Killing Rate Constants. The susceptible population model (Equation 1), initial parameter estimates, and final parameter estimates are presented here. Susceptible model equation (1) reproduced here for easier reader reference:

$$\frac{dS}{dt} = \left(g_S\left[1 - \frac{S+R}{Nmax}\right] - k_S\frac{C}{C+EC_{50S}}\right)S$$

Initial Killing Pharmacodynamic Model of Isolate 34 Meropenem Monotherapy Experiments

Table H.9: Initial Parameter Estimates for 34 Meropenem Monotherapy

Parameter	g_S	Nmax	k_S	EC_{50S}
Value	3.43	2.34E+11	7.56	2.28
Lower Bound	2.84	6.31E+10	5.85	0
Upper Bound	4.01	4.04E+11	9.28	6.57
Type	User-defined	User-defined	User-defined	User-defined
Source of estimate	Growth model final parameter	Growth model final parameter	Average of pairwise k_S data	Average of pairwise EC_{50S} data
Source of bounds	Growth model univariate 95% confidence interval	Growth model univariate 95% confidence interval	95% confidence interval of mean	95% confidence interval of mean

Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L)

Table H.10: Final Parameter Estimates for 34 Meropenem Monotherapy

Parameter	Estimate	SE	CV %	Lower univariate CI	Upper univariate CI
g_S	3.43	0.25	7	2.91	3.94
Nmax	2.26E+11	7.01E+10	31	8.45E+10	3.68E+11
k_S	7.28	0.37	5	6.53	8.03
EC_{50S}	1.67	0.30	18	1.06	2.28

SE – Standard Error; CV – Coefficient of Variation; CI – 95% confidence interval; Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L)

Initial Killing Pharmacodynamic Model of Isolate 22 Meropenem Monotherapy Experiments

Table H.11: Initial Parameter Estimates for 22 Meropenem Monotherapy

Parameter	g_S	Nmax	k_S	EC_{50S}
Value	3.69	5.14E+10	8.71	8.293
Lower Bound	3.05	1.78E+10	8.44	7.075
Upper Bound	4.34	8.50E+10	8.97	9.511
Type	User-defined	User-defined	User-defined	User-defined
Source of estimate	Growth model final parameter	Growth model final parameter	Average of pairwise k_S data	Average of pairwise EC_{50S} data
Source of bounds	Growth model univariate 95% confidence interval	Growth model univariate 95% confidence interval	95% confidence interval of mean	95% confidence interval of mean

Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L)

Table H.12: Final Parameter Estimates for 22 Meropenem Monotherapy

Parameter	Estimate	SE	CV %	Lower univariate CI	Upper univariate CI
g_S	3.37	0.50	15	2.34	4.40
Nmax	5.43E+10	3.00E+10	55	-6.95E+09	1.16E+11
k_S	8.44	0.99	12	6.42	10.46
EC_{50S}	7.08	3.00	42	0.93	13.22

SE – Standard Error; CV – Coefficient of Variation; CI – 95% confidence interval; Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L)

Initial Killing Pharmacodynamic Model of Isolate 24 Meropenem Monotherapy Experiments

Table H.13: Initial Parameter Estimates for 24 Meropenem Monotherapy

Parameter	g_S	Nmax	k_S	EC_{50S}
Value	1.71	4.33E+10	3.61	0.911
Lower Bound	1.40	1.94E+09	3.52	0.00
Upper Bound	2.03	8.84E+10	3.69	2.43
Type	User-defined	User-defined	User-defined	User-defined
Source of estimate	Growth model final parameter	Growth model final parameter	Average of pairwise k_S data	Average of pairwise EC_{50S} data
Source of bounds	Growth model univariate 95% confidence interval	Growth model univariate 95% confidence interval	95% confidence interval of mean	95% confidence interval of mean

Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L)

Table H.14: Final Parameter Estimates for 24 Meropenem Monotherapy

Parameter	Estimate	SE	CV %	Lower univariate CI	Upper univariate CI
g_S	1.99	0.15	8	1.68	2.30
Nmax	4.03E+10	1.21E+10	30	1.58E+10	6.48E+10
k_S	3.69	0.18	5	3.33	4.05
EC_{50S}	0.08	0.33	420	-0.59	0.75

SE – Standard Error; CV – Coefficient of Variation; CI – 95% confidence interval; Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L)

Initial Killing Pharmacodynamic Model of Isolate 44 Meropenem Monotherapy Experiments

Table H.15: Initial Parameter Estimates for 44 Meropenem Monotherapy

Parameter	g_S	Nmax	k_S	EC_{50S}
Value	2.27	1.20E+11	6.44	29.49
Lower Bound	1.65	1.29E+10	4.01	1
Upper Bound	2.88	2.27E+11	8.67	64.547
Type	User-defined	User-defined	User-defined	User-defined
Source of estimate	Growth model final parameter	Growth model final parameter	Average of pairwise k_S data	Average of pairwise EC_{50S} data
Source of bounds	Growth model univariate 95% confidence interval	Growth model univariate 95% confidence interval	95% confidence interval of mean	95% confidence interval of mean

Units of g are h^{-1}; Units of Nmax are CFU/mL; Units of k are h^{-1}; Units of EC50 are mg/L

Table H.16: Final Parameter Estimates for 44 Meropenem Monotherapy

Parameter	Estimate	SE	CV %	Lower univariate CI	Upper univariate CI
g_S	2.49	0.31	12	1.87	3.12
Nmax	1.16E+11	5.81E+10	49	-1.46E+09	2.34E+11
k_S	4.10	1.81	44	0.43	7.77
EC_{50S}	38.34	51.38	134	-65.87	142.54

SE – Standard Error; CV – Coefficient of Variation; CI – 95% confidence interval; Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L)

Pharmacodynamic Mathematical Model of the More Susceptible Population to Polymyxin B Monotherapy

Model input data for all mathematical models can be found in Appendix F. Phoenix® 8.1 Win Nonlin® code utilized for each model can be found in Appendix G. Determination of initial parameter estimates for the polymyxin B susceptible models are discussed in Determining Initial Pharmacodynamic Model Estimates for Susceptible Population Killing Rate Constants. The susceptible population model (Equation 1), initial parameter estimates, and final parameter estimates are presented here. Susceptible model equation (1) reproduced here for easier reader reference:

$$\frac{dS}{dt} = \left(g_S\left[1 - \frac{S+R}{Nmax}\right] - k_S\frac{C}{C + EC_{50S}}\right)S$$

Initial Killing Pharmacodynamic Model of Isolate 34 Polymyxin B Monotherapy Experiments

Table H.17: Initial Parameter Estimates for 34 Polymyxin B Monotherapy

Parameter	g_S	Nmax	k_S	EC_{50S}
Value	3.43	2.34E+11	10.60	0.235
Lower Bound	2.84	6.31E+10	8.99	0.011
Upper Bound	4.01	4.04E+11	12.22	0.460
Type	User-defined	User-defined	User-defined	User-defined
Source of estimate	Growth model final parameter	Growth model final parameter	Average of pairwise k_S data	Average of pairwise EC_{50S} data
Source of bounds	Growth model univariate 95% confidence interval	Growth model univariate 95% confidence interval	95% confidence interval of mean	95% confidence interval of mean

Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L)

Table H.18: Final Parameter Estimates for 34 Polymyxin B Monotherapy

Parameter	Estimate	SE	CV %	Lower univariate CI	Upper univariate CI
g_S	4.01	0.42	11	3.15	4.86
Nmax	4.04E+11	1.60E+11	40	8.18E+10	7.27E+11
k_S	9.78	0.54	6	8.70	10.86
EC_{50S}	0.05	0.01	19	0.03	0.07

SE – Standard Error; CV – Coefficient of Variation; CI – 95% confidence interval; Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L)

Initial Killing Pharmacodynamic Model of Isolate 22 Polymyxin B Monotherapy Experiments

Table H.19: Initial Parameter Estimates for 22 Polymyxin B Monotherapy

Parameter	g_S	Nmax	k_S	EC_{50S}
Value	3.69	5.14E+10	23.7	0.142
Lower Bound	3.05	1.78E+10	10.39	0.004
Upper Bound	4.34	8.50E+10	37.01	0.280
Type	User-defined	User-defined	User-defined	User-defined
Source of estimate	Growth model final parameter	Growth model final parameter	Average of pairwise k_S data	Average of pairwise EC_{50S} data
Source of bounds	Growth model univariate 95% confidence interval	Growth model univariate 95% confidence interval	95% confidence interval of mean	95% confidence interval of mean

Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L)

Table H.20: Final Parameter Estimates for 22 Polymyxin B Monotherapy

Parameter	Estimate	SE	CV %	Lower univariate CI	Upper univariate CI
g_S	3.66	0.39	11	2.84	4.48
Nmax	5.24E+10	2.08E+10	40	9.01E+09	9.57E+10
k_S	14.31	2.27	16	9.59	19.03
EC_{50S}	0.05	0.03	49	0.00	0.11

SE – Standard Error; CV – Coefficient of Variation; CI – 95% confidence interval; Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L)

Initial Killing Pharmacodynamic Model of Isolate 24 Polymyxin B Monotherapy Experiments

Table H.21: Initial Parameter Estimates for 24 Polymyxin B Monotherapy

Parameter	g_S	Nmax	k_S	EC_{50S}
Value	1.71	4.33E+10	8.82	0.039
Lower Bound	1.40	1.94E+09	7.83	0.006
Upper Bound	2.03	8.84E+10	9.81	0.071
Type	User-defined	User-defined	User-defined	User-defined
Source of estimate	Growth model final parameter	Growth model final parameter	Average of pairwise k_S data	Average of pairwise EC_{50S} data
Source of bounds	Growth model univariate 95% confidence interval	Growth model univariate 95% confidence interval	95% confidence interval of mean	95% confidence interval of mean

Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L)

Table H.22: Final Parameter Estimates for 24 Polymyxin B Monotherapy

Parameter	Estimate	SE	CV %	Lower univariate CI	Upper univariate CI
g_S	2.03	0.18	9	1.66	2.40
Nmax	4.04E+10	1.39E+10	35	1.17E+10	6.92E+10
k_S	7.83	0.81	10	6.16	9.50
EC_{50S}	0.06	0.03	43	0.01	0.11

SE – Standard Error; CV – Coefficient of Variation; CI – 95% confidence interval; Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L)

Initial Killing Pharmacodynamic Model of Isolate 44 Polymyxin B Monotherapy Experiments

Table H.23: Initial Parameter Estimates for 44 Polymyxin B Monotherapy

Parameter	g_S	Nmax	k_S	EC_{50S}
Value	2.27	1.20E+11	10.55	0.041
Lower Bound	1.65	1.29E+10	9.73	0.011
Upper Bound	2.88	2.27E+11	11.36	0.108
Type	User-defined	User-defined	User-defined	User-defined
Source of estimate	Growth model final parameter	Growth model final parameter	Average of pairwise k_S data	Average of pairwise EC_{50S} data
Source of bounds	Growth model univariate 95% confidence interval	Growth model univariate 95% confidence interval	95% confidence interval of mean	95% confidence interval of mean

Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L)

Table H.24: Final Parameter Estimates for 44 Polymyxin B Monotherapy

Parameter	Estimate	SE	CV %	Lower univariate CI	Upper univariate CI
g_S	2.61	0.27	10	2.07	3.16
Nmax	1.14E+11	4.26E+10	37	2.87E+10	2.00E+11
k_S	10.71	0.71	7	9.28	12.15
EC_{50S}	0.03	0.01	42	0.00	0.05

SE – Standard Error; CV – Coefficient of Variation; CI – 95% confidence interval; Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L)

Pharmacodynamic Mathematical Model of Meropenem Monotherapy

Model input data for all mathematical models can be found in Appendix F. Phoenix® 8.1 Win Nonlin® code utilized for each model can be found in Appendix G. Determination of initial parameter estimates for the meropenem models are discussed in Full Model Initial Estimates of Parameters. The two population models (Equations 1 and 2), initial parameter estimates, and final parameter estimates are presented here. Susceptible model equation (1) and resistant model equation (2) reproduced here for easier reader reference:

$$\frac{dS}{dt} = \left(g_S\left[1 - \frac{S+R}{Nmax}\right] - k_S\frac{C}{C+EC_{50S}}\right)S$$

$$\frac{dR}{dt} = \left(g_R\left[1 - \frac{S+R}{Nmax}\right] - k_R\frac{C}{C+EC_{50R}}\right)R$$

Pharmacodynamic Model of Isolate 34 Meropenem Monotherapy Experiments

According to the protocol discussed in Pharmacodynamic Mathematical Modeling, equation (1) and equation (11) were utilized for a total of 6 parameters, 13 time-kill experiments and 24 simultaneously solved differential equations.

Table H.25: Full Model Initial Parameter Estimates for 34 Meropenem Monotherapy

Parameter	g_S	g_R	Nmax	k_S	EC_{50S}	k'_R
Value	3.43	2.39	2.34E+11	7.28	1.67	0.548941
Lower Bound	2.84	1.75	6.31E+10	6.53	0.69	0.325514
Upper Bound	4.01	3.03	4.04E+11	8.03	2.65	0.772367
Type	User-defined	User-defined	User-defined	User-defined	User-defined	User-defined
Source of estimate	Growth control model fit	Growth control model fit	Growth control model fit	Net effect model fit	Net effect model fit	Average of pairwise k_R data
Source of bounds	Univar. bounds	Planar bounds	Univar. bounds	Planar bounds	Planar bounds	Lowest and Greatest k_R / EC_{50R}

Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L); k' (L/mg*h^{-1})

Table H.26: Full Model Final Parameter Estimates for 34 Meropenem Monotherapy

Parameter	Estimate	SE	CV %	Lower univariate CI	Upper univariate CI
g_S	4.01	0.50	12	3.01	5.01
g_R	2.94	0.70	24	1.55	4.34
Nmax	1.93E+11	9.37E+10	49	4.88E+09	3.81E+11
k_S	6.53	0.56	9	5.40	7.66
EC_{50S}	0.94	0.42	45	0.10	1.78
k'_R	0.54	0.17	32	0.20	0.89

SE – Standard Error; CV – Coefficient of Variation; CI – 95% confidence interval; Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L); k' (L/mg*h^{-1})

Table H.27: Full Model Final Secondary Parameter Estimates for 34 Meropenem Monotherapy

Parameter	Estimate	SE	CV %
SC_S (mg/L)	1.49	0.52	35
SC_R (mg/L)	5.43	0.46	8
d_S (min)	10.4	1.29	12
d_R (min)	14.1	3.33	24

SE – Standard Error; CV – Coefficient of Variation; SC – Stationary Concentration; d – doubling time

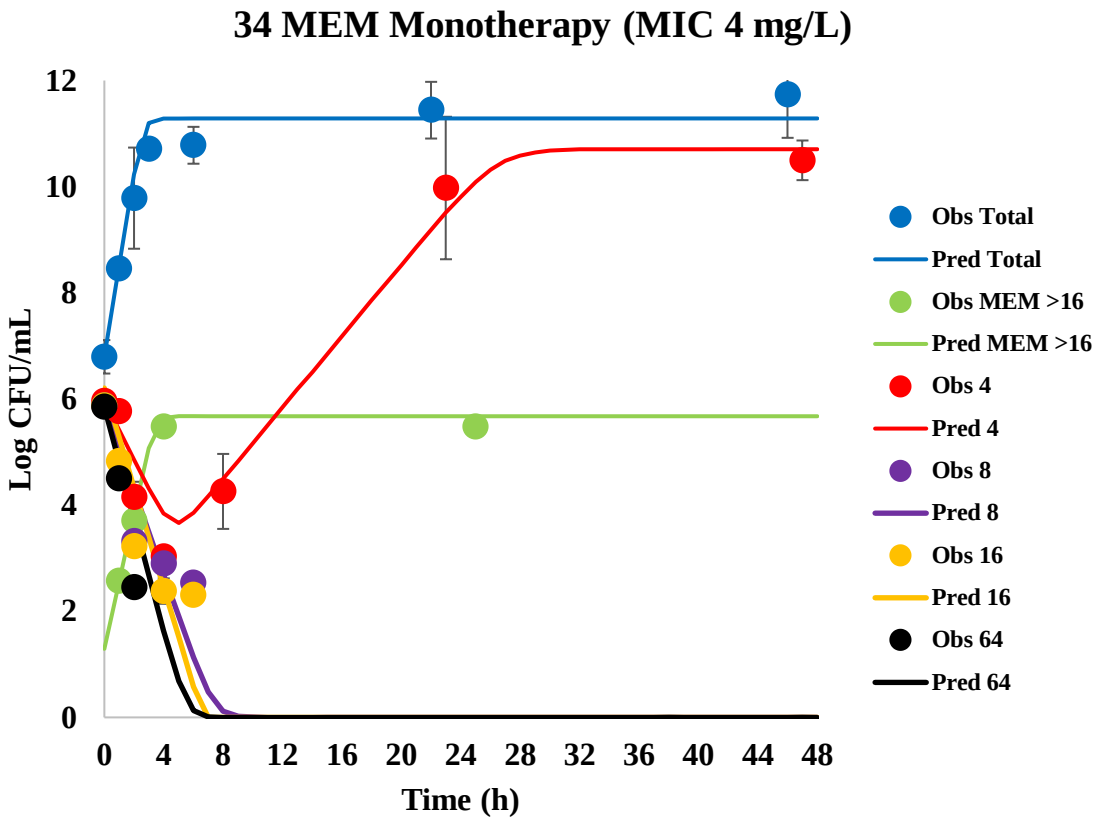

Figure H.5: Predicted and Observed Time-kill Data for Meropenem against Isolate 34. Observed data represent geometric means with standard deviations of at least 2 time-kill experiments except for green data which was only performed once. Note that some error bars are smaller than the symbol. Blue data represent the sum of both populations. Green data represent the subpopulation with a meropenem MIC > 16 mg/L. Other data indicate the total colony count with the given concentration of meropenem present in the time-kill flask.

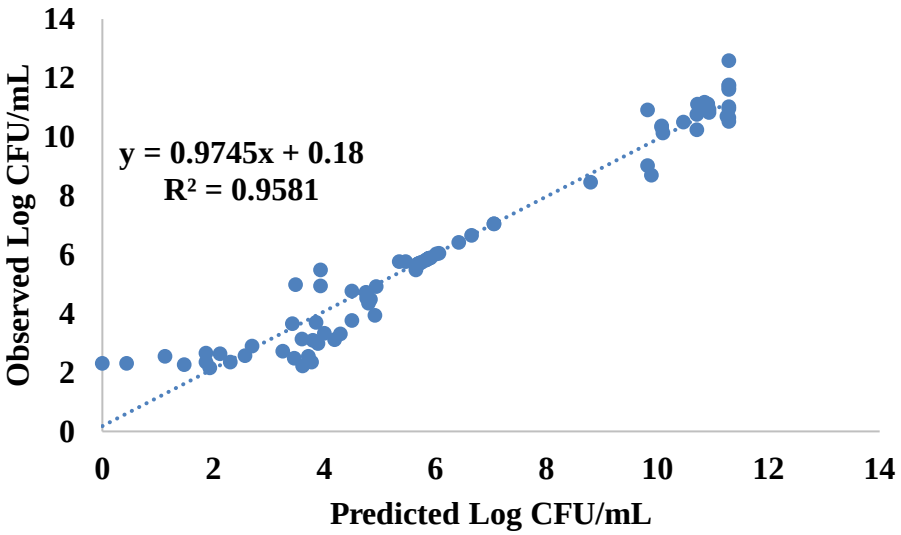

Figure H.6: Observed vs. Predicted Data for Meropenem Monotherapy Model against Isolate 34.

Pharmacodynamic Model of Isolate 22 Meropenem Monotherapy Experiments

According to the protocol discussed in Pharmacodynamic Mathematical Modeling, equation (1) and equation (11) were utilized for a total of 6 parameters, 13 time-kill experiments and 22 simultaneously solved differential equations.

Table H.28: Full Model Initial Parameter Estimates for 22 Meropenem Monotherapy

Parameter	g_S	g_R	Nmax	k_S	EC_{50S}	k'_R
Value	3.69	2.06	5.14E+10	8.44	7.08	0.10
Lower Bound	3.05	1.3	1.78E+10	6.42	0.93	0.02
Upper Bound	4.34	2.83	8.5E+10	10.46	13.22	0.18
Type	User-defined	User-defined	User-defined	User-defined	User-defined	User-defined
Source of estimate	Growth control model fit	Growth control model fit	Growth control model fit	Net effect model fit	Net effect model fit	Average of pairwise k_R data
Source of bounds	Univar. bounds	Planar bounds	Univar. bounds	Planar bounds	Planar bounds	Lowest and Greatest k_R / EC_{50R}

Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC_{50} (mg/L); k' (L/mg*h^{-1})

Table H.29: Full Model Final Parameter Estimates for 22 Meropenem Monotherapy

Parameter	Estimate	SE	CV %	Lower univariate CI	Upper univariate CI
g_S	3.05	0.45	15	2.15	3.95
g_R	1.65	0.15	9	1.35	1.95
Nmax	7.8E+10	3.30E+10	42	1.2E+10	1.4E+11
k_S	6.65	0.61	9	5.44	7.87
EC_{50S}	1.44	0.70	48	0.05	2.84
k'_R	0.05	0.01	20	0.03	0.06

SE – Standard Error; CV – Coefficient of Variation; CI – 95% confidence interval; Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC_{50} (mg/L); k' (L/mg*h^{-1})

Table H.30: Full Model Final Secondary Parameter Estimates for 22 Meropenem Monotherapy

Parameter	Estimate	SE	CV %
SC_S (mg/L)	1.22	0.48	39
SC_R (mg/L)	36.0	4.07	11
d_S (min)	13.6	2.01	15
d_R (min)	25.2	2.28	9

SE – Standard Error; CV – Coefficient of Variation; SC – Stationary Concentration; d – doubling time

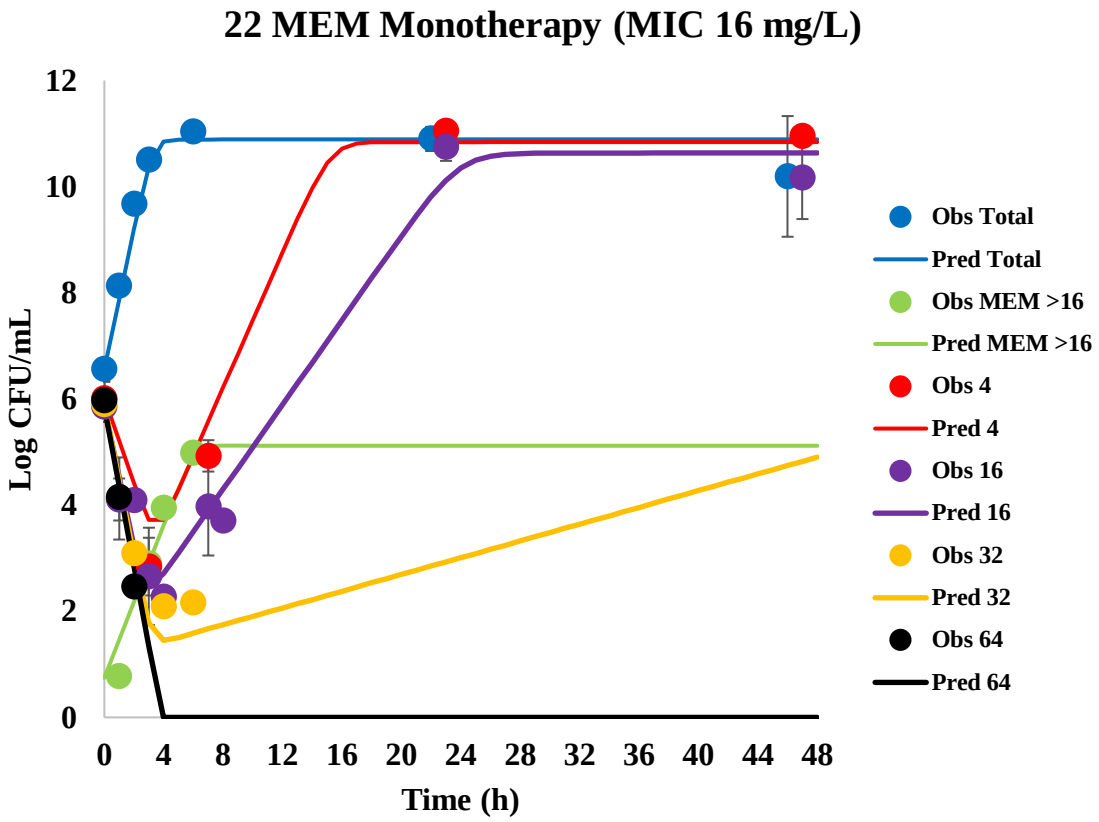

Figure H.7: Predicted and Observed Time-kill Data for Meropenem against Isolate 22. Observed data represent geometric means with standard deviations of at least 2 time-kill experiments except for green data which was only performed once. Note that some error bars are smaller than the symbol. Blue data represent the sum of both populations. Green data represent the subpopulation with a meropenem MIC > 16 mg/L. Other data indicate the total colony count with the given concentration of meropenem present in the time-kill flask.

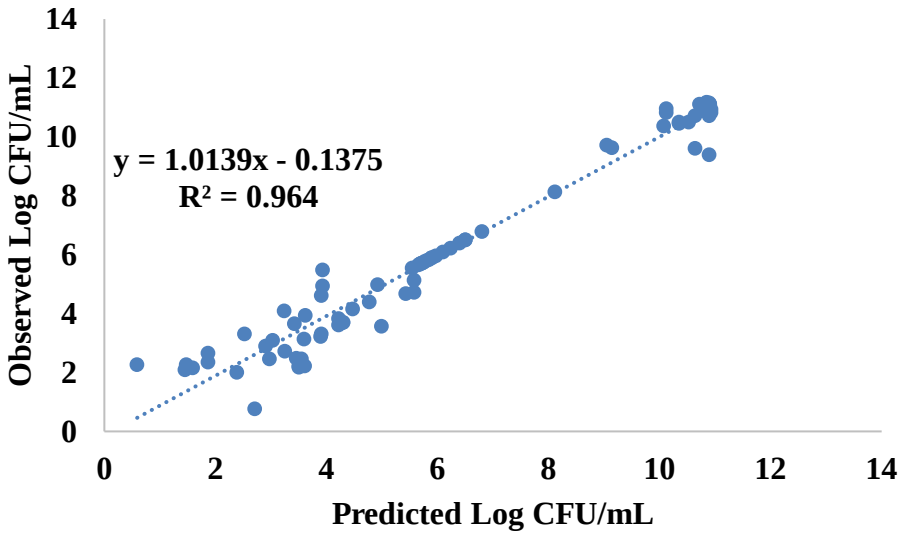

Figure H.8: Observed vs. Predicted Data for Meropenem Monotherapy Model against Isolate 22.

Pharmacodynamic Model of Isolate 24 Meropenem Monotherapy Experiments

According to the protocol discussed in Pharmacodynamic Mathematical Modeling, equation (10) and equation (11) were utilized for a total of 5 parameters, 13 time-kill experiments and 23 simultaneously solved differential equations.

Table H.31: Full Model Initial Parameter Estimates for 24 Meropenem Monotherapy

Parameter	g_S	g_R	Nmax	k_S	k'_R
Value	1.71	1.22	4.03E+10	3.69	0.010
Lower Bound	1.4	0.63	1.58E+10	3.12	0.003
Upper Bound	2.03	1.82	6.48E+10	4.26	0.017
Type	User-defined	User-defined	User-defined	User-defined	User-defined
Source of estimate	Growth control model fit	Growth control model fit	Growth control model fit	Net effect model fit	Average of pairwise k_R data
Source of bounds	Univar. bounds	Planar bounds	Univar. bounds	Planar bounds	Lowest and Greatest k_R / EC_{50R}

Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); k' ($L/mg*h^{-1}$)

Table H.32: Full Model Final Parameter Estimates for 24 Meropenem Monotherapy

Parameter	Estimate	SE	CV %	Lower univariate CI	Upper univariate CI
g_S	2.03	0.25	13	1.52	2.54
g_R	1.70	0.07	4	1.56	1.83
Nmax	5.2E+10	2.06E+10	40	1.1E+10	9.3E+10
k_S	3.72	0.30	8	3.13	4.31
k'_R	0.013	0.000	5	0.012	0.014

SE – Standard Error; CV – Coefficient of Variation; CI – 95% confidence interval; Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); k' ($L/mg*h^{-1}$)

Table H.33: Full Model Final Secondary Parameter Estimates for 24 Meropenem Monotherapy

Parameter	Estimate	SE	CV %
SC_S (mg/L)	-	-	-
SC_R (mg/L)	131	2.42	2
d_S (min)	20.5	2.57	13
d_R (min)	24.5	0.96	4

SE – Standard Error; CV – Coefficient of Variation; SC – Stationary Concentration; d – doubling time

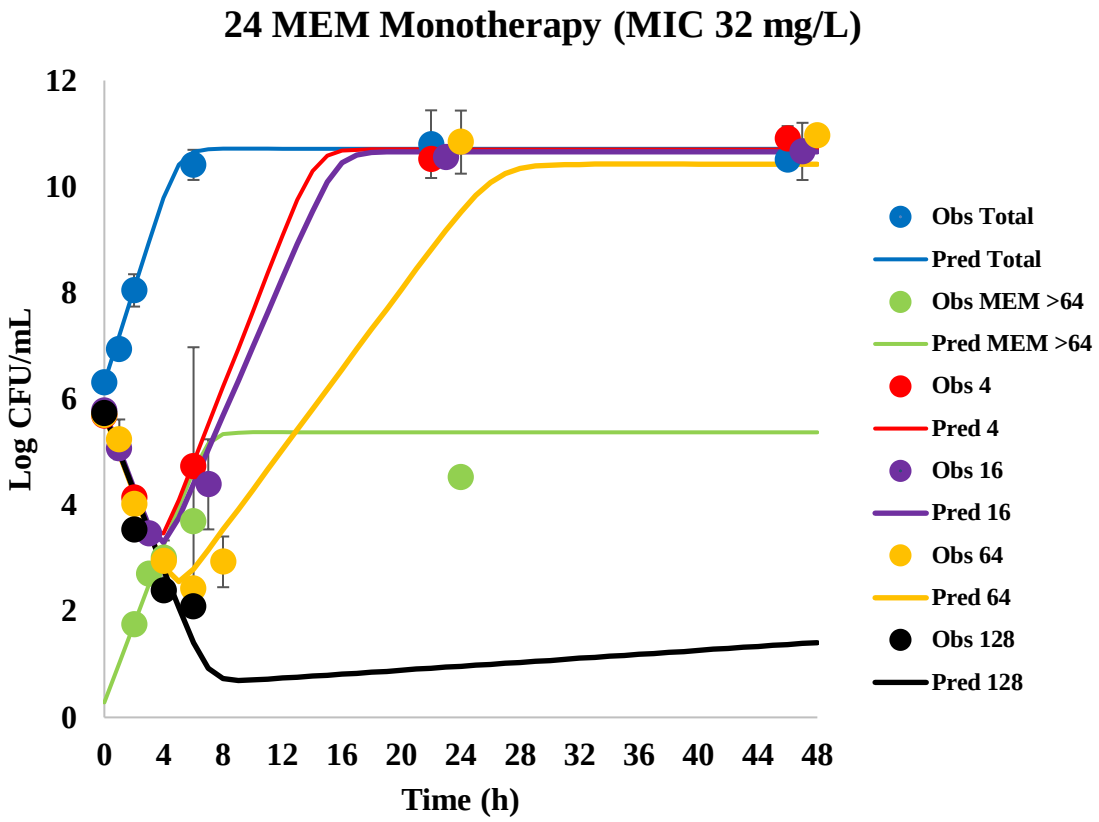

Figure H.9: Predicted and Observed Time-kill Data for Meropenem against Isolate 24. Observed data represent geometric means with standard deviations of at least 2 time-kill experiments except for green data which was only performed once. Note that some error bars are smaller than the symbol. Blue data represent the sum of both populations. Green data represent the subpopulation with a meropenem MIC > 64 mg/L. Other data indicate the total colony count with the given concentration of meropenem present in the time-kill flask.

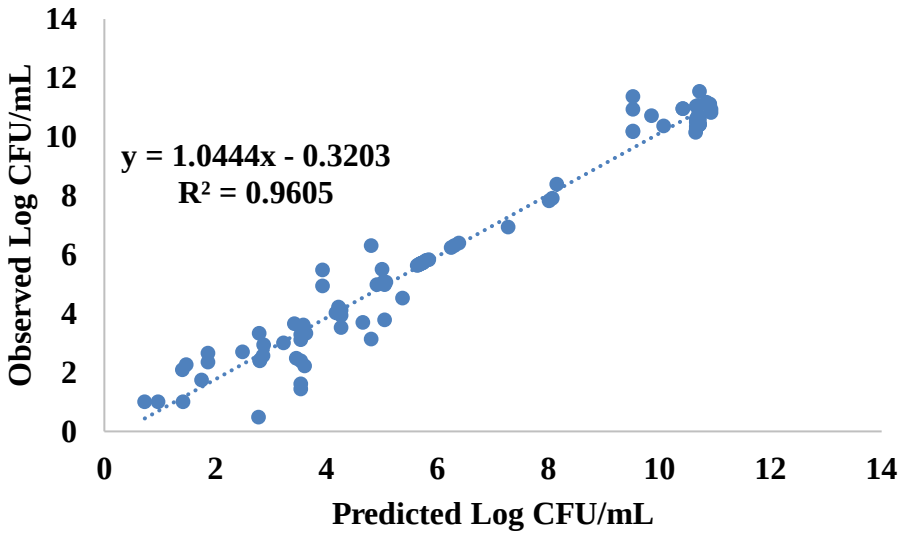

Figure H.10: Observed vs. Predicted Data for Meropenem Monotherapy Model against Isolate 24**.**

Pharmacodynamic Model of Isolate 44 Meropenem Monotherapy Experiments

According to the protocol discussed in Pharmacodynamic Mathematical Modeling, equation (1) and equation (11) were utilized for a total of 5 parameters, 13 time-kill experiments and 24 simultaneously solved differential equations.

Table H.34: Full Model Initial Parameter Estimates for 44 Meropenem Monotherapy

Parameter	g_S	g_R	Nmax	k_S	EC_{50S}
Value	2.55	2.27	1.20E+11	4.1	38.340
Lower Bound	1.97	1.28	1.29E+10	0.43	1.000
Upper Bound	3.13	3.26	2.27E+11	7.77	205.010
Type	User-defined	User-defined	User-defined	User-defined	User-defined
Source of estimate	Growth control model fit	Growth control model fit	Growth control model fit	Net effect model fit	Net effect model fit
Source of bounds	Univar. bounds	Planar bounds	Univar. bounds	Planar bounds	Planar bounds

Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC_{50} (mg/L)

Table H.35: Full Model Final Parameter Estimates for 44 Meropenem Monotherapy

Parameter	Estimate	SE	CV %	Lower univariate CI	Upper univariate CI
g_S	2.68	0.20	8	2.26	3.09
g_R	1.58	0.14	9	1.31	1.85
Nmax	7.0E+10	1.52E+10	22	4.0E+10	1.0E+11
k_S	6.07	0.96	16	4.15	7.99
EC_{50S}	66.60	25.11	38	16.35	116.85

SE – Standard Error; CV – Coefficient of Variation; CI – 95% confidence interval; Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC_{50} (mg/L)

Table H.36: Full Model Final Secondary Parameter Estimates for 44 Meropenem Monotherapy

Parameter	Estimate	SE	CV %
SC_S (mg/L)	52.5	3.42	7
SC_R (mg/L)	-	-	-
d_S (min)	15.6	1.19	8
d_R (min)	26.3	2.26	9

SE – Standard Error; CV – Coefficient of Variation; SC – Stationary Concentration; d – doubling time

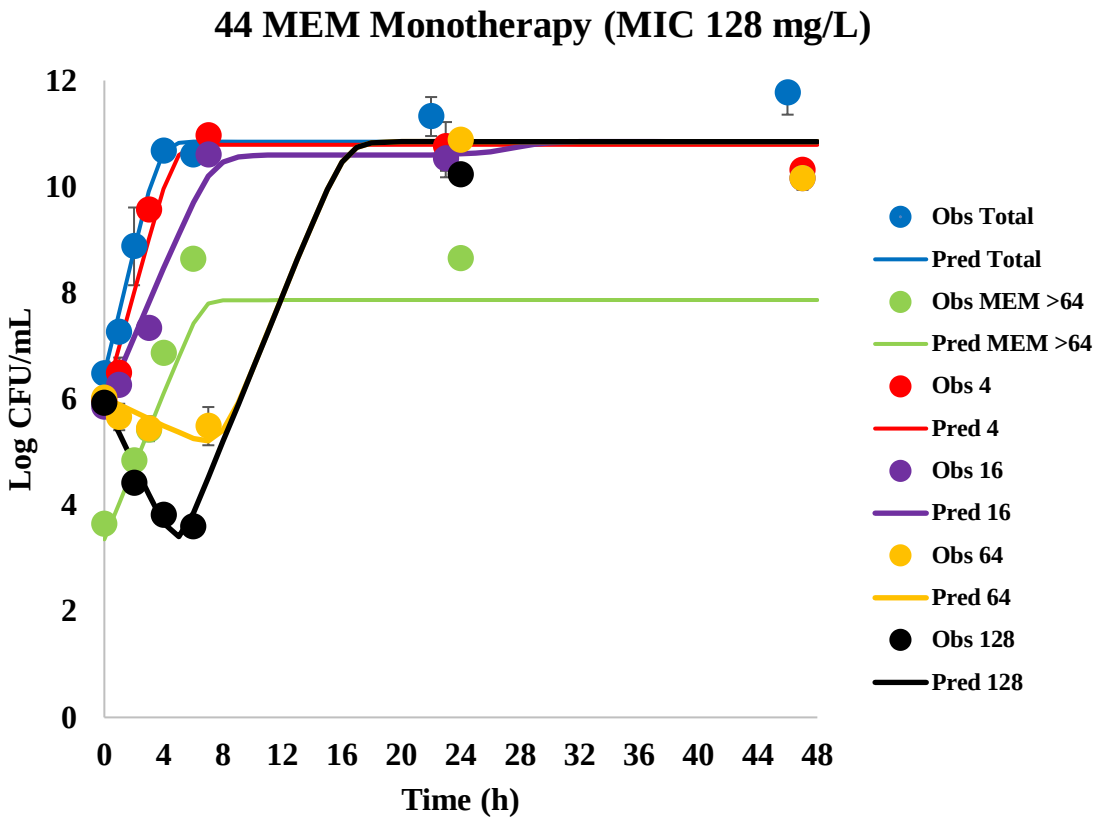

Figure H.11: Predicted and Observed Time-kill Data for Meropenem against Isolate 44. Observed data represent geometric means with standard deviations of at least 2 time-kill experiments except for green data which was only performed once. Note that some error bars are smaller than the symbol. Blue data represent the sum of both populations. Green data represent the subpopulation with a meropenem MIC > 64 mg/L. Other data indicate the total colony count with the given concentration of meropenem present in the time-kill flask.

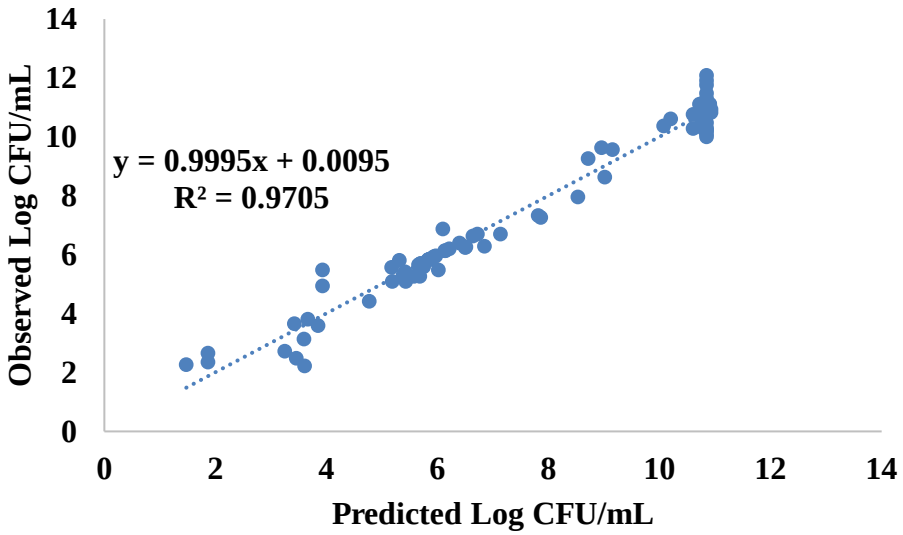

Figure H.12: Observed vs. Predicted Data for Meropenem Monotherapy Model against Isolate 44**.**

Pharmacodynamic Mathematical Model of Polymyxin B Monotherapy

Model input data for all mathematical models can be found in Appendix F. Phoenix® 8.1 Win Nonlin® code utilized for each model can be found in Appendix G. Determination of initial parameter estimates for the polymyxin B models are discussed in Full Model Initial Estimates of Parameters. The two population models (Equations 1 and 2), initial parameter estimates, and final parameter estimates are presented here. Susceptible model equation (1) and resistant model equation (2) reproduced here for easier reader reference:

$$\frac{dS}{dt} = \left(g_S\left[1 - \frac{S+R}{Nmax}\right] - k_S\frac{C}{C + EC_{50S}}\right)S$$

$$\frac{dR}{dt} = \left(g_R\left[1 - \frac{S+R}{Nmax}\right] - k_R\frac{C}{C + EC_{50R}}\right)R$$

Pharmacodynamic Model of Isolate 34 Polymyxin B Monotherapy Experiments

According to the protocol discussed in Pharmacodynamic Mathematical Modeling, equation (1) and equation (11) were utilized for a total of 6 parameters, 19 time-kill experiments and 36 simultaneously solved differential equations.

Table H.37: Full Model Initial Parameter Estimates for 34 Polymyxin B Monotherapy

Parameter	g_S	g_R	Nmax	k_S	EC_{50S}	k'_R
Value	3.43	1.37	2.34E+11	9.78	0.05	0.03
Lower Bound	2.84	0.79	6.31E+10	8.06	0.02	0.01
Upper Bound	4.01	1.94	4.04E+11	11.50	0.08	0.05
Type	User-defined	User-defined	User-defined	User-defined	User-defined	User-defined
Source of estimate	Growth control model fit	Growth control model fit	Growth control model fit	Net effect model fit	Net effect model fit	Average of pairwise k_R data
Source of bounds	Univar. bounds	Planar bounds	Univar. bounds	Planar bounds	Planar bounds	Lowest and Greatest k_R / EC_{50R}

Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L); k' (L/mg*h^{-1})

Table H.38: Full Model Final Parameter Estimates for 34 Polymyxin B Monotherapy

Parameter	Estimate	SE	CV %	Lower univariate CI	Upper univariate CI
g_S	3.92	0.44	11	3.04	4.80
g_R	1.22	0.05	4	1.11	1.32
Nmax	1.01E+11	2.19E+10	22	5.76E+10	1.44E+11
k_S	11.18	0.70	6	9.80	12.56
EC_{50S}	0.07	0.01	17	0.05	0.10
k'_R	0.08	0.02	22	0.05	0.12

SE – Standard Error; CV – Coefficient of Variation; CI – 95% confidence interval; Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L); k' (L/mg*h^{-1})

Table H.39: Full Model Final Secondary Parameter Estimates for 34 Polymyxin B Monotherapy

Parameter	Estimate	SE	CV %
SC_S (mg/L)	0.04	0.00	10
SC_R (mg/L)	14.8	2.80	19
d_S (min)	10.6	1.20	11
d_R (min)	34.2	1.45	4

SE – Standard Error; CV – Coefficient of Variation; SC – Stationary Concentration; d – doubling time

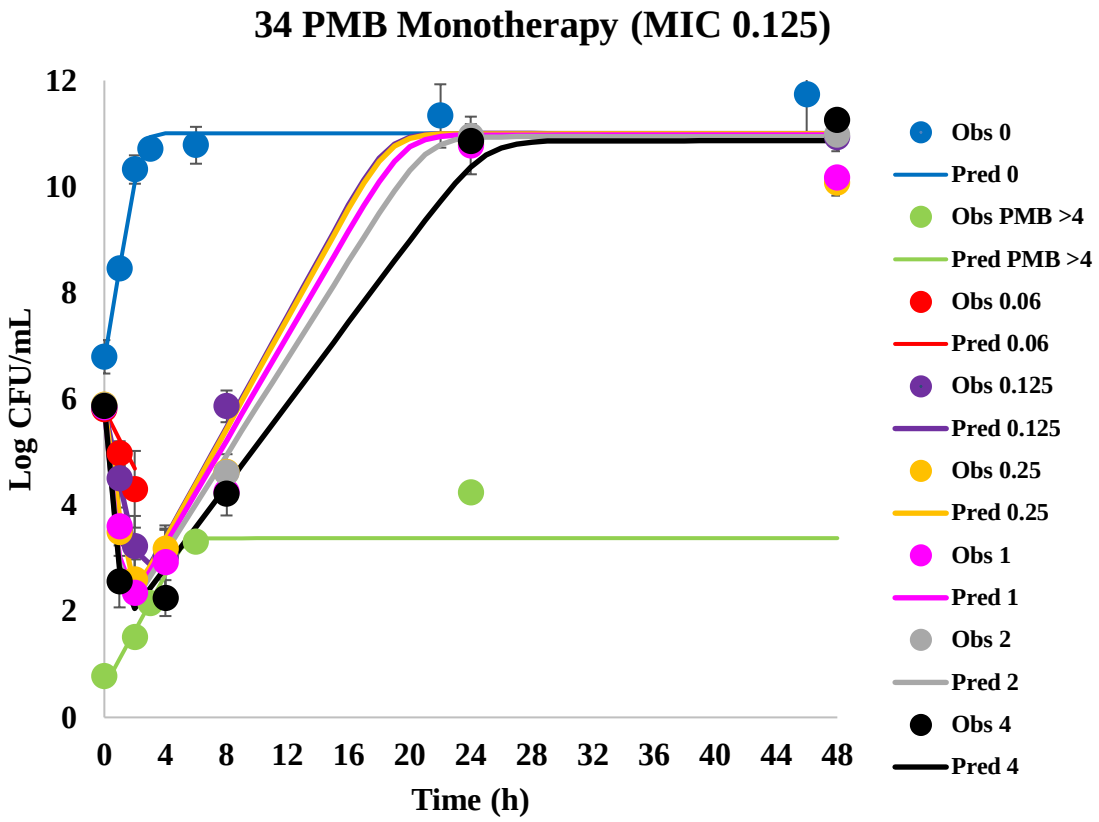

Figure H.13: Predicted and Observed Time-kill Data for Polymyxin B against Isolate 34. Observed data represent geometric means with standard deviations of at least 2 time-kill experiments except for green data which was only performed once. Note that some error bars are smaller than the symbol. Blue data represent the sum of both populations. Green data represent the subpopulation with a polymyxin B MIC > 4 mg/L. Other data indicate the total colony count with the given concentration of polymyxin B present in the time-kill flask.

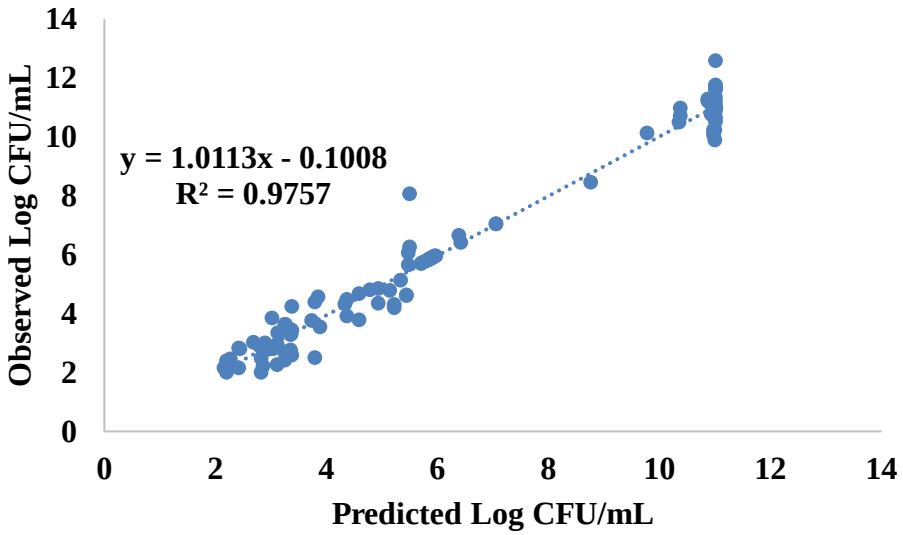

Figure H.14: Observed vs. Predicted Data for Polymyxin B Monotherapy Model against Isolate 34.

Pharmacodynamic Model of Isolate 22 Polymyxin B Monotherapy Experiments

According to the protocol discussed in Pharmacodynamic Mathematical Modeling, equation (10) and equation (11) were utilized for a total of 5 parameters, 16 time-kill experiments and 28 simultaneously solved differential equations.

Table H.40: Full Model Initial Parameter Estimates for 22 Polymyxin B Monotherapy

Parameter	g_S	g_R	Nmax	k_S	k'_R
Value	3.69	1.52	5.14E+10	14.31	0.03
Lower Bound	3.05	0.31	1.78E+10	6.63	0.00
Upper Bound	4.34	2.74	8.50E+10	21.99	0.05
Type	User-defined	User-defined	User-defined	User-defined	User-defined
Source of estimate	Growth control model fit	Growth control model fit	Growth control model fit	Net effect model fit	Average of pairwise k_R data
Source of bounds	Univar. bounds	Planar bounds	Univar. bounds	Planar bounds	Lowest and Greatest k_R / EC_{50R}

Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); k' (L/mg*h^{-1})

Table H.41: Full Model Final Parameter Estimates for 22 Polymyxin B Monotherapy

Parameter	Estimate	SE	CV %	Lower univariate CI	Upper univariate CI
g_S	3.67	0.42	11	2.84	4.50
g_R	1.19	0.06	5	1.07	1.32
Nmax	8.5E+10	2.2E+10	26	4.1E+10	1.3E+11
k_S	8.83	0.62	7	7.58	10.07
k'_R	0.05	0.02	39	0.01	0.09

SE – Standard Error; CV – Coefficient of Variation; CI – 95% confidence interval; Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); k' (L/mg*h^{-1})

Table H.42: Full Model Final Secondary Parameter Estimates for 22 Polymyxin B Monotherapy

Parameter	Estimate	SE	CV %
SC_S (mg/L)	-	-	-
SC_R (mg/L)	23.7	8.21	35
d_S (min)	11.3	1.28	11
d_R (min)	34.8	1.82	5

SE – Standard Error; CV – Coefficient of Variation; SC – Stationary Concentration; d – doubling time

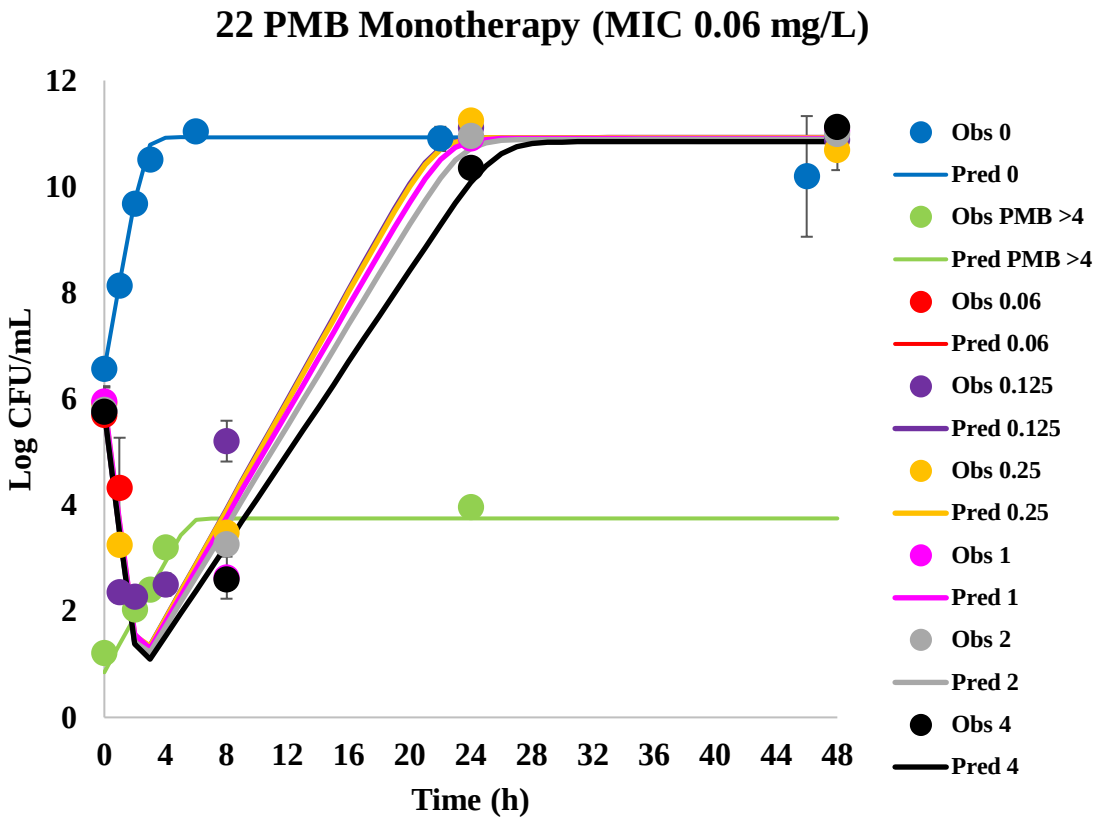

Figure H.15: Predicted and Observed Time-kill Data for Polymyxin B against Isolate 22. Observed data represent geometric means with standard deviations of at least 2 time-kill experiments except for green data which was only performed once. Note that some error bars are smaller than the symbol. Blue data represent the sum of both populations. Green data represent the subpopulation with a polymyxin B MIC > 4 mg/L. Other data indicate the total colony count with the given concentration of polymyxin B present in the time-kill flask.

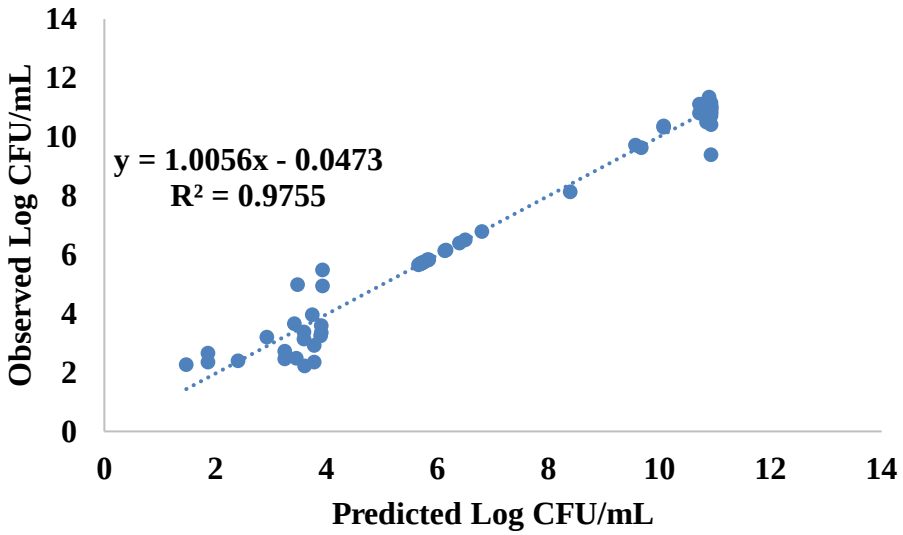

Figure H.16: Observed vs. Predicted Data for Polymyxin B Monotherapy Model against Isolate 22.

Pharmacodynamic Model of Isolate 24 Polymyxin B Monotherapy Experiments

According to the protocol discussed in Pharmacodynamic Mathematical Modeling, equation (1) and equation (11) were utilized for a total of 6 parameters, 18 time-kill experiments and 34 simultaneously solved differential equations.

Table H.43: Full Model Initial Parameter Estimates for 24 Polymyxin B Monotherapy

Parameter	g_S	g_R	Nmax	k_S	EC_{50S}	k'_R
Value	1.71	1.22	4.33E+10	7.83	0.06	0.10
Lower Bound	1.40	0.86	1.94E+09	5.14	0.01	0.01
Upper Bound	2.03	1.59	8.84E+10	10.53	0.11	0.25
Type	User-defined	User-defined	User-defined	User-defined	User-defined	User-defined
Source of estimate	Growth control model fit	Growth control model fit	Growth control model fit	Net effect model fit	Net effect model fit	Average of pairwise k_R data
Source of bounds	Univar. bounds	Planar bounds	Univar. bounds	Planar bounds	Planar bounds	Lowest and Greatest k_R / EC_{50R}

Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L); k' (L/mg*h^{-1})

Table H.44: Full Model Final Parameter Estimates for 24 Polymyxin B Monotherapy

Parameter	Estimate	SE	CV %	Lower univariate CI	Upper univariate CI
g_S	3.92	0.44	11	3.04	4.80
g_R	1.22	0.05	4	1.11	1.32
Nmax	1.01E+11	2.19E+10	22	5.76E+10	1.44E+11
k_S	11.18	0.70	6	9.80	12.56
EC_{50S}	0.07	0.01	17	0.05	0.10
k'_R	0.08	0.02	22	0.05	0.12

SE – Standard Error; CV – Coefficient of Variation; CI – 95% confidence interval; Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); EC50 (mg/L); k' (L/mg*h^{-1})

Table H.45: Full Model Final Secondary Parameter Estimates for 24 Polymyxin B Monotherapy

Parameter	Estimate	SE	CV %
SC_S (mg/L)	0.02	0.01	31
SC_R (mg/L)	9.32	0.80	9
d_S (min)	20.5	2.36	11
d_R (min)	40.1	1.20	3

SE – Standard Error; CV – Coefficient of Variation; SC – Stationary Concentration; d – doubling time

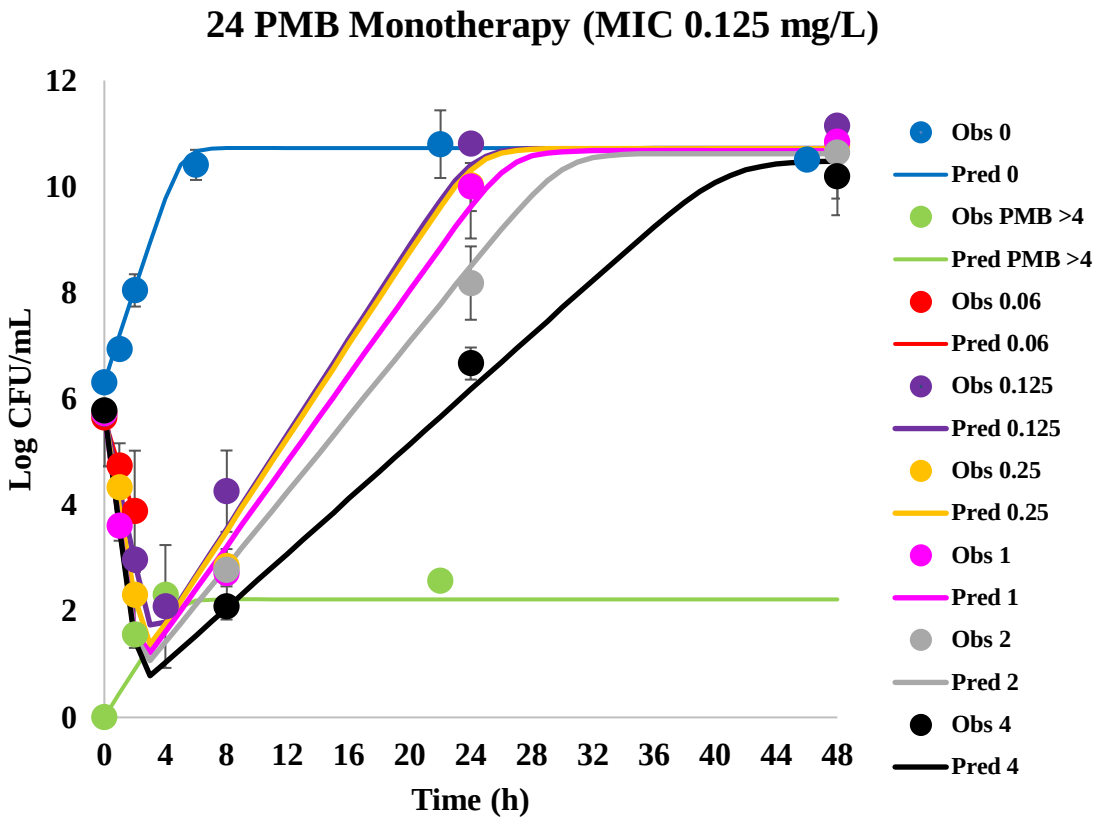

Figure H.17: Predicted and Observed Time-kill Data for Polymyxin B against Isolate 24. Observed data represent geometric means with standard deviations of at least 2 time-kill experiments except for green data which was only performed once. Note that some error bars are smaller than the symbol. Blue data represent the sum of both populations. Green data represent the subpopulation with a polymyxin B MIC > 4 mg/L. Other data indicate the total colony count with the given concentration of polymyxin B present in the time-kill flask.

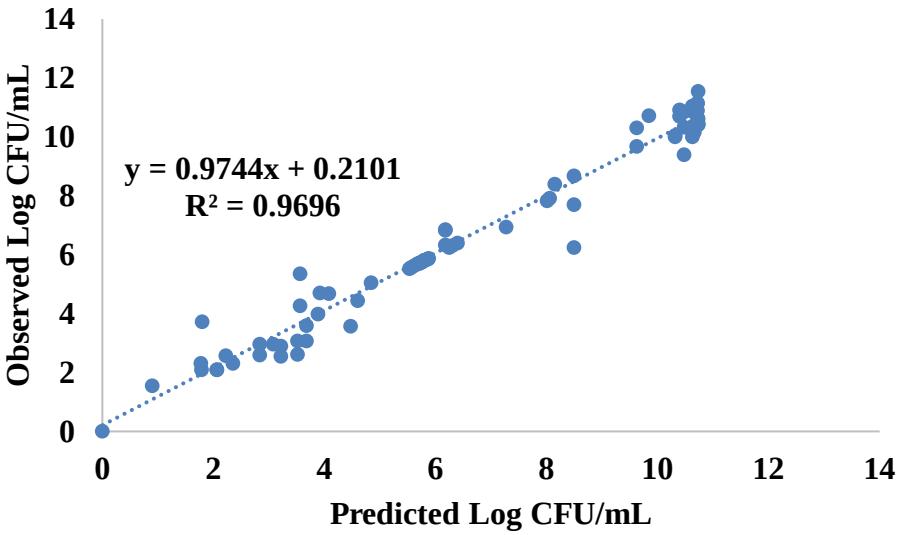

Figure H.18: Observed vs. Predicted Data for Polymyxin B Monotherapy Model against Isolate 24**.**

Pharmacodynamic Model of Isolate 44 Polymyxin B Monotherapy Experiments

According to the protocol discussed in Pharmacodynamic Mathematical Modeling, equation (10) and equation (11) were utilized for a total of 5 parameters, 25 time-kill experiments and 43 simultaneously solved differential equations.

Table H.46: Full Model Initial Parameter Estimates for 44 Polymyxin B Monotherapy

Parameter	g_S	g_R	Nmax	k_S	k'_R
Value	2.63	1.26	1.15E+11	10.75	0.02
Lower Bound	1.75	0.73	2.9E+10	8.46	0.00
Upper Bound	3.50	1.80	2.02E+11	13.04	0.20
Type	User-defined	User-defined	User-defined	User-defined	User-defined
Source of estimate	Growth control model fit	Growth control model fit	Growth control model fit	Net effect model fit	Average of pairwise k_R data
Source of bounds	Univar. bounds	Planar bounds	Univar. bounds	Planar bounds	Lowest and Greatest k_R / EC_{50R}

Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); k' ($L/mg*h^{-1}$)

Table H.47: Full Model Final Parameter Estimates for 44 Polymyxin B Monotherapy

Parameter	Estimate	SE	CV %	Lower univariate CI	Upper univariate CI
g_S	2.65	0.58	22	1.51	3.80
g_R	0.79	0.04	5	0.71	0.87
Nmax	5.4E+10	2.6E+10	48	2.3E+09	1.1E+11
k_S	9.23	1.04	11	7.18	11.29
k'_R	0.04	0.01	39	0.01	0.07

SE – Standard Error; CV – Coefficient of Variation; CI – 95% confidence interval; Units: g (h^{-1}); Nmax (CFU/mL); k (h^{-1}); k' ($L/mg*h^{-1}$)

Table H.48: Full Model Final Secondary Parameter Estimates for 44 Polymyxin B Monotherapy

Parameter	Estimate	SE	CV %
SC_S (mg/L)	-	-	-
SC_R (mg/L)	19.7	6.71	34
d_S (min)	15.8	3.45	22
d_R (min)	52.7	2.67	5

SE – Standard Error; CV – Coefficient of Variation; SC – Stationary Concentration; d – doubling time

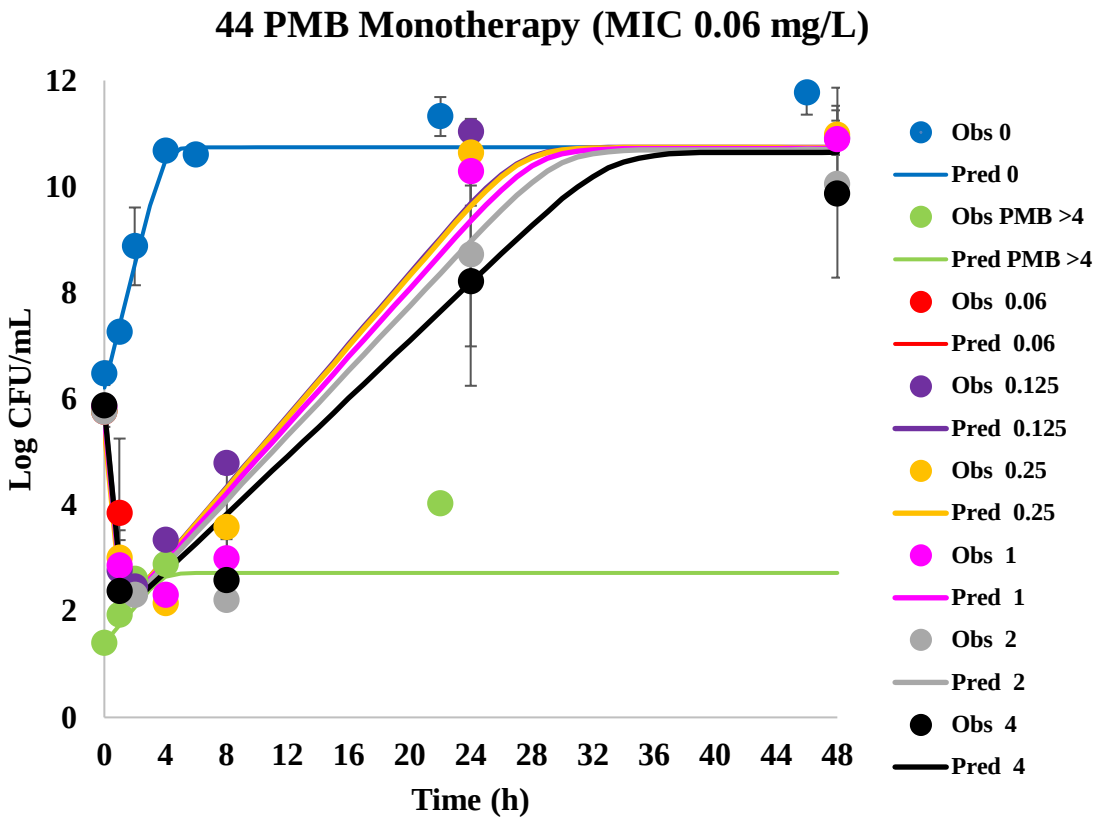

Figure H.19: Predicted and Observed Time-kill Data for Polymyxin B against Isolate 44. Observed data represent geometric means with standard deviations of at least 2 time-kill experiments except for green data which was only performed once. Note that some error bars are smaller than the symbol. Blue data represent the sum of both populations. Green data represent the subpopulation with a polymyxin B MIC > 4 mg/L. Other data indicate the total colony count with the given concentration of polymyxin B present in the time-kill flask.

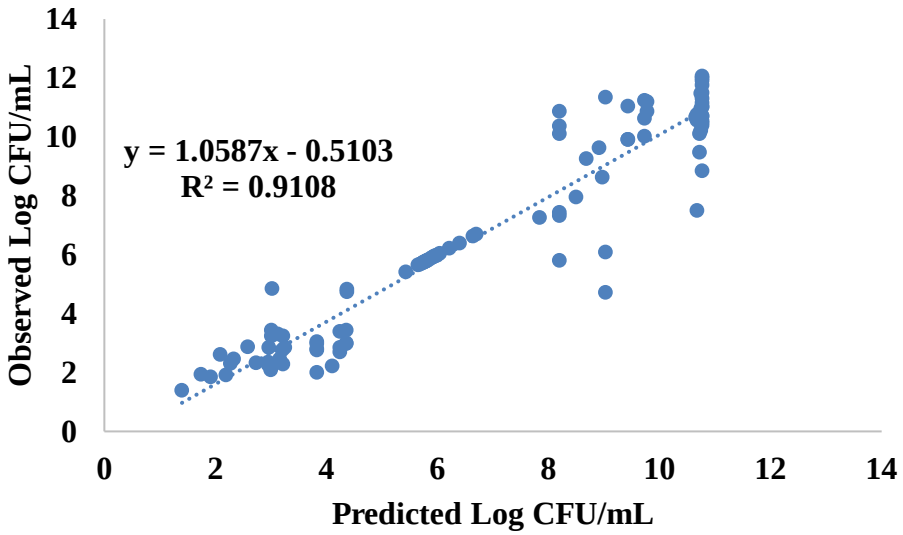

Figure H.20: Observed vs. Predicted Data for Polymyxin B Monotherapy Model against Isolate 44**.**

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VITA

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EDUCATION

Doctor of Pharmacy, University of Kentucky, Lexington, KY
May, 2016

Master of Science in Pharmaceutical Science, University of Kentucky, Lexington, KY
May 2016
Major field: Infectious Disease
Thesis: *In Vitro Activity of Polymyxin B and Meropenem Alone and in Combination against Carbapenem-resistant Enterobacteriaceae*

PROFESSIONAL POSITIONS HELD

University of Kentucky HealthCare Clinical Pharmacist, Lexington, KY

Lexington Compounding Pharmacy Consultant Pharmacist, Lexington, KY

University of Kentucky HealthCare Pharmacy Intern, Lexington, KY

PUBLICATIONS

Kulengowski B, Clark JA, Burgess DS. Antimicrobial activity against carbapenem-resistant *Enterobacteriaceae* that produce Verona integron-encoded metallo-beta-lactamase or *Klebsiella pneumoniae* carbapenemase. *Diagnostic Microbiology and Infectious Disease*. Under review.

Kulengowski B, Burgess DS. Imipenem/relebactam activity against carbapenem-resistant *Enterobacteriaceae* at a tertiary referral academic medical center. *Journal of Antimicrobial Chemotherapy*. Under review.

Haffler ZJ, **Kulengowski B,** Burgess DS. Evaluation of an automated system for determining antimicrobial susceptibility against carbapenem-resistant *Enterobacteriaceae* compared to broth microdilution. *International Journal of Antimicrobial Agents*. Under review.

Kulengowski B, Clark JA, Burgess DS. Effect of increasing amikacin MIC on the activity of amikacin and meropenem in combination against carbapenem-resistant *Enterobacteriaceae*. *Diagnostic Microbiology and Infectious Disease.* 2018. 92(3):262-266.

Kulengowski B, Clark JA, Burgess DS. Staggering the administration of polymyxin B and meropenem in time-kill against carbapenem-resistant *Enterobacteriaceae* exhibiting a wide range of meropenem MICs. *Diagnostic Microbiology and Infectious Disease*. 2018. In press.

Kulengowski B, Ribes JA, Burgess DS. Polymyxin B Etest® compared to gold-standard broth microdilution in carbapenem-resistant *Enterobacteriaceae* exhibiting a wide range of polymyxin B MICs. *Clinical Microbiology and Infection.* 2018. 25(1):92-95.

Kulengowski B, Rutter WC, Campion JJ, Lee GC, Feola DJ, Burgess DS. Effect of the degree of meropenem MIC on the killing activity of meropenem in combination with amikacin or polymyxin B against MBL- and KPC-producing *Enterobacter cloacae*. *Diagnostic Microbiology and Infectious Disease.* 2018. 92(3):262-266.

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Landon J, Gao X, **Kulengowski B**, Neathery JK, Liu K. Impact of pore size characteristics on the electrosorption capacity of carbon xerogel electrodes for capacitive deionization. *J Electrochem Soc.* 2012; 159:61-66.

SCHOLASTIC HONORS

Tetraphase Pharmaceuticals, Inc. Grant	2018
Achaogen Grant	2018
ID Week 2018 Trainee Travel Grant	2018
Merck Investigator Studies Program Travel Award	2018
ASM Infectious Disease Travel Award	2018
Pharmaceutical Sciences Excellence in Graduate Achievement Fellowship	2018
Presidential Fellowship Nomination	2018
Merck Investigator Studies Program Grant	2017
Peter G. Glavanos, Jr. Scholarship	2017
Joseph V. Swintosky Scholarship	2017
S. Elizabeth Helton Memorial Scholarship	2017
Pharmaceutical Sciences Excellence in Graduate Achievement Fellowship	2017
Presidential Fellowship Nomination	2017
ASM Infectious Disease Travel Award	2017
ID Week 2017 Trainee Travel Grant	2017
Astronaut Scholarship Foundation Scholarship	2012
University of Kentucky Commonwealth Scholarship	2009-2013
Ralph and Janice Young Scholarship	2009-2012
National Eagle Scout Scholarship	2009