OPTIMIZING ANTIBIOTIC DOSING REGIMENS FOR THE TREATMENT INFECTION CAUSED BY CARBAPENEM RESISTANCE ENTEROBACTERIACEAE IN THAILAND: THE STUDY OF MOLECULAR RESISTANCE MECHANISMS, *IN VITRO* ACTIVITY OF MONOTHERAPY AND COMBINATION THERAPY, AND TREATMENT OUTCOMES

By

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A Thesis Submitted in Partial Fulfillment of the Requirements

for Doctor of Philosophy CLINICAL PHARMACY

Department of CLINICAL PHARMACY

Graduate School, Silpakorn University

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แบบแผนกำหนดการใช้ยาที่เหมาะสม สำหรับต้านเชื้อ Enterobacteriaceae ที่ดื้อต่อยา carbapenem: การศึกษากลไกการดื้อยาระดับอณูวิทยา ฤทธิ์ของยาต้านจุลชีพชนิดเดี่ยวและคู่ผสม และ ผลลัพธ์ทางการรักษา

โดย
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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปรัชญาดุษฎีบัณฑิต
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CAPT. PARNRADA NULSOPAPON : OPTIMIZING ANTIBIOTIC DOSING REGIMENS FOR THE TREATMENT INFECTION CAUSED BY CARBAPENEM RESISTANCE ENTEROBACTERIACEAE IN THAILAND: THE STUDY OF MOLECULAR RESISTANCE MECHANISMS, *IN VITRO* ACTIVITY OF MONOTHERAPY AND COMBINATION THERAPY, AND TREATMENT OUTCOMES THESIS ADVISOR : ASSOCIATE PROFESSOR. DR. WICHAI SANTIMALEEWORAGUN

Carbapenem-resistant Enterobacterales (CRE) is a hospital-acquired pathogen with a high mortality rate and limited effective treatment options. The main objective was to design the optimal combination regimens for the treatment of infections caused by CRE. The methods were divided into 3 sub-studies. Firstly, an *in vitro* study aimed to design the optimal antibiotic options. Broth microdilution/E-test method and checkerboard method were performed for the mono- and combined activities, respectively. The molecular study was demonstrated to describe the types of carbapenemase and *mcr-1* genes detected by polymerase chain reaction (PCR). Secondly, a Pharmacokinetic/Pharmacodynamic (PK/PD) study aimed to design the optimal antibiotic dosing regimens. Monte Carlo simulation was used to establish the optimal antimicrobial dosing regimens achieving the ≥ 90% of a probability of target attainment (PTA) and ≥ 90% of a cumulative fraction of response (CFR). Finally, a clinical study is used to evaluate the treatment outcomes. A quasi-experimental study was conducted to compare mortality rates between patients received the optimal combination regimens (prospective group) and the usual care (retrospective group). A total of 199 CRE clinical isolates were collected, including 49 CRE clinical isolates from Phramongkutklao hospital and 150 CRE clinical isolates from the bacterial culture bank of the Department of Medical Sciences Ministry of Public Health in health region V. The proportion of CRE clinical isolates were Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) (81.91%; n =163), Carbapenem-resistant *Escherichia coli* (CREC) (16.58%; n = 33) and Carbapenem-resistant *Enterobacter cloacae* (CREclo) (1.51%; n =3). Most CRE isolates were resistant to aztreonam, fosfomycin, ceftazidime/avibactam, tigecycline, and colistin, whereas they were highly susceptible to aminoglycosides. Most detected carbapenemases were NDM (36.18%; n = 72), OXA-48 (45.73%; n = 91) and NDM plus OXA-48 (15.58%; n = 31); five *mcr-1* genes also carried bla_{OXA-48}. Only 49 CRE clinical isolates from Phramongkutklao hospital performed synergistic testing, the results showed amikacin combination with fosfomycin (44%) had a greater synergy rate than other combination regimens. The optimal antibiotic-dosing regimens showed that high-dosing antibiotic regimens achieved higher PTA and CFR targets than usual regimens. Combination regimens also reached greater CFR targets than single regimens. In the clinical study, 88 patients (40 prospective patients and 48 retrospective patients) were included. The 14-day mortality rate was lower than in prospective patients compared to retrospective patients (17.50% *vs* 37.50%, respectively; *p*-value = 0.038). Risk factors associated with 14-day mortality were receiving intervention (PK/PD dosing protocol) and vassopresor use. Optimizing doses of antibiotic combination regimens may be an optimal option for the treatment. Further studies with larger sample sizes are required.

มหาวิทยาลัยศิลปากร

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CHAPTER I
INTRODUCTION

1.1 Background information and Rationale for this study

In the 21st century, antibiotic resistance is one of the major problems because of the global burden. Resistant pathogens are non-susceptible to available antibiotics which caused clinical high mortality (clinical impact) and high budget (economic impact), whereas new antibiotics in drug development are fewer [1, 2]. Carbapenem-Resistant Enterobacteriaceae (CRE) are categorized into one of the critical groups in WHO lists [3]. In Thailand, the spread of CRE has risen continuously since 2011 from 0.5% to 3.1% in *Escherichia coli* and from 0.7% to 11.5% in *Klebsiella pneumoniae* [4]. CRE seems likely to be difficult to treat, especially from NDM types which are often detected in Thailand [5-7].

Diverse actions are designed to address antibiotic resistance with limited resources, known as antimicrobial stewardship programs (ASPs). IDSA recommended that dose-optimization by using pharmacokinetics/pharmacodynamics (PK/PD) application is one of the supplemental strategies [8, 9]. The benefit of the strategy is to reduce inappropriate antibiotic use and provide minimum resistance as well as maximum success in clinical treatment [10]. Optimization of antibiotics consisted of optimal antibiotic options and optimal antibiotic dosing. For the antibiotic options, the second-line antibiotic groups preserved for multi-drug resistance have a role in the CRE treatment [11]. The strategy that increases effective antibiotic options is combination therapy (combined with the two agents) [12]. However, current evidence is not reported which the optimal combination therapy is. The reasons may be that combined antibiotics can vary among different sites of infection, causative pathogens, the patterns of local antimicrobial susceptibility, and patient comorbidity [13]. For antibiotic dosing, the probability is used to evaluate the optimal regimens by using Monte Carlo simulation [10]. Several studies used the advanced statistical technique for antibiotic dosing in monotherapy. The methods were proposed to

determine the optimal regimens by using the calculating probability of antibiotic dosing with Monte Carlo simulation [14].

The treatment of any infections caused by CRE needed further investigation because of differences in local resistance, resistance mechanisms, and PK/PD parameters. Integrated an *in vitro* study of antibiotic activities with PK/PD analysis was used to design the optimal antibiotic combination regimens to combat CRE. The anticipated result is to fill the limited data on the appropriate antibiotic regimens for the individual patient in Thailand.

1.2 Objectives of the research

1.2.1 Primary objectives

To design the optimal antibiotic combination regimens for the treatment of infection caused by CRE

1.2.2 Secondary objective

1) To describe the CRE resistance characteristics.
2) To investigate the *in vitro* mono and combined antibiotic activities against CRE
3) To evaluate the treatment outcomes in patients with CRE infections treated with the optimal antibiotic combination regimens

1.3 Hypothesis

1.3.1 The optimal combination regimens for the CRE treatment may improve treatment outcomes in an individual patient.

1.3.2 The CRE resistance characteristics are various and different.

1.3.3 Colistin-containing regimens have synergistic activity.

1.3.4 *In vitro* mono- and combined antibiotic activities against CRE based on CRE resistance characteristics.

1.4. Scope of the study

The study included three parts – an *in vitro* study, a PK/PD study, and a clinical study.

1.4.1 *In vitro* study

Any CRE isolates from the department of medical sciences in health region V, Samut Songkhram, and Phramongkutklao hospital are determined the mono- antibiotic activity against CRE. Furthermore, any CRE isolates from Phramongkutklao hospital are determined both mono- and combined antibiotic activity against CRE. A group of antibiotics consisted of carbapenems (meropenem and imipenem), aminoglycosides (amikacin and gentamicin), colistin, fosfomycin, tigecycline and ceftazidime/avibactam.

1.4.2 PK/PD study

Application of PK/PD analysis by using Monte Carlo simulation is used to design the optimal combination regimens in Thai critically ill patients.

1.4.3 Clinical study

Any patients who are infected caused by CRE and admitted to Phramongkutklao hospital.

1.5 Research gap

There are difficult to conclude which optimal antibiotic combination regimens for the treated infection caused by CRE in Thailand due to geographical variation in antibiotic resistance. The majority of previous studies reported over-presentation of KPC carbapenemase type, whereas most carbapenemase types in Thailand were NDM or OXA-48.

1.6 Research question

Which are the optimal antibiotic combination regimens for CRE treatment in Thai patients?

1.7 Impact or anticipated outcomes

17.1 Patients

- Increase combination antibiotic regimens for the CRE treatments
- Individualized patients are received optimal combination antibiotic regimens for Individualized treatment, leading to improve treatment outcomes.

1.7.2 Healthcare professionals

- Support decision of treatment and/or treatment plans

1.7.3 Hospitals

- Support hospital policy related to antibiotic stewardship programs (ASPs)
- Reduce and prevent antibiotic resistance in the hospital

CHAPTER II

Review of the Literature

2.1 Overview of CRE

In the 21st century, antibiotic resistance is one of the major problems because of a global burden. Available effective antibiotics are limited, whereas new antibiotics in drug development are fewer. High mortality (clinical impact) and high budget (economic impact) are the direct impacts of antibiotic resistance [1, 2]. As the result, World Health Organization (WHO) prioritized antibiotic-resistant pathogen lists for the first research and development of new antibiotics. Not only *Acinetobacter baumannii and Pseudomonas aeruginosa* but also Carbapenem-Resistant Enterobacterales (CRE) are categorized into the critical groups [3]. The definition of CRE may be different. In 2015, Centers for Disease Control and Prevention (CDC) definition of CRE were Enterobacterales being resistant to one of the carbapenems (not including intermediate) [15], whereas carbapenem non-susceptibility was defined based on Clinical and Laboratory Standards Institute (CLSI) in 2020 [16].

Over the last decade, CRE have trended to increase continuously throughout several regions such as the United States of America, Europe and Asia [17-19]. In Thailand, National Antimicrobial Resistance Surveillance Thailand (NARST) data showed that the spread of CRE have been risen continuously since 2011 from 0.5% to 3.1% in *E. Coli* and from 0.7% to 13.3% in *K. pneumoniae.* Thus, CRE are categorized into the first group of resistant bacteria for national surveillance [20].

CRE are known as “nightmare bacteria” because of their virulence. Soontaros S et al in 2019 showed patient-infected CRE were more mortality risk than patient-infected Carbapenem-Susceptible Enterobacterales (CSE) (pooled-adjusted odds ratio 2.85, 95% confidence interval 1.88 to 4.30) [21]. Generally, CRE were multi-drug resistance (MDR) which found two main resistance mechanisms – producing carbapenemase (CP-CRE) (e.g. KPC, NDM, OXA-48) or non-producing carbapenemase (non-CP-CRE) (e.g. lux pump or porin changes). The spread of resistance mechanisms of CP-CRE has also varied in different regions [7-10]. In a comparison of the two

groups, CP-CRE may be more virulent than the non-CP-CRE groups because of higher mortality. Additionally, higher Minimum Inhibitory Concentrations (MICs) are more detected in CP-CRE than non-CP-CRE [22]. Among CP-CRE, the distribution of carbapenem MICs in NDM is higher than KPC and OXA-48 [23]. Thus, NDM types seem likely to be difficult to treat compared with others. Unfortunately, NDM types are the major types detected in Thailand [5-7].

2.2 Antimicrobial Stewardship Programs (ASPs)

At this time, antibiotic options for the treated CRE infections are limited. The rationale for limited options is overuse and misuse of available antibiotics, whereas insufficient newly developed antibiotics are in a pipeline. Healthcare professionals have been forced to wisely use antibiotic agents and to strongly weigh the benefits and risks [24]. As a result, selecting optimizing antibiotics should be precise decision-making to combat the CRE treatment.

Antimicrobial stewardship programs (ASPs) are designed to address antibiotic resistance in CRE with limited resources. Dose-optimization by using pharmacokinetic/pharmacodynamic (PK/PD) application is a supplemental strategy for ASPs which still need further evidence to support recommendations [8, 9]. The strategy is used for an individual patient. There are two approaches to optimizing antibiotic agents, including optimizing antibiotic options and optimizing antibiotic dosing. The benefit of the strategy is to reduce inappropriate antibiotic use and provide minimum resistance as well as maximum the success of clinical treatment in clinical routine practice [10]. Furthermore, pharmacists are one of the key persons to drive the success of interventions by providing antibiotic information to infectious disease physicians [8, 9].

2.3 Optimizing antibiotic options for the treatment of CRE

2.3.1 Overview of optimal antibiotic options

Effective antibiotic options for the treatment of CRE have been limited. As previous mentions, CRE seem to be difficult to treat because of multi-drug resistance

and higher carbapenem MICs. The antibiotics for the treated CRE are divided into new antibiotic groups and classic antibiotic groups [11]. In the new antibiotic group, most antibiotics are developed to combat KPC-producing Enterobacterales, whereas NDM- or OXA-48 producing Enterobacterales are fewer. Only two antibiotics (aztreonam and ceftazidime/avibactam) have the activity against NDM-producing Enterobacterales. In the classic antibiotic group, the last-resorted antibiotics (e.g. carbapenem, tigecycline, colistin, aminoglycosides and fosfomycin) preserved for multi-drug resistance have a role in the treatment [12].

2.3.2 Monotherapy *vs* combination therapy

Currently, several studies showed that combination therapy is more effective than monotherapy in clinical studies. In 2014, Falagas ME et al conducted a systematic review to assess the effectiveness of the antibiotic agents for treating patients; the results displayed that monotherapy had higher mortality and more treatment failure than combination therapy (0-80% for mortality in monotherapy and 0-67% for mortality in combination therapy; 40% for treatment failure in monotherapy and 16.7% for treatment failure in combination therapy, respectively) [25]. In 2017, Karaiskos I et al reviewed any current evidence of the treatment of XDR gram-negative bacteria from 1970 to 2017 (n = 2,972). The results showed that combination therapy and monotherapy had lower mortality rates than inappropriate therapy (33.7%, 42.3%, and 57%, respectively) [26]. In 2019, Schmid A et al conducted a systematic review and meta-analysis to compare monotherapy and combination therapy. In subgroup analysis of bloodstream infection (BSI) patients infected carbapenemase-producing Enterobacterales revealed lower mortality in combination therapy than monotherapy (Relative Risk (RR) = 0.61, 95% CI 0.45–0.85; p= 0.03; I^2 = 21%, p-value = 0.026) [27]. Overall, combination therapy may be a potential strategy to overcome CRE infections in the antibiotic resistance era.

2.3.3. The antibiotic combination therapy for the CRE treatment

2.3.3.1 *In vitro* study

Before being applied combination therapy in the clinical study, *in vitro* synergistic activity against CRE is firstly evaluated. Generally, antibiotic-based

regimens from in vitro studies used to combat CRE are diverse depending on the definition of each study as follows: a) aminoglycoside-based regimens b) carbapenem-based regimens c) colistin-based regimens d) fosfomycin-based regimens and e) tigecycline-based regimens Interesting, CRE isolates producing KPC were predominant revealed *in vitro* studies, whereas NDM or OXA-48 did not. Table 1 showed *in vitro* studies of each antibiotic-based regimen in CRE isolates.

Overall, the synergistic effect varied in these studies because of heterogeneity in methodologies and differences in carbapenemase types. Thus, it is difficult to conclude which antibiotic-based combination regimens should be used as the optimal antibiotic option against CRE.

Table 1 *In vitro* study of synergistic testing against Carbapenem-resistance Enterobacterales (CRE) isolates

Author (year) [Ref]	Total isolates	Types of bacteria	Carbapenemase resistance mechanism	Synergistic testing method	Antibiotic combination regimens (%synergy)
a) Aminoglycoside-based regimens					**% Synergy = 0%-33.33%**
Yim H, et al (2011) [28]	43	KP, EC	Not report	Time-kill	Amikacin + tigecycline (33.33%)
Gaibani P, et al (2017) [29]	13	KP, EC	KPC	Checkerboard	Gentamicin + ceftazidime-avibactam (0%) [indifferent]
Fredborg M, et al (2017) [30]	10	KP, EC	NDM, VIM	Time-kill, Checkerboard	Amikacin + meropenem (20%) Amikacin + gentamcin (0%)
Oliva A, et al (2017) [31]	33	KP	Not report	Time-kill	Amikacin + gentamcin (58.60%)
Del Bono V, et al (2017) [32]	19	KP	KPC	Time-kill	Gentamicin + tigecycline (0%)
Brennan-Krohn T, et al (2017) [33]	10	KP, EC	KPC-2, KPC-3	Checkerboard	Gentamicin + minocycline (5%)
b) Carbapenem-based regimens					**% Synergy = 10%-100%**
Wiskirchen DE, et al (2011) [34]	5	KP	KPC-2, KPC-3	Time-kill	Meropenem + tigecycline (80%)
Bulik CC & Nicolau DP (2011) [35]	1	KP	KPC-3	Time-kill	Ertapenem + doripenem (100%)
Poirel L, et al (2016) [36]	20	KP	KPC-2, OXA-48, NDM, NDM+OXA-48	Time-kill, Checkerboard	Meropenem + imipenem (40%) Meropenem + ertapenem (40%) Meropenem + doripenem (15%) Imipenem + doripenem (20%) Ertapenem + doripenem (15%)

Author (year) [Ref]	Total isolates	Types of bacteria	Carbapenemase resistance mechanism	Synergistic testing method	Antibiotic combination regimens (%synergy)
Fredborg M, et al (2017) [30]	10	KP, EC	NDM, VIM	Time-kill, Checkerboard	Meropenem + tobramycin (20%): Meropenem + imipenem (20%) Meropenem + doripenem (60%) Ertapenem + meropenem (80%)
Oliva A, et al (2017) [31]	32	KP	KPC	Time-kill	Ertapenem + meropenem (91.6%)
Gaibani P, et al (2017) [29]	13	KP, EC	KPC	Checkerboard	Meropenem + ertapenem (31%) Meropenem + ceftazidime-avibactam (100%) Imipenem + ceftazidime-avibactam (100%) Ertapenem + ceftazidime-avibactam (84.62%)
Brennan-Krohn T, et al (2017) [33]	10	KP, EC	KPC-2, KPC-3	Checkerboard	Meropenem + minocycline (10%)
Nath S, et al (2018) [37]	4	KP	KPC-2, KPC-3	Time-kill	Meropenem + ceftazidime-avibactam (66.67%)
c) Colistin-based regimens					**% Synergy = 0%-75%**
Betts JW, et al (2014) [38]	18	KP, EC, Enterobacter spp., *Serratia marcescens*	KPC, NDM, VIM	Time-kill, Checkerboard	Colistin + tigecycline (38.89%) **synergy only EC, KP, Enterobacter spp., excepting *Serratia marcescens*.
Gaibani P, et al (2014) [29]	17	KP	KPC-3	Time-kill, Checkerboard	Colistin + meropenem (0.06%) Colistin + tigecycline (11.76%)
Kulengowski B, et al (2017) [39]	4	KP	KPC	Time-kill	Colistin + meropenem (75%)

Author (year) [Ref]	Total isolates	Types of bacteria	Carbapenemase resistance mechanism	Synergistic testing method	Antibiotic combination regimens (%synergy)
Oliva A, et al (2017) [31]	33	KP	Not report	Time-kill	Colistin + meropenem (46.1%)
Del Bono V, et al (2017) [32]	19	KP	KPC	Time-kill	Colistin + meropenem (0%)
Brennan-Krohn T, et al (2017) [33]	10	KP, EC	KPC-2, KPC-3	Checkerboard	Colistin + minocycline (30%) Colistin + gentamicin (25%) Colistin + meropenem (10%)
d) Fosfomycin-based regimens					**% Synergy = 0%-42%**
Evren E, et al (2013) [40]	12	KP	OXA-48	Checkerboard	Fosfomycin + imipenem (42%) Fosfomycin + meropenem (33%) Fosfomycin + colistin (0%) [fully antagonist = 100%]
Fredborg M, et al (2017) [30]	10	KP, EC	NDM, VIM	Time-kill, Checkerboard	Fosfomycin + meropenem (20%)
e) Tigecycline-based regimens					**% Synergy = 8%**
Gaibani P, et al (2017) [29]	13	KP, EC	KPC	Checkerboard	Tigecycline + ceftazidime-avibactam (8%)

Abbreviations: KP, *K. pneumoniae;* EC, *E. Coli*

2.3.3.2 Clinical study

2.2.3.2.1 Antibiotic combination regimens

Similar to *in vitro* study, three antibiotic-based regimens are often used in routine practice, including carbapenem-based regimens, aminoglycoside-based regimens, colistin-based regimens and tigecycline-based regimens.

a) Carbapenem-based regimens

Two potential strategies for the treatment of CRE infections consist of dual carbapenems therapy (DCT) and high-dose prolonged infusion of carbapenem combination regimens [41]. For DCT, the common regimens are the combination of ertapenem 1 g every 24 h with an infusion time of 30-60 minutes before a prolonged infusion (3 hours) of high-dose meropenem (2 g every 8 hours) [26, 41]. The synergy mechanism is that ertapenem had a high affinity to carbapenemase; thus, other carbapenems with high concentration are easy to hydrolyze carbapenemase [42]. Referring to the reviewed study of Sheu et al, patients receiving DCT had a greater clinical cure rate and lower mortality rate than patients receiving non-DCT. However, DCT had the synergistic activity against KPC- or OXA-48 producing Enterobacterales but no synergistic activity against MBL-types [41].

For high-dose prolonged-infusion of carbapenems (2 g every 8 hours with an infusion time of 3 hours), current evidence provided that carbapenem-based regimens had lower mortality than carbapenem-sparing regimens (29.5% and 34.6%, respectively) [26]. Referring to the reviewed study of Rodriguez-Bano J et al, MICs of meropenem and imipenem are related to clinical cure rates. High rates of clinical cure were observed at meropenem MICs ≤ 4 μg/mL (69%) compared with meropenem MICs ≤ 8 μg/mL (29%) [11]. Thus, the carbapenem-based regimens with high-dose prolonged-infusion have benefited when meropenem MICs may not exceed 8 μg/mL (MICs ≤ 8 μg/mL) [26].

b) Aminoglycoside-based regimens

Aminoglycosides have a role as adjunctive antibiotics for the combination therapy. The synergistic mechanism is that aminoglycosides

affect protein synthesis. When inhibition of protein synthesis occurs, the expression of β-lactamase enzymes (e.g. KPC or NDM) may decrease. Additionally, aminoglycosides also interfere with the permeabilizing of the outer membrane of the pathogens affecting an increase in the penetration of carbapenems at the target site [43].

c) Colistin-based regimens

The regimens are effective to treat multi-drug resistant strains and are preferably combined with other regimens such as carbapenem, tigecycline, aminoglycosides, and fosfomycin [41, 45]. The synergistic mechanisms are that colistin may disrupt the cell membrane of the pathogens which may allow to increase in the penetration of other antibiotics as well as may disrupt the target sites of antimicrobial resistance mechanisms (e.g. efflux pumps). A systematic review and meta-analysis included observational studies and randomized controlled trials (RCTs) showed that the colistin alone are significantly higher mortality than colistin combination regimens (OR = 1.58; 95% CI 1.03-2.42 for colistin-carbapenem regimen, OR = 1.57; 95% CI 1.06-2.32 for colistin-tigecycline regimen, colistin-aminoglycoside regimen or colistin-fosfomycin regimen). In a subgroup analysis of *K. pneumonia* with BSI, colistin alone is significantly higher mortality than colistin-tigecycline regimen or colistin-aminoglycoside regimen (OR = 2.09; 95% CI 1.21-3.60) [46]. However, higher doses of colistin (more than 200 mg/day) which are necessary for the treatment CRE infections are associated with nephrotoxicity. Thus, patients treated with colistin should be concerned with nephrotoxicity, particularly colistin-aminoglycoside regimen [11, 24, 45].

d) Tigecycline-containing regimens

Tigecycline can be combined with carbapenem, aminoglycosides, or colistin. In 2014, Falagas ME et al reported that tigecycline-gentamicin had lower 28/30-day mortality than other regimens (0-50% for tigecycline-gentamicin, 0-64% for colistin- tigecycline, 0-67% for colistin-carbapenem and 40-61% for colistin-gentamicin) [13]. In 2016 Ni W et al conducted a systematic review and meta-analysis of tigecycline-containing regimens which included controlled trials

or cohort studies. The results showed that monotherapy had higher 30-day mortality than monotherapy (OR = 1.83; 95% CI 1.07- 3.12; P = 0.03). Additionally, standard dose tigecycline had differed significantly in mortality at ICU from high-dose tigecycline regimens (loading dose 200 mg, maintenance dose 100 mg every 12 hours) (OR, 12.48; 95% CI, 2.06–75.43; P = 0.006) [47]. As the result, tigecycline-based regimens with high-dose tigecycline seem to be a suitable agent for high-risk patients. The benefit of the regimen is non-nephrotoxic [41].

2.2.3.2.2 Monotherapy *vs* Combination therapy

Gutierrez-Gutierrez B, et al. conducted an INCREMENT study in 2017, The research study was a large retrospective cohort study. The results showed that overall mortality was not different between patients receiving appropriate combination therapy (35%; n = 47 of 135) and appropriate monotherapy (41%; n = 85 of 208) (HR 0.76, 95% CI 0.53–1.08, P = 0.12; adjusted HR 1.63, 95% CI 0.67–3.91, P = 0.28) for treatment patients with BSIs. In subgroup analysis, combination therapy (24%; n = 17 of 72) and monotherapy (20%; n = 21 of 105) were not different in mortality in low-risk patients (HR 1.18, 95% CI 0.62–2.23, P = 0.61; adjusted HR 1.21, 95% CI 0.56–2.56, P = 0.62). Nevertheless, combination therapy (48%; n = 30 of 63) had significantly lower mortality than monotherapy (62%; n = 64 of 103) (HR 0.60, 95% CI 0.39–0.93, P = 0.02; adjusted HR 0.56, 95% CI 0.34–0.91, P = 0.02) in high-risk patients. Furthermore, difference of combination regimens was analyzed and compared with colistin monotherapy. The results showed that combination regimens included aminoglycosides, tigecycline, colistin and carbapenem had lower 30-day mortality than colistin monotherapy (adjusted HR = 0.42, 95% CI 0.20–0.88 for aminoglycoside included regimen, adjusted HR = 0.45, 95% CI 0.23–0.86 for tigecycline included regimen, adjusted HR = 0.47, 95% CI 0.24–0.92 for colistin included regimen and adjusted HR = 0.56, 95% CI 0.26–1.23 for carbapenem included regimen, respectively) [44].

In Thailand, clinical outcomes are not clear because of fewer studies and no specific combination regimens. In 2016, Thamlikitkul V and

Popum S. conducted a clinical study to monitor the effectiveness and safety of colistin regimens for the treatment of any infections caused by gram-negative bacteria at Siriraj hospital from 2005 to 2016. Approximately, one-quarter of patients (22.45%; n = 31 of 138) infected Entetobacterales. For the treatment regimens, colistin combined with carbapenem is the most concomitant antibiotic regimen (50%). The clinical outcomes at the end of treatment found that the colistin-combination regimens are favorable outcomes (71.7%), non-favorable outcomes (5.1%), and death (23.2%). Additionally, overall 30-day mortality is 39.9% [48]. Similar to the study of Prawang A et al, they conducted a retrospective cohort study during 2016-2018. Patients are bacteremia with non-carbapenem-resistant *Klebsiella pneumoniae* (non-CRKP), carbapenem-resistant *Klebsiella pneumoniae* (CRKP) and colistin-resistant *Klebsiella pneumoniae* (CoRKP). The result showed that antibiotic combination therapy is favorable for 14-day survival rates outcomes. Most combination regimens included colistin-based regimens and aminoglycoside-based regimens [49].

As mentioned above, it should be emphasized that combination therapy has more effectiveness than monotherapy for the CRE treatment, especially in critically ill patients (high-risk mortality), whereas monotherapy may be an alternative option for the CRE treatment in non-critically ill patients (low-risk mortality). However, there is not concluded in the clinical study which effective antibiotic options for the CRE treatment in combination therapy are. Further studies are needed to investigate.

2.4 Optimizing antibiotic dosing for the treatment of CRE

2.4.1 Overview of Optimal Antibiotic Dosing

Optimal antibiotic dosing is a key consideration after selecting appropriate antibiotic choices for the CRE treatment because of being relevant to clinical outcomes [10]. Inappropriate antibiotic dosing causes suboptimal conditions or toxicity, followed by the emergence of antibiotic resistance and followed by poorer clinical outcomes. Mathematics and statistics are useful to design the optimal

antibiotic regimens in uncertain situations, such as critically ill, severe sepsis, or septic shock [10]. The treatment in the situation may be difficult to predict the clinical outcomes because of pathophysiological changes in the patients and alterations of pharmacokinetic parameters, affecting to change the properties of antibiotics to partition into water or fat (hydrophilic or lipophilic) [50, 51]. The results of change have impacted on bacterial susceptibility and treatment outcomes [52].

MICs are a key success for antibiotic dosing. They are associated with the relationship between antibiotic options for the CRE treatment and clinical outcomes. A measurement of MICs is performed by several methods such as an automated system, broth microdilution (BMD), and gradient method (E-test method). The Clinical & Laboratory Standards Institute (CLSI) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines are mostly used to interpret (Table 2) [53-55].

Table 2 Susceptibility breakpoints guidelines of antibiotics

Guidelines [56, 57]	**EUCAST**		**CLSI**		
Antibiotics	**S**	**R**	**S**	**I**	**R**
MER	≤ 2	> 8	≤ 1	2	≥ 4
IMP	≤ 2	> 4	≤ 1	2	≥ 4
AMI	≤ 8	> 16	≤ 16	32	≥ 64
GEN	≤ 2	> 4	≤ 4	8	≥ 16
TGC	≤ 0.5	> 0.5	-	-	-
COL	≤ 2	> 2	-	-	-
FOS	≤ 32	> 32	≤ 64*	128*	≥ 256*
AZT	≤ 2	> 4	≤ 4	8	≥ 16
CZA	≤ 8/4	> 8/4	≤ 8/4	-	≥ 16/4

Abbreviations: CLSI, Clinical & Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; MER, meropenem; IMP, imipenem; AMI, amikacin; GEN, gentamicin; COL, colistin; TGC, tigecycline; FOS, Fosfomycin; AZT, aztreonam, CZA, ceftazidime/avibactam; S, susceptible; I, Intermediate; R, resistant

* Fosfomycin is used for treating UTIs.

Application of PK/PD by using Monte Carlo simulation is required to determine the optimal antibiotic dosing achieved in PK/PD target among critically ill patients as well as to predict the probability of clinical success of treatment. Three parts of interactions, including appropriate antibiotics consisting of antibiotic regimens and PK/PD index and target, the patients consisting of pharmacokinetic parameters, and bacteria consisting of MICs, are needed to simulate [10]. Achievement in a Probability of target attainment (PTA) or a cumulative fraction of response (CFR) values ≥ 90% represent the optimal antibiotic dosing regimens.

2.4.2 Pharmacokinetics/pharmacodynamics of antibiotics

Focusing on classic antibiotic groups, the PK parameters and PK/PD index/target of the antibiotic options are as follows:

2.4.2.1 Carbapenems (meropenem, imipenem)

2.4.2.1.1 PK/PD of carbapenems

Carbapenems are one of the beta-lactam groups. The mechanism of action is to bind to penicillin-binding proteins (PBPs) which prevent completing transpeptidation (cross-linking) of peptidoglycan strands, preventing the bacterial cell wall synthesis [58].

The pharmacokinetics of meropenem include 2% of protein binding, 0.18-0.30 (L/Kg) of volume of distribution, 1 hour of half-life, and 70% of excretion unchanged by renal [58, 59].

The pharmacokinetics of imipenem include 20% of protein binding, 0.20-0.23 (L/Kg) of volume of distribution, 1 hour of half-life, and 60-70 (with cilastatin) of excretion unchanged by renal [58, 60]

The optimal PK/PD index of carbapenem is T>MIC [61]. The recommendation of the PK/PD target for carbapenems in critically ill patients is 100% *f* T>MIC. In critically ill patients, the beta-lactam target is 100% *f* T>MIC which is the minimum to achieve clinical efficacy in ICU [62].

2.4.2.1.2 Carbapenems with Monte Carlo simulation

In 2013, Jaruratanasirikul S et al conducted Thai population pharmacokinetics and assess the probability of target attainment (PTA) for imipenem in VAP patients. PK/PD index and target of the study are 20% *f*T>MIC and 40% *f*T>MIC. The study found that all imipenem regimens (0.5 g q 6 hours infusion 30 minutes, 0.5 g q 6 hours infusion 2 hours, and 1 g q 6 hours infusion 2 hours) achieved in the target (PTA ≥ 90%) at MICs ≤ 2 μg/mL. For pathogens with MICs of 4 μg/mL, the PTA of 40% *f*T>MIC following administration of 1 g every 6 hours (infusion 2 hours) was 98.75%. None of any regimens achieved the target at MICs ≥ 8 μg/mL [63].

In 2015, Jaruratanasirikul S et al conducted Thai population pharmacokinetics and assess the probability of target attainment (PTA) for meropenem in critically ill patients. PK/PD index and target of the study are 40% *f*T>MIC and 80% *f*T>MIC. For pathogens with MICs of 2 and 4 g/ml, the PTA of 80% *f*T>MIC following administration of 2 g every 8 hours (infusion 4 hours) were 94.72% and 84.32%, respectively [64]. Similar to the study of Thunyapituk N et al in 2019, PK/PD target and index are 40% *f*T>MIC, 75% *f*T>MIC, and 100% *f*T>MIC. The study found that all meropenem regimens achieved the target (PTA ≥ 90%) at MICs ≤ 4 μg/mL. The continuous infusion regimens - 1 g every 6 hours (continuous infusion), 1 g every 8 hours (continuous infusion) and 2 g q 8 hours (continuous infusion) achieved in the target (PTA ≥ 90%) at MICs ≤ 8 μg/mL [65].

Overall, previous results found that prolonged infusion and continuous infusion have a benefit for the CRE treatment, particularly higher MICs.

2.4.2.2 Aminoglycosides (amikacin, gentamicin)

2.4.2.2.1 PK/PD of aminoglycosides

Aminoglycosides have a role in the CRE treatment as adjunctive agents [11]. Mechanisms of action of aminoglycosides are inhibition of ribosomal translocation where the peptidyl-tRNA moves from the A-site to the P-site; they can also disrupt the integrity of bacterial cell membrane [58].

The pharmacokinetics of aminoglycosides includes 0-30% of protein binding, < 0.3 (L/Kg) of volume of distribution, 2-3 hour of half-life, and 94-98% of excretion unchanged by renal [58, 66, 67]

PK/PD index and target for amikacin are *f*Cmax:MIC at least 8 – 10. The usual dose recommendations of aminoglycosides consist of 5 to 7 mg/kg/day for gentamicin and 15 to 20 mg/kg/day for amikacin [11], whereas the alternative dose recommendations for the CRE treatment are 10 to 15 mg/kg/day for gentamicin and 25 to 30 mg/kg/day for amikacin [11, 68].

2.4.2.2.2 Aminoglycosides with Monte Carlo simulation

In 2008, Rea R.S., et al showed conducted a population pharmacokinetic study and pharmacokinetic simulation. One hundred two patients (n = 102) (body weight 81.4 ± 30.3 kg and creatinine clearance (CrCL) 2.2 ± 1.9 mg/dL) included to develop pharmacokinetic model and use the final model for simulation. At C_{max}/MIC ≥ 10, aminoglycoside dosage regimens ranged from 5 to 30 mg/kg, 15-30 mg/kg and 30 mg/kg achieved the PTA target (> 90%) at MICs of 0.5, 1 and 2 µg/mL, respectively [45].

In 2016, Roger et al evaluated whether high amikacin and high gentamicin doses were achieved in the targets for ICU patients with severe sepsis (30 mg/kg amikacin or 8 mg/kg gentamicin). The targeted concentrations for amikacin were peak ≥60 µg/mL and trough 2.5 µg/mL, whereas for gentamicin were peak ≥ 30 µg/mL and trough 0.5 µg/mL; the target PK/PD ratio was 10×MIC. Severe sepsis patients (n = 63) had median (IQR) SOFA at the initiation of aminoglycoside therapy were 7 (4–10). The results at the first dose showed that 36 of 47 patients (77%) achieved the target (peak concentrations ≥ 60 µg/mL) of amikacin when received more than 30 mg/kg of amikacin, whereas one of 16 patients (6%) achieved in the target (peak concentrations ≥ 30 µg/mL) received more than 8 mg/kg of amikacin. At the target PK/PD ratio, 59 of 63 patients (94%) achieved the target. As a result, the recommended doses of amikacin and gentamicin for severe sepsis patients were 30 mg/kg and 8 mg/kg, respectively.

In 2017, Kato H et al conducted pharmacokinetics and pharmacodynamics for an optimal initial dosing regimen. The results recommended that the initial total daily dose of amikacin required to achieve Cmax/MIC ≥ 8 μg/mL was 15 mg/kg daily, and also recommended 15 mg/kg/day as the maintenance dosage for amikacin MICs ≤ 4 μg/mL. For amikacin MICs ≥ 8 μg/mL, amikacin monotherapy regimens should not be considered [69].

2.4.2.3 Tigecycline

2.4.2.3.1 PK/PD of aminoglycosides

Tigecycline is a semisynthetic glycylglycine derivative. A mechanism of action is bacterial protein synthesis inhibition by binding to the 30S ribosomal subunit. Compared with tetracyclines, they exhibit bacteriostatic activity, but tigecycline has five times higher binding than tetracyclines. [11, 24, 45].

The pharmacokinetics of tigecycline include 71-89% of protein binding and 7-9 (L/Kg) of volume of distribution. The half-life of multiple doses is approximately 37 to 67 hours [70]. The tigecycline elimination included two routes; the major route is feces (containing 58.6 ± 4.5% ~ 59% of the dose) by biliary/fecal excretion and another route is urines (containing 33.2 ± 1.9% ~ 33% of the dose) by unchanged tigecycline [71, 72].

The optimal PK/PD index of colistin is AUC/MIC [61]. PK/PD index and target for tigecycline are $fAUC_{0-24}$/MIC ≥ 0.9 [73].

2.4.2.3.2 Tigecycline with Monte Carlo simulation

In 2017, Xie J, et al assessed PTA and CFR of tigecycline for CRE from their population PK/PD study which included 10 critically ill patients (n =10; APACHE II score at admission 20 (13–22)). Three PK/PD indices and targets were used in the PK/PD study as follows: AUC_{0-24}/MIC = 4.5 for HAP, AUC_{0-24}/MIC = 6.96 for cIAI, and AUC_{0-24}/MIC = 17.9 for cSSSI. The tigecycline dosing regimens were 1) 100 mg loading dose followed by 50 mg maintenance dose every 12 hours, 2) 200 mg loading dose followed by 100 mg maintenance dose every 12 hours, 3) 300 mg loading dose followed by 50 mg maintenance dose every 12 hours and 4) 400 mg

loading dose followed by 200 mg maintenance dose every 12 hours. The PTA was simulated on day 5th (120-144 hours). The PTA results showed at AUC_{0-24}/MIC = 4.5 and 6.96, all dosing regimens in normal weight achieved PTA of 90% at MICs of ≤ 4 μg/mL, whereas AUC_{0-24}/MIC = 17.9, all dosing regimens in normal weight achieved PTA of 90% at MICs of ≤ 1 μg/mL. Furthermore, the PTA of obese patients was lower than normal-weight patients. High dose tigecycline (200 mg q 12 hours) met achieved PTA of 90% for AUC_{0-24}/MIC = 4.5 and 6.96 at MICs of ≤ 4 μg/mL and AUC_{0-24}/MIC = 17.9 at MICs of ≤ 2 μg/mL, respectively. For CFR, tigecycline dosing regimens ranged of 100-200 mg q 12 hours met CFR of 90% at AUC_{0-24}/MIC = 4.5, 6.96 and 17.9 for *Klebsiella pneumoniae* and *Escherichia coli* [74].

In 2018, Ni w et al simulated PK/PD of tigecycline to evaluate PTA in CRKP-HAP. The PK/PD index and target was $fAUC_{0-24h}$/MIC ≥ 0.9. Only tigecycline maintenance dosing regimens are used to simulate as follows: 1) 50 mg every 12 hours 2) 75 mg every 12 hours and 3) 100 mg every 12 hours. One-hundred sixty-four (n = 164) CRKP isolates were included in the study. Their carbapenemase types consisted of KPC (n = 145 of 164; 88.4%), NDM (n = 13 of 164; 7.95%), IMP (n = 4 of 164; 2.4%) and OXA-48-like (0%). Furthermore, MIC_{50} and MIC_{90} for KPC-producing isolates were 1 μg/mL and 2 μg/mL, whereas MIC_{50} and MIC_{90} for non-KPC-producing isolates were 1 μg/mL and 1 μg/mL. The PK/PD results found all tigecycline dosing regimens reached a PTA of 90% at MICs ≤ 0.5 μg/mL. Only 75 mg and 100 mg twice daily reached PTA of 90% at MICs ≤ 1 μg/mL (MIC_{50}), whereas none of the tigecycline dosing regimens reached PTA of 90% at MICs ≤ 2 μg/mL (MIC_{90}). For CFR, only tigecycline 100 mg q 12 hours met CFR of 90% for CP-KP [75].

2.4.2.4 Colistin

2.4.2.4.1 PK/PD of colistin

Colistin or polymyxin E is one of the groups of polymyxin antibiotics. The prodrug of colistin is colistimethate (CMS). A mechanism of action is to disrupt cell membrane permeability (by charge alteration).

The pharmacokinetics of colistin includes 59-74% of protein binding in critically ill patients, 12.4 L of volume of distribution, and 3 hour of half-life. The optimal PK/PD index of colistin is AUC/MIC [61]. At steady state, the recommendation of the PK/PD target for colistin is an area under the plasma concentration-time curve across 24 hours ($AUC_{ss,24\ hr}$) of ~50 mg hour/L which equals a target average steady-state plasma concentration ($C_{ss,avg}$) of ~2 μg/mL for total drug [76].

2.4.2.4.2 Colistin with Monte Carlo simulation

In 2019, Jitaree et al conducted a study to describe the optimal dosage regimen of colistin for the treatment of CRE in critically ill patients. The PK/PD targets were *f*AUC/MIC ≥ 25. Additionally, they determined the dose ranges based on creatinine clearance (CrCL ≥80, 51 to 79, 30 to 50, 11 to 29, and ≤10 mL/min). At a MIC of 0.5 μg/mL, the recommended dose of colistin included 150 mg every 12 hours, 114 mg every 12 hours, 150 mg every 24 hours, 60 mg every 24 hours for CrCL ≥80, 51 to 79, 30 to 50, and ≤ 11 mL/min, respectively. At a MIC of 2 μg/mL, the recommended dose of colistin included 180 mg every 8 hours, 150 mg every 12 hours, and 120 mg every 24 hours for CrCL 51 to 79, 29 to 50, and ≤ 10 mL/min, respectively. At a MIC of 8 μg/mL, the recommended dose of colistin included 150 mg every 8 hours and 180 mg every 12 hours for CrCL 11 to 29 and ≤ 10 mL/min, respectively. At MIC 16 μg/mL, the recommended dose of colistin included 180 mg every 8 hours for CrCL ≤ 10 mL/min. At a MIC of ≥ 32 μg/mL, all regimens were not recommended [77].

2.4.2.5 Fosfomycin

2.4.2.5.1 PK/PD of fosfomycin

Fosfomycin is a bactericidal antibiotic agent. The mechanism of action is cell wall inhibition which interferes with the formation of the peptidoglycan precursor UDP N-acetylmuramic acid.

The pharmacokinetics of fosfomycin includes very low protein binding, 2.73 ± 0.41 (L/Kg) of volume of distribution, 1.54 ± 0.40 hour of the half-life, and 93 - 99% of excretion unchanged by renal [78].

The optimal PK/PD index of fosfomycin is unclear depending on the microorganism. AUC/MIC is appropriate for *Pseudomonas aeruginosa, E. coli* and *Proteus spp.,* whereas T > MIC is appropriate for *Staphylococcus aureus* and *Enterococcus spp.* [79].

2.4.2.5.2 Fosfomycin with Monte carlo simulation

In 2016, Albiero J et al published evidence that compared combination therapy (fosfomycin-carbapenem) with monotherapy. At MIC_{50} and MIC_{90} of fosfomycin equal to 64 and 512 µg/mL, respectively. Only fosfomycin regimens of 4 g every 8 hours (12 g/day), 6 g every 6 hours (24 g/day), and 8 g every 8 hours (24 g/day) as a 0.5 and 3- hours infusion achieved the PTA target at MICs ≤ 64 of µg/mL [80]. In 2019, Rodríguez-Gascón et al showed 24 g/d (every 6-8 hours) achieved the ≥90% PTA target at MICs ≤ 16 µg/mL [81]. In 2020, Leelawattanachai P et al simulated fosfomycin dosing regimens with NDM-positive isolates. The PK/PD target and index were $fAUC_{0-24}$ = 21.5. The results showed 24 g/day (8 g infusion 1 h every 8 hours) in patients with CrCL ≥ 50 mL/min) met the ≥90% PTA target at MICs ≤ 128 µg/mL. Furthermore, infusion times between 1 hour and 4 hours were not different in the PTA [82].

2.4.2.6 Ceftazidime/avibactam

2.4.2.6.1 PK/PD of ceftazidime/avibactam

Ceftazidime-avibactam is one of the ß-lactam and ß-lactamase inhibitors (BLBIs) combined with ceftazidime (a broad-spectrum cephalosporin) and avibactam (a novel ß-lactamase inhibitor). Avibactam has activities against KPC and some OXA-producing isolates, but it has no activity against MBL-producing isolates [83].

The pharmacokinetics of ceftazidime included low volume distributions at a steady-state (14.3 L; range 10.8-17.1), 10% of protein

binding, 1.76-1.98 hours of the half-life, and 80-90% of excretion in the urine, whereas the pharmacokinetics of avibactam included low oral bioavailability (7%), low volume distributions at steady state (15-25 L), low protein binding (5.7-8.2%), 1.49-1.59 hours of the half-life and 97% of excretion in the urine as unchanged form. Ceftazidime-avibactam is administrated by parenteral due to low oral bioavailability. The recommended dosing regimens were 2.5 h infusion 2-3 hours every 8 hours. Nonetheless, it is eliminated by renal clearance; dose adjustment is required in patients with CrCL ≤ 50 ml/min.

The targeted PK/PD indices and targets were 100%*f*T > MIC for ceftazidime (CAZ) and fC_{trough} exceeding 1 μg/mL for 100% of the dosing interval (100%*f*T> 1 μg/mL) [84].

2.4.2.6.2 Ceftazidime-avibactam with Monte Carlo simulation

In 2018, Stein GE et al determined PTA from Monte Carlo simulations. The 2.5 g infusion 2 h every 8 hours of CZA dosing regimens achieved PTA (>90%) at MICs ≤ 16 μg/mL in patients with CrCL 51–130 mL/min and 131-190 mL/min. Nonetheless, the 1.25 g infusion 2 h every 8 hours of CZA dosing regimens achieved PTA (>90%) at MICs ≤ 16 μg/mL in patients with CrCL 31–50 mL/min [84].

2.5 Risk factors for mortality in patients with the CRE infections

There were mortality-associated risk factors in patients infected with CRE such as BSI, type of antibiotic regimens, Charlson Comorbidity Index (CCI), or severity of infection (Table 3). Understanding risk factors for mortality in patients with CRE infection could be beneficial in planning either the treatment or preventable factors for a death decrease in the high-risk patients

Table 3 Risk factors for mortality in patients with the CRE infections

Author (year) [Ref]	Objective	Study design	Variables	Results related to mortality
Matin A, et al (2018) [85]	To investigate the association between CRE and CSE infections and mortality among hospitalized adult patients	Systematic review and meta-analysis	- BSI - Monotherapy *vs* combination therapy	Mortality - BSIs caused by CRE vs CSE (OR, 3.65; 95% CI, 2.11–6.30) - Monotherapy vs combination therapy (OR, 2.19; 95% CI, 1.00–4.80)
Schmid A, et al (2019) [27]	To investigate the effect of combination antimicrobial therapy *vs* monotherapy against multi-resistant Gram-negative bacteria on mortality as a primary outcome and cure rate as secondary outcome	Systematic review and meta-analysis	- Monotherapy *vs* combination therapy	Mortality - Monotherapy vs combination therapy (RR 0.83, CI 0.73–0.93, P = 0.002, I^2 =24%)
Lin YT, et al 2019 [86]	To investigate the outcome of patients who received different antimicrobial therapy in BSI-CRE in 16 hospitals in Taiwan	Retrospective study	- Charlson Comorbidity Index - Colistin monotherapy	14-day mortality 1) In univariate analysis (p < 0.20) - Chronic respiratory failure with mechanical ventilator (HR, 2.14; 95% CI, 0.87–5.24; P = 0.096) - Malignancy (HR, 2.29; 95% CI, 0.94–5.62; P = 0.069) - Charlson Comorbidity Index (HR, 1.11; 95% CI, 0.96–1.29;

Author (year) [Ref]	Objective	Study design	Variables	Results related to mortality
				P = 0.147) - Surgical drain (HR, 1.93; 95% CI, 0.74–5.02; P = 0.180) - Septic shock (HR, 2.08; 95% CI, 0.80–5.42; P = 0.134) - APACHE II score (HR, 1.07; 95% CI, 1.01–1.13; P = 0.024) - Colistin monotherapy (HR, 4.05; 95% CI, 1.65–9.96; P = 0.002) - Antibiotic monotherapy (HR, 0.25; 95% CI, 0.07–0.86; P = 0.028) 2. In multivariate analysis, - Charlson Comorbidity Index (HR, 1.21; 95% CI, 1.03–1.42; P = .022) - Colistin monotherapy (HR, 5.57; 95% CI, 2.13–14.61; P < 0.001)
Chotiprasitsakul D, et al [87]	To study the epidemiology of CRE, and to compare risk factors and related mortality between nonsusceptibility to ertapenem alone Enterobacteriaceae (NSEE),	Retrospective study	- Chronic kidney disease - Hematologic malignancies - SOFA score	30-day mortality 1) In univariate analysis (p < 0.10) - Non-susceptibility to ertapenem alone (HR, 0.43; 95% CI, 0.10–1.90; P= 0.27) - Chronic kidney disease (HR, 5.68; 95% CI, 1.99–16.25; P = 0.001) - Solid tumor (HR, 0.31; 95% CI, 0.09–1.08; P = 0.07)

Author (year) [Ref]	Objective	Study design	Variables	Results related to mortality
	with non-susceptibility to other carbapenems (imipenem, meropenem, or doripenem) Enterobacteriaceae (NSOCE) at a tertiary care hospital in Thailand.			- Hematologic malignancies (HR, 6.95; 95%CI, 2.61–18.50; P < 0.001) - SOFA score (HR, 1.28; 95%CI, 1.16–1.41; P<0.001) - ICU at the time of culture (HR, 2.46; 95%CI, 0.93–6.47, P = 0.07) - On mechanical ventilation (HR, 2.57; 95%CI, 0.84–7.88, P = 0.10) - Primary bacteremia (HR, 3.33; 95%CI, 1.23–9.03, P =0.02) - In vitro active empiric treatment in the first 3 days of infection (HR, 1.97; 95%CI, 0.56–6.84; P =0.29) 2) In multi-variate analysis (p < 0.10) - Chronic kidney disease (HR, 4.12; 95% CI, 1.02–16.63; P = 0.046) - Hematologic malignancies (HR, 6.84; 95%CI, 1.86–25.12; P = 0.004) - SOFA score (HR, 1.22; 95%CI, 1.06–1.41; P = 0.007)
Giannella M, et al (2018) [88]	To evaluate the impact of high-dose (HD) arbapenem-based combination therapy	Retrospective study	- Chronic kidney disease - Septic shock - Colistin-resistant strain	14-day mortality - Charlson comorbidity index (HR 1.31, 95%CI 1.20–1.43, P < 0.001)

Author (year) [Ref]	Objective	Study design	Variables	Results related to mortality
	on clinical outcome in patients with monomicrobial carbapenem-resistant *Klebsiella pneumoniae* (CR-KP) bloodstream-infection (BSI) in Italy		- Admission to a surgical ward	- Septic shock at BSI onset (HR 3.14, 95%CI 2.19–4.50, P < 0.001) - Isolation of a colistin-resistant strain (HR 1.52, 95%CI 1.02–2.24, P < 0.001) - Admission to a surgical ward (HR 0.44, 95%CI 0.25–-0.78, P = 0.005)
Gutierrez-Gutierrez, B et al (2017) [89]	To investigate the effect of appropriate therapy and of appropriate combination therapy on mortality of patients with bloodstream infections (BSIs) due to CPE.	Retrospective cohort study	- Source other than urinary or biliary tracts - Charlson comorbidity index score - Pitt bacteraemia score - Severe sepsis or septic shock - Appropriate therapy (started in ≤5 days after infection) - High-mortality-risk centre	1. In univariate regression (p <0.2) - OXA-type carbapenemase (HR 1.43; 95%CI 1.00- 2.05; P = 0.05) - Nosocomial acquisition (HR 1.83; 95%CI 1.06- 3.16; P = 0.03) - Source other than urinary or biliary tracts† (HR 2.12; 95%CI 1.37- 3.29; P = 0·0009) - ICU admission (HR 1.55; 95%CI 1.16–2.08; P =0.003) - Charlson comorbidity index score (HR 1.10; 95%CI 1.05–1.16; P <0·0001) - Mechanical ventilation (HR 1.76; 95%CI 1.32–2.34; P<0·0001) - Mental status: not alert (HR 2.45; 95%CI 1.82–3.29;

Author (year) [Ref]	Objective	Study design	Variables	Results related to mortality
				P<0·0001) - Chronic kidney disease (HR 1·33; 95%CI 0.97–1.84; P = 0·08) - Chronic liver disease (HR 1·58; 95%CI 1·08–2·31; P = 0·02) - Leukaemia or metastatic cancer (HR 1·61; 95%CI 1.12–2.31; P = 0·009) - Pitt bacteraemia score (HR 1.17; 95%CI 1·13–1·22; P <0·0001) - Severe sepsis or septic shock (HR 3·87; 95%CI 2.78–5.39; P<0·0001) - Early appropriate therapy (started in ≤2 days after infection) (HR 0·84; 95%CI 0·59–1·21; P = 0·35) - Appropriate therapy (started in ≤5 days after infection) (HR 0.44; 95%CI 0.33–0.61; P <0·0001) - High-mortality-risk centre (HR 2.25; 95%CI 1·69–2·99; P <0·0001) 2. In multivariate Cox regression - Source other than urinary or biliary tracts (HR 1.72; 95%CI 1.09–2.72; P = 0·02) - Charlson comorbidity index score (HR 1.13; 95%CI 1.07–

Author (year) [Ref]	Objective	Study design	Variables	Results related to mortality
				1.20; P<0·0001) - Pitt bacteraemia score (HR 1·09; 95%CI 1.04–1.15; P= 0·0003) - Severe sepsis or septic shock (HR 3·11; 95%CI 2·14–4·51; P<0·0001) - Appropriate therapy (started in ≤5 days after infection) (HR 0·45; 95%CI 0·33–0·62; P <0·0001) - High-mortality-risk centre (HR 2.37; 95%CI 1.74–3.22; P <0.0001)

Abbreviations: CRE, Carbapenem-Resistant Enterobacterales; CSE, Carbapenem-susceptible Enterobacterales; BSI, Bloodstream infections

CHAPTER III

Research methodology

3.1 Conceptual framework

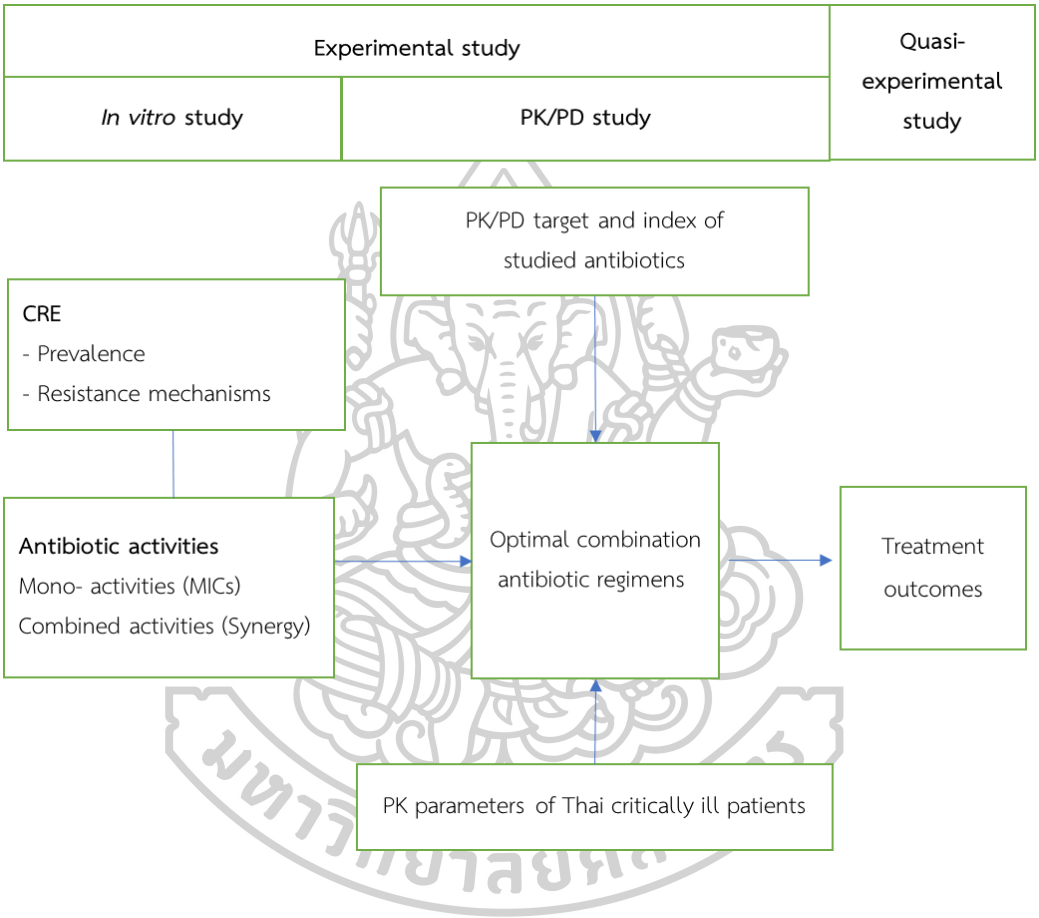

Figure 1 Conceptual framework

3.2 Research design

Experimental studies and quasi-experimental studies were approached in the study. Experimental studies are investigated in the laboratory setting and using a computer for simulation; they contain two sub-study designs, including *in vitro* study and PK/PD study. Additionally, a quasi-experimental study with an intervention group (prospective phase) and a control group (retrospective phase) was designed to evaluate treatment outcomes of optimal combination regimens.

3.3 Population and sample sizes

3.3.1 Experimental study

3.3.1.1 *In vitro* study

3.3.1.1.1 Population

Any CRE clinical isolates are obtained from 17 hospitals (n = 17). The majority of CRE clinical isolates are collected from two settings, including Phramongkutklao hospital and the bacterial culture bank of the department of medical sciences in health region V (which was obtained from 16 western hospitals in health region V, Thailand). Lists of hospitals stratified by hospital levels are shown in Table 4.

Table 4 Lists of hospitals stratified by hospital levels

No.	Hospital levels	No	Hospital name
1	Super-tertiary hospital (University hospitals): 1 hospital	1.1	Phramongkutklao hospital
2	Advance-level Hospital (A): 3 hospitals	2.1	Nakhon Pathom hospital
		2.2	Ratchaburi hospital
		2.3	Samut Sakhon hospital
3	Standard-level Hospital (S): 8 hospitals	3.1	BanPong hospital
		3.2	Banphaeo general hospital
		3.3	Chaophrayayommarat hospital
		3.4	Huahin hospital
		3.5	Phanhon Phon Phayuha Sena hospital
		3.6	Phra Chomklao Phetchaburi hospital
		3.7	Prachuap Khiri Khan hospital

Table 4 Lists of hospitals stratified by hospital levels

No.	Hospital levels	No	Hospital name
		3.8	Somdetphraphutthaloetla hospital
4	Middle-level Hospital (M1): 5 hospitals	4.1	Damnoen Saduak hospital
		4.2	Krathumbaen hospital
		4.3	Makarak hospital
		4.4	Photharam hospital
		4.5	Somdet Phra Sangkharat 17th Hospital

3.3.1.1.2 Samples

1) Eligible criteria

1.1) CRE clinical isolates are obtained from the bacterial culture bank of the department of medical sciences in health region V from 1/7/2019 to 31/10/2020.

1.2) CRE clinical isolates from Phramongkutklao hospital are obtained from the sterile sites and non-sterile sites with clinical symptoms followed by CDC/NHSN Surveillance Definitions for Specific Types of Infections from 1/1/2018 to 30/4/2020 [90].

3.3.1.1.3 Sample size calculations

The estimation of the sample size is determined by the single population proportion formula. If the proportion is larger than 5%, the finite population correction formula is used to calculate the sample size as follows:

$$n' = \frac{NZ^2\ P\ (1\text{-}P)}{d^2(N\text{-}1) + Z^2\ P\ (1\text{-}P)}$$

where n’ = Sample size with finite population correction

N = Population size

Z = Z statistic a level of confidence

P = Prevalence proportion (one proportion)

d = Precision proportion

However, when the prevalence is below 10% (the proportion (P) = 0.1), Naing et al suggested that the precision (d) should be half of P because the assumption of sample size determination is a normal approximation. Checking assumption, n'P and n'(1-P) must be greater than 5 [91].

Calculation of sample sizes, a level of confidence were 95% CI (Z = 1.96; two-tailed alpha = 0.0250, type I error would be 5%), P = the prevalence of CRE from previous study at Rajavithi Hospital in 2015 (P = 5.8% = 0.058), n/N = 411/7,039 [92], d = a half of P (d = 0.029) are calculated with the formula as follows:

$$n' = \frac{7{,}039 \times 1.96^2 \times 0.058 \times (1-0.058)}{0.029^2(7{,}039-1) + 1.96^2\ 0.058 \times (1-0.058)}$$

$$n' = 241.06 \sim 242$$

The expected total CRE isolates should be at least 242 isolates (n' ~ 242). Accept assumption for normal approximation because n'P and n'(1-P) are greater than 5 (n'P = 13.98 and n'(1-P) = 13.17).

Nonetheless, the total CRE clinical isolates in a real-world situation were obtained only 199 isolates. All CRE clinical isolates (n = 49) from Phramongkutklao hospital were collected, whereas 150 CRE clinical isolates from the bacterial culture bank of the department of medical sciences in health region V would be sampled. Calculation of a level of confidence were 92.32% CI (Z = 1.77; two-tailed alpha = 0.0384). Accept assumption for normal approximation because n'P and n'(1-P) are greater than 5 (n'P = 11.48 and n'(1-P) = 10.81). Thus, type I error would be 7.68%.

3.3.1.1.4 Sampling technique

Only CRE clinical isolates from the bacterial culture bank of the department of medical sciences in health region V are sampled, whereas all CRE clinical isolates from Phramongkutklao hospital were collected due to a few isolates. Proportional sampling is a systematic sampling technique that will be divided a population into subpopulations in a multicenter study. The number of isolates in each hospital (each stratum) will be calculated with the formula [93]:

$$n_i = \frac{N_i}{N} \times n$$

where n_i = The number of CRE isolates of each hospital

n = The expected total CRE samples (n = 150)

N_i = The total CRE isolates of each hospital from bacterial culture bank

N = The total CRE isolates from bacterial culture bank (n = 190)

3.3.1.2 Pharmacokinetics and pharmacodynamic study (PK/PD study)

In the case of PK/PD experiments on the computer, the eligible criteria of population pharmacokinetic study are selected from a literature review as follows:

1) The studies are the population pharmacokinetic studies from Thai critically ill patients (If none of the studies, the studies from Asian critically ill patients and/or the highest number of critically ill patients will be considered, respectively).

2) The studies analyzed the results based on the compartment model.

3) The studies have sufficient pharmacokinetic parameters, including

- Clearance (Cl), volume of distribution of the central compartment (V_c) for one-compartment model

- Clearance (Cl), volume of distribution of the central compartment (V_c), intercompartment clearance (Q), and volume of the peripheral compartment (V_p) for two-compartment model

3.3.2 Quasi-Experimental study

3.3.2.1 Population

Any patients have been diagnosed with any diseases caused by CRE infection by ID physicians at Phramongkutklao hospital.

3.3.2.2 Samples

3.3.2.2.1 Inclusion criteria

1) Any patients have been diagnosed with any diseases caused by CRE infection by ID physicians at Phramongkutklao hospital from 1/10/2019 to 31/1/2022

2) Any patients aged ≥ 18 years

3) Any patients have ≥ 2 of the signs and symptoms of SIRS [94].

- Fever (temperature > 38 °C) or
  hypothermia (temperature < 36°C)
- Tachypnea (heart rate > 90 beats per minute)
- Respiratory rate > 20 beats per minute
  or $Paco_2$ < 32 mm Hg (4.3 kPa)
- White blood cell count > 12,000 cells/mL
  or White blood cell count < 4,000 cells/mL

3.3.2.2.2 Exclusion criteria

1) Patients are breastfeeding or pregnant.

2) Patients are insufficient or incomplete information on the medical electronic record such as patients transferred.

3.3.2.2 Sample size calculations

The estimation of the sample size is determined by the formula [95].

$$n = \frac{\left[Z_{\alpha/2} + Z_{\beta}\right]^2 X \left[\pi_1(1-\pi_1) + \pi_2(1-\pi_2)\right]}{\left[\pi_1-\pi_2\right]^2}$$

where n = sample sizes per groups

$Z_{\alpha/2}$, Z_{β} = Z statistic a level of confidence

π_1 = Proportion of group 1

π_2 = Proportion of group 2

$\pi_1-\pi_2$ = the difference in proportions

The samples are divided into two groups – intervention and control groups. Calculation of sample sizes per groups, a level of confidence ($Z_{\alpha/2}$) = 1.96 at 5% (95% CI), desired power (Z_{β}) = 0.84 (80%). In 2019, Prawang A et al study conducted a clinical study at Pramongkutklao hospital. All-cause 14-day mortality rate from the patients infected with CRKP was 40% (π_1) [49]. For this study, the goal is to reduce the all-cause 14-day mortality to 25%, resulting all-cause 14-day mortality rate would be 15% (π_2).

$$n = \frac{\left[1.96 + 0.84\right]^2 X \left[0.40 \times (1 - 0.40) + 0.15 \times (1 - 0.15)\right]}{\left[0.40 - 0.15\right]^2}$$

$$n = 46.0992 \sim 47$$

drop out 10%: $n = 4.60992 \sim 5$

$$n \sim 47 + 5 = 51$$

As described above, two groups will be studied, corresponding to a total of 102 participants ($n_{total/group} = 51$)

3.4 Research procedure

This study consisted of three steps based on the study design, including *in vitro* study, PK/PD study, and clinical study.

3.4.1 *In vitro* study

3.4.1.1 Tools/reagents

3.4.1.1.1 Tools/reagents for phenotyping testing

a) General preparations

- Disposable gloves
- 70% Ethanol

b) For culture medium preparations

- MacConkey agar (Clinical Diagnostics Ltd., Part, Bangkok, Thailand and Oxoid Ltd, Basingstoke, Hampshire, England)
- Blood agar (Clinical Diagnostics Ltd., Part, Bangkok, Thailand)
- Muller Hinton Agar power (Himedia®, Himedia Laboratories Pvt. Ltd., India)
- Cation-adjusted Mueller Hinton Broth (CAMHB or MHB II) (BBL™ Mueller Hinton II Broth (Cation-Adjusted), Becton Dickinson and Company, France)
- Tryptic Soy Broth powder (Oxoid Ltd, Hampshire, England)
- Agar-agar powder (TM media, Titan Biotech Ltd., New Delhi India and Himedia®, Himedia Laboratories Pvt. Ltd., India)
- Sterile distilled water
- Laboratory bottle in size of 2 L
- Petri disk

c) For antibiotic stock solution

- Meropenem powder

- Imipenem/cilastatin powder
- Amikacin powder
- Gentamicin powder
- CMS/Colistin powder
- Fosfomycin powder
- G-6-P solution
- Aztreonam powder
- Tigecycline powder
- Sterile water for injection 10 mL
- Eppendorf 2 mL
- A syringe of 5 ml with a needle of 18”
- 0.22-µm membrane filter

d) For antibiotic susceptibility testing

- Normal saline powder (Univar ®, Ajax Finechem Pty Ltd, Taren Point, Australia
- Fosfomycin E-test strips
- Ceftazidime-avibactam E-test strips
- Lab test tube
- U-type 96 well cell culture plate (SPL, Gyeonggi-do, Korea)
- Commercial 96 well plates for an automated system (DKMGN plates)
- 1 µL inoculation loops
- Cotton Swab Sterile
- Forceps
- Pipets and pipet tips
- Vortex

3.4.1.1.2 Tools/reagents for genotyping testing

a) General preparations

- Disposable gloves
- 70% Ethanol

b) For DNA extraction

- Proteinase K Solution
- RNase A Solution
- Digestion Solution
- Lysis Solution
- Wash Buffer I (concentrated)
- Wash Buffer II (concentrated)
- Elution Buffer (10 mM Tris-Cl, pH 9.0, 0.1 mM EDTA)
- GeneJET Genomic DNA Purification Columns
- Collection Tubes
- Microcentrifuge tubes (Eppendorf 2 mL)
- Dry bath of heating up to 56 °C
- Vortex

c) For Polymerase chain reaction (PCR) testing

- 2X PCR master mix (JumpStart™ REDTaq® ReadyMix™ Reaction Mix for PCR, 100 reactions, Sigma-Aldrich®, Merck Ltd, Darmstadt, Germany)
- Carbapenemase primers (100 nmol RxnReady® Oligos, RxnReady® Primer Pools, Integrated DNA Technologies, Inc., Iowa, USA)
- *Mcr-1* primers (100 nmol RxnReady® Oligos, RxnReady® Primer Pools, Integrated DNA Technologies, Inc., Iowa, USA)
- ERIC primers (100 nmol RxnReady® Oligos, RxnReady® Primer Pools, Integrated DNA Technologies, Inc., Iowa, USA)
- Microcentrifuge tubes (Eppendorf 2 mL)
- Microcentrifuge tube for PCR (Eppendorf 0.2 mL)
- Pipets and pipet tips
- Vortex
- PCR thermal cycler

d) For gel electrophoresis

- 1% agarose gel
- Tris base, acetic acid, and EDTA (TAE solution)
- 1% Ethidium bromide
- Loading dye
- Pipets and pipet tips
- Electrophoresis equipment
- UV light

3.4.1.2 Phenotyping testing

3.4.1.2.1 Culture media preparations

(1) Cation-adjusted Mueller Hinton Broth (CAMHB) for antimicrobial susceptibility testing with broth microdilution method (2X) and synergistic testing (4X) with checkerboard method [96].

- For broth microdilution method (2X), weight 44 gm of the CAMHB powder in a laboratory bottle in size of 2 L and add 1 L of distilled water to the container
- For synergistic testing (4X), weight 88 gm of the CAMHB powder in a laboratory bottle in size of 2 L and add 1 L of distilled water to the container
- Agitate the medium (the mixed solution)
- Boil 1 minute to dissolve the medium completely
- Autoclave at 121°C, pressure 15 Ibf/in^2, 15 minutes
- Cool to room temperature
- Store the medium at 2-8 °C

(2) Mueller-Hinton Agar (MHA) for antimicrobial susceptibility testing with gradient-method [97].

- Weight 38 gm of the MHA powder in a laboratory bottle in size of 2 L
- Add 1 L of distilled water to the container

- Agitate the medium (the mixed solution)
- Autoclave at 121°C, pressure 15 Ibf/in^2, 15 minutes
- Cool to 45-50 °C
- Gently mix well before pouring into sterile Petri dishes (~ 20 ml)
- Cool to room temperature
- Store the MHA plates at 2-8 °C

(3) Tryptic soy broth agar (TSA) for bacterial isolation and cultivation

- Weight 24 gm of tryptic soy broth powder in a laboratory bottle in size of 2 L
- Weight 15 gm of agar powder and add it to the Erlenmeyer-flask
- Add 1 L of distilled water to the container
- Agitate the medium (the mixed solution)
- Autoclave at 121°C, pressure 15 Ibf/in^2, 15 minutes
- Cool to 45-50 °C
- Gently mix well before pouring into sterile Petri dishes (~ 20 ml)
- Cool to room temperature
- Store the TSA plates at 2-8 °C

3.4.1.2.2 Reagent preparations

3.4.1.2.2.1 0.9% NSS solution

- Weight 0.9 g of antibiotic powder in a laboratory bottle in size of 250 mL
- Add 100 mL of distilled water to the container
- Mix the solution well
- Autoclave at 121°C, pressure 15 Ibf/in^2, 15 minutes
- Cool to room temperature

- Store the solution at 2-8 ºC

3.4.1.2.2 Antibiotic solution preparations

An antibiotic stock solution is prepared for antibiotic susceptibility testing with broth microdilution and synergistic testing with the checkboard method. Each antibiotic stock solution is determined the final concentration by weight to volume as follows:

1) Meropenem stock solution

- A desired working meropenem stock solution = 12,500 µg/mL
- Weight 0.0625 g of antibiotic powder in a conical tube of 15 ml
- Add 5 mL of distilled water to the container
- Mix well using vortex so that the antibiotic powder goes into the solution
- Filter into Eppendorf of 2 mL using a syringe with a needle and 0.22-µm membrane filter for sterilization of antibiotic stock solution
- Label the desired concentration
- Seal the Eppendorf using parafilm

2) Imipenem/cilastatin stock solution

- A desired working Imipenem/cilastatin stock solution = 12,500 µg/mL
- Weight 0.125 g of antibiotic powder in a conical tube of 15 ml
- Add 5 mL of distilled water to the container
- Mix well using vortex so that the antibiotic powder goes into the solution
- Filter into Eppendorf of 2 mL using a syringe with needle and 0.22-µm membrane filter for sterilization of antibiotic stock solution

- Label the desired concentration
- Seal the Eppendorf using parafilm

3) Amikacin stock solution

- A desired working amikacin stock solution = 12,500 µg/mL
- Weight 0.0625 g of antibiotic powder in a conical tube of 15 ml
- Add 5 mL of distilled water to the container
- Mix well using vortex so that the antibiotic powder goes into the solution
- Filter into Eppendorf of 2 mL using a syringe with a needle and 0.22-µm membrane filter for sterilization of antibiotic stock solution
- Label the desired concentration
- Seal the eppendorf using parafilm

4) Gentamicin stock solution

- A desired working gentamicin stock solution = 50,000 µg/mL
- Weight 0.0625 g of antibiotic powder in a conical tube of 15 ml
- Add 5 mL of distilled water to the container
- Mix well using vortex so that the antibiotic powder goes into the solution
- Filter into Eppendorf of 2 mL using a syringe with a needle and 0.22-µm membrane filter for sterilization of antibiotic stock solution
- Label the desired concentration
- Seal the Eppendorf using parafilm

5) CMS/Colistin stock solutio

- A desired working CMS/colistin stock solution = 5,290 µg/mL

- Weight 0.1024 g g of antibiotic powder in a conical tube of 15 ml
- Add 10 mL of distilled water to the container
- Mix well using vortex so that the antibiotic powder goes into the solution
- Filter into Eppendorf of 2 mL using a syringe with a needle and 0.22-μm membrane filter for sterilization of antibiotic stock solution
- Label the desired concentration
- Seal the Eppendorf using parafilm

6) Tigecycline stock solution

- A desired working tigecycline stock solution = 12, 500 μg/mL
- Weight 0.0625 g of antibiotic powder in a conical tube of 15 ml
- Add 5 mL of distilled water to the container
- Mix well using vortex so that the antibiotic powder goes into the solution
- Filter into Eppendorf of 2 mL using a syringe with a needle and 0.22-μm membrane filter for sterilization of antibiotic stock solution
- Label the desired concentration
- Seal the eppendorf using parafilm

7) Fosfomycin stock solution

- A desired working fosfomycin stock solution = 50,000 μg/mL
- Weight 0.0625 g of antibiotic powder in a conical tube of 15 ml
- Add 5 mL of distilled water to the container
- Mix well using vortex so that the antibiotic powder goes into the solution

- Filter into Eppendorf of 2 mL using a syringe with a needle and 0.22-μm membrane filter for sterilization of antibiotic stock solution
- Label the desired concentration
- Seal the eppendorf using parafilm

8) G-6-P stock solution

- A desired working fosfomycin stock solution = 500,000 μg/mL
- Weight 1 g of antibiotic powder in a conical tube of 15 ml
- Add 2 mL of distilled water to the container
- Mix well using vortex so that the antibiotic powder goes into the solution
- Filter into Eppendorf of 2 mL using a syringe with a needle and 0.22-μm membrane filter for sterilization of antibiotic stock solution
- Label the desired concentration
- Seal the eppendorf using parafilm

9) Aztreonam stock solution

- A desired working fosfomycin stock solution = 12, 500 μg/mL
- Weight 0.0625 g of antibiotic powder in a conical tube of 15 ml
- Add 5 mL of distilled water to the container
- Mix well using vortex so that the antibiotic powder goes into the solution
- Filter into Eppendorf of 2 mL using a syringe with a needle and 0.22-μm membrane filter for sterilization of antibiotic stock solution
- Label the desired concentration
- Seal the eppendorf using parafilm

3.4.1.2.3 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) is a test used to determine each antibiotic activity (mono antibiotic activity testing).

1) Subculture CRE isolates

Before antimicrobial susceptibility testing, CRE isolates are a subculture for bacterial growth and identifying type of bacteria.

- Firstly, after getting samples, isolate CRE isolates by transferring stocked skim milk at -80 ºC on the surface of sheep blood agar or tryptic soy agar (culture medium used for bacterial growth). Then, incubate them at 37 ℃ for 10-12 hours
- Secondly, pick up 3-5 bacterial colonies from the sheep blood agar plate into the MacConkey agar plate (culture medium used for identifying CRE) by streaking them directly on the surface of the medium. Then, incubate them at 37 ℃ for 10-12 hours. To identify CRE isolates (*K. pneumoniae*, *E. coli*, *E. cloacae*), the morphology of colonies on MacConkey agar appeared large and mucoid-pink.
- Before determining antimicrobial susceptibility, all strains were isolated again on sheep blood agar. Then, incubate them at 37 ℃ for 6 hours.

2) Prepare bacterial inoculum preparation of 0.5 McFarland by direct colony suspension method [98]

- Select at least 3-5 colonies from the last sheep blood agar plates
- Pick up these isolated colonies with a sterile loop
- Suspended to 0.9% in normal saline
- Adjust the suspension by adding more bacterial colonies or 0.9% sodium chloride solution until the mixed suspension

achieves turbidity equivalent to 0.5 McFarland standard which provides a density of a bacterial suspension with ~ 10^8 colony forming units (CFU/mL) (CLSI recommendation: "Should use adequate light to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines")

3) Perform MICs

3.1) Broth microdilution method

Only CRE clinical isolates collected from Pramongkutklao hospitals (n =49) were performed MICs using broth microdilution method.

3.1.1) Prepared two-fold serial dilutions of antibiotics

Twelve dilutions of each studied antibiotic using the two-fold serial dilution method were prepared as follows:

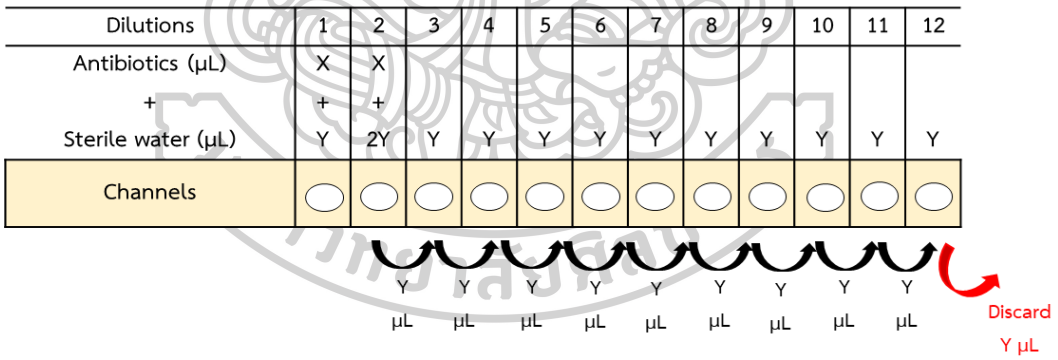

Figure 2 Twelve dilutions of each studied antibiotic using the two-fold serial dilution method

3.1.1.1) Meropenem

- Desired range of two-fold serial dilutions (0.125 to 256 µg/mL for meropenem)
- Prepare a serial of two-fold dilution of meropenem and imipenem for 25 CRE strains (n = 25) as follows:

- Use the micropipette to dispense 102.4 µL of the meropenem stock solution to the first and second channels of multichannel reservoirs
- Use the micropipette to dispense 2.5 ml of the sterile water to all the channels across a row of multichannel reservoirs
- Gently mix the solution in the first and second channels
- Use the micropipette to continually carry out 2.5 ml of the meropenem solution from the second channels to the next channels
- Discard 2.5 ml at the last channels of multichannel reservoirs

3.1.1.2) Imipenem

- Desired range of two-fold serial dilutions (0.125 to 256 µg/mL for imipenem)
- Prepare a serial of two-fold dilution of imipenem for 25 CRE strains (n = 25) as follows:

- Use the micropipette to dispense 102.4 µL of the imipenem stock solution to the first and second channels of multichannel reservoirs
- Use the micropipette to dispense 2.5 ml of the sterile water to all the channels across a row of multichannel reservoirs

- Gently mix the solution in the first and second channels
- Use the micropipette to continually carry out 2.5 ml of the meropenem solution from the second channel to the next channels
- Discard 2.5 mL at the last channels of multichannel reservoirs

3.1.1.3) Colistin

- Desired range of two-fold serial dilutions (0.0625 to 128 µg/mL for colistin)
- Prepare a serial of two-fold dilution of colistin for 25 CRE strains (n = 25) as follows:
  - Use the micropipette to dispense 120.98 µL of the colistin stock solution to the first and second channels of multichannel reservoirs
  - Use the micropipette to dispense 2.5 ml of the sterile water to all the channels across a row of multichannel reservoirs
  - Gently mix the solution in the first and second channels
  - Use the micropipette to continually carry out 2.5 ml of the colistin solution from the second channel to the next channels
  - Discard 2.5 ml at the last channels of

multichannel reservoirs

3.1.1.4) Tigecycline

- Desired range of two-fold serial dilutions (0.03125 to 64 µg/mL for tigecycline)
- Prepare a serial of two-fold dilution of tigecycline for 25 CRE strains (n = 25) as follows:
  - Use the micropipette to dispense 25.6 µL of the tigecycline stock solution to the first and second channels of multichannel reservoirs
  - Use the micropipette to dispense 2.5 ml of the sterile water to all the channels across a row of multichannel reservoirs
  - Gently mix the solution in the first and second channels
  - Use the micropipette to continually carry out 2.5 ml of the tigecycline solution from the second channel to the next channels
  - Discard 2.5 ml at the last channels of multichannel reservoirs

3.1.1.5) Amikacin

- Desired range of two-fold serial dilutions (0.0625 to 128 µg/mL for amikacin)
- Prepare a serial of two-fold dilution of amikacin for 25 CRE strains (n = 25)

- Use the micropipette to dispense 51.2 µL of the amikacin stock solution to the first and second channels of multichannel reservoirs
- Use the micropipette to dispense 2.5 ml of the sterile water to all the channels across a row of multichannel reservoirs
- Gently mix the solution in the first and second channels
- Use the micropipette to continually carry out 2.5 ml of the amikacin solution from the second channel to the next channels
- Discard 2.5 ml at the last channels of multichannel reservoirs

3.1.1.6) Gentamicin

- Desired range of two-fold serial dilutions (0.03125 to 64 µg/mL for gentamicin)
- Prepare a serial of two-fold dilution of gentamicin for 25 CRE strains (n = 25) as follows:

- Use the micropipette to dispense 6.4 µL of the gentamicin stock solution to the first and second channels of multichannel reservoirs
- Use the micropipette to dispense 2.5 ml of the sterile water to all the channels across a row of multichannel reservoirs

- Gently mix the solution in the first and second channels
- Use the micropipette to continually carry out 2.5 ml of the gentamicin solution from the second channel to the next channels
- Discard 2.5 ml at the last channels of multichannel reservoirs

3.1.1.7) Aztreonam

- Desired range of two-fold serial dilutions (0.0625 to 128 µg/mL for aztreonam)
- Prepare a serial of two-fold dilution of aztreonam for 25 CRE strains (n = 25) as follows:
  - Use the micropipette to dispense 51.2 µL of the aztreonam stock solution to the first and second channels of multichannel reservoirs
  - Use the micropipette to dispense 2.5 ml of the sterile water to all the channels across a row of multichannel reservoirs
  - Gently mix the solution in the first and second channels
  - Use the micropipette to continually carry out 2.5 ml of the aztreonam solution from the second channel to the next channels
  - Discard 2.5 ml at the last channels of multichannel reservoirs

3.1.2) Prepared the final inoculum

- Mix 0.5 McFarland bacterial suspension 5 µL with cation-adjust MHB II 5 mL in a ratio of 1:1000

3.1.3) Dispense the solution and incubation

- Dispense 50 µL of two-fold serial antibiotic solution across each column of 96 microwell plates
- Dispense 50 µL of the final inoculum of each isolate across each row of 96 microwell plates
- Incubate the 96 microwell plates for 18-20 hours at 35 $^{\circ}$C

3.1.4) Read and Interpreting the results

- After these isolates were incubated, read the MICs value at the first well that precipitate did not present
- Interpreting the results following CLSI or EUCAST guidelines

3.2) Gradient method for fosfomycin and ceftazidime-avibactam [99]

- Swab a lawn of bacteria from bacterial inoculum with 0.5 McFarland suspension by using a cotton strip on the surface of MHA agar plates
- Place E-test strips onto each MHA agar plates
- Incubate the plates for 18 hours at 35 $^{\circ}$C
- Read the MICs value at the point that contains the inhibition zone intersected by the scale on the E strips
- Interpreting the results following CLSI guidelines or EUCAST guideline

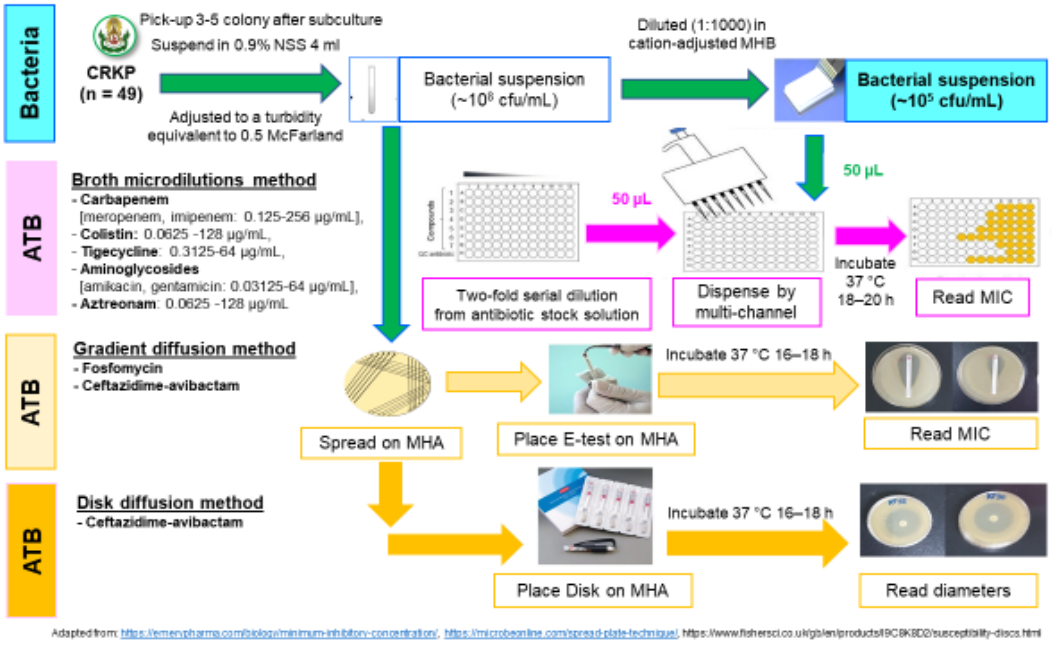

Figure 3 Antimicrobial susceptibility testing with broth microdilution and gradient method for Pharmongkutklao hospital

3.3) Broth microdilution method using DKMGN plate (Sensititre™)

Only CRE clinical isolates collected from Health Region V hospitals (n = 150) were performed MICs using broth microdilution method with DKMGN plate.

- Add 30 µL of 0.5 McFarland bacterial suspension to cation-adjust MHB II 11 mL
- Vortex the mixture
- Add 50 µL of the mixture to each of the DKMGN well plates
- Seal the DKMGN well plates
- Incubate the DKMGN well plates for 18-20 hours at 35 $^{\circ}$C

- Read the MICs value at the first well that precipitate did not present
- Interpreting the results following CLSI or EUCAST guidelines/

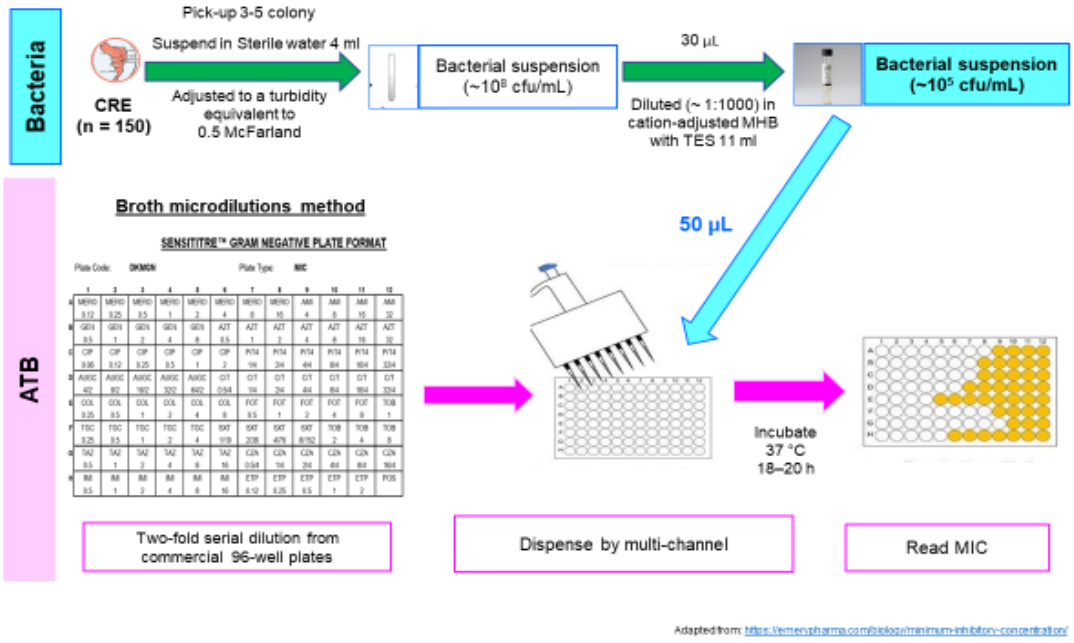

Figure 4 Antimicrobial susceptibility testing with Broth microdilution method using DKMGN plate for Health Region V hospitals

3.4.1.2.4 Synergistic testing

After determining the MICs of studied antibiotics, eleven antibiotic combinations were performed with the synergistic effect by checkerboard technique. These antibiotic-based combinations consisted of amikacin-based regimens, gentamicin-based regimens, colistin-based regimens, fosfomycin-based regimens, and triple-based regimens. Table showed all antibiotic combination regimens.

1) Amikacin-based regimens

For amikacin-based regimens consisted of amikacin combined with fosfomycin and amikacin combined with tigecycline.

1.1) Amikacin-fosfomycin

Eighteen CRE clinical isolates (n = 18) which had fosfomycin MICs of 12-256 µg/mL were included to synergistic testing.

- Desired range of two-fold serial dilutions (0.5 to 32 µg/mL for amikacin and 1 to 1,024 µg/mL for fosfomycin)

- Prepare a serial of two-fold dilution of amikacin

  - Use the micropipette to dispense 102.4 µL of the amikacin stock solution to the first and second channels of multichannel reservoirs
  - Use the micropipette to dispense 10 mL of the sterile water to all the channels across a row of multichannel reservoirs
  - Gently mix the solution in the first and second channels
  - Use the micropipette to continually carry out 10 mL of the amikacin solution from the second channel to the next channels
  - Discard 10 mL at the last channels of multichannel reservoirs

- Prepare a serial of two-fold dilution of fosfomycin

  - Use the micropipette to dispense 1.31 mL of the fosfomycin stock solution to the first and second channels of multichannel reservoirs
  - Use the micropipette to dispense 16 mL of the sterile water to all the channels across a row of multichannel reservoirs
  - Gently mix the solution in the first and second channels

- Use the micropipette to continually carry out 16 mL of the fosfomycin solution from the second channel to the next channels
- Discard 16 mL at the last channels of multichannel reservoirs

- Prepare the final inoculum in each channel of multichannel reservoirs
  - Use the micropipette to mix 0.5 McFarland bacterial suspension 21 µL with cation-adjust MHB II 21 mL in a ratio of 1:1000
- Dispense the solution to 96 microwell plate
  - Dispense 25 µL of two-fold serial amikacin solution across each well from row A to row G
  - Dispense 25 µL of two-fold serial fosfomycin solution across each well from column 1 to column 11
  - Dispense 25 µL of 0.9% NSS solution into each row H wells and each column 12 wells
  - Dispense 50 µL of the final inoculum to each of the 96 wells
- Incubate the 96-microwell plate for 18-20 hours at 35 ^{O}C
- Read and interpret the results
  - After these isolates were incubated, read the MICs value of each antibiotic at the first well that precipitate did not present
  - Calculate FICI values

$$FICI = \frac{MIC\ A_{\text{combination A and B}}}{MIC\ A_{\text{alone}}} + \frac{MIC\ B_{\text{combination A and B}}}{MIC\ B_{\text{alone}}}$$

- Interpret the results following FICI values
  - FICI values ≤ 0.5 are classified as synergy
  - FICI values > 0.5 to 1 are classified as additive
  - FICI values > 1 to 4 are classified as indifferent
  - FICI values > 4 are classified as antagonism

	Row (Drug A)		1	2	3	4	5	6	7	8	9	10	11	12
Amikacin MICs (µg/dl)	32	A												
	16	B												
	8	C												
	4	D												
	2	E												
	1	F												
	0.5	G												
	control	H												
			1,024	512	256	128	64	32	16	8	4	2	1	control
			Column (Drug B): Fosfomycin MICs (µg/dl)											

Figure 5 Checkerboard synergy testing for amikacin-fosfomycin combination

1.2) Amikacin-tigecycline

All CRE clinical isolates (n = 49) were included in synergistic testing.

- Desired range of two-fold serial dilutions (0.0625 to 64 µg/mL for amikacin and 0.125 to 8 µg/mL for tigecycline)
- Prepare a serial of two-fold dilution of amikacin
  - Use the micropipette to dispense 307.2 µL of the amikacin stock solution to the first and second channels of multichannel reservoirs

- Use the micropipette to dispense 15 mL of the sterile water to all the channels across a row of multichannel reservoirs
- Gently mix the solution in the first and second channels
- Use the micropipette to continually carry out 15 mL of the amikacin solution from the second channel to the next channels
- Discard 15 mL at the last channels of multichannel reservoirs

- Prepare a serial of two-fold dilution of tigecycline
  - Use the micropipette to dispense 32 µL of the tigecycline stock solution to the first and second channels of multichannel reservoirs
  - Use the micropipette to dispense 25 mL of the sterile water to all the channels across a row of multichannel reservoirs
  - Gently mix the solution in the first and second channels
  - Use the micropipette to continually carry out 25 mL of the tigecycline solution from the second channel to the next channels
  - Discard 25 mL at the last channels of multichannel reservoirs

- Prepare the final inoculum in each channel of multichannel reservoirs
  - Use the micropipette to mix 0.5 McFarland bacterial suspension 15 µL with cation-adjust MHB II 15 mL in a ratio of 1:1000

- Dispense the solution to 96 microwell plate
    - Dispense 25 µL of two-fold serial tigecycline solution across each well from row A to row G
    - Dispense 25 µL of two-fold serial amikacin solution across each well from column 1 to column 11
    - Dispense 25 µL of 0.9% NSS solution into each row H wells and each column 12 wells
    - Dispense 50 µL of the final inoculum to each of the 96 wells
- Incubate the 96-microwell plate for 18-20 hours at 35 ^{o}C
- Read and interpret the results
    - After these isolates were incubated, read the MICs value of each antibiotic at the first well that precipitate did not present
    - Calculate FICI values

$$FICI = \frac{MIC\ A_{\text{combination A and B}}}{MIC\ A_{\text{alone}}} + \frac{MIC\ B_{\text{combination A and B}}}{MIC\ B_{\text{alone}}}$$

    - Interpret the results following FICI values
        - FICI values ≤ 0.5 are classified as synergy
        - FICI values > 0.5 to 1 are classified as additive
        - FICI values > 1 to 4 are classified as indifferent
        - FICI values > 4 are classified as antagonism

	Row (Drug A)		1	2	3	4	5	6	7	8	9	10	11	12
Tigecycline MICs (µg/dl)	8	A												
	4	B												
	2	C												
	1	D												
	0.5	E												
	0.25	F												
	0.125	G												
	control	H												
			64	32	16	8	4	2	1	0.5	0.25	0.125	0.0625	control
			Column (Drug B): Amikacin MICs (µg/dl)											

Figure 6 Checkerboard synergy testing for amikacin-tigecycline combination

2) Gentamicin-based regimens

For gentamicin-based regimens consisted of gentamicin combined with fosfomycin and gentamicin combined with tigecycline.

2.1) Gentamicin-fosfomycin

Eighteen CRE clinical isolates (n = 18) which had fosfomycin MICs of 12-256 µg/mL were included in synergistic testing.

- Desired range of two-fold serial dilutions (0.25 to 16 µg/mL for gentamicin and 1 to 1,024 µg/mL for fosfomycin)

- Prepare a serial of two-fold dilution of gentamicin

- Use the micropipette to dispense 12.8 µL of the gentamicin stock solution to the first and second channels of multichannel reservoirs
- Use the micropipette to dispense 10 mL of the sterile water to all the channels across a row of multichannel reservoirs
- Gently mix the solution in the first and second channels

- Use the micropipette to continually carry out 10 mL of the gentamicin solution from the second channel to the next channels
- Discard 10 mL at the last channels of multichannel reservoirs

- Prepare a serial of two-fold dilution of fosfomycin
  - Use the micropipette to dispense 1.31 mL of the fosfomycin stock solution to the first and second channels of multichannel reservoirs
  - Use the micropipette to dispense 16 mL of the sterile water to all the channels across a row of multichannel reservoirs
  - Gently mix the solution in the first and second channels
  - Use the micropipette to continually carry out 16 mL of the fosfomycin solution from the second channel to the next channels
  - Discard 16 mL at the last channels of multichannel reservoirs

- Prepare the final inoculum in each channel of multichannel reservoirs
  - Use the micropipette to mix 0.5 McFarland bacterial suspension 21 µL with cation-adjust MHB II 21 mL in a ratio of 1:1000

- Dispense the solution to 96 microwell plate
  - Dispense 25 µL of two-fold serial gentamicin solution across each well from row A to row G

- Dispense 25 µL of two-fold serial fosfomycin solution across each well from column 1 to column 11
- Dispense 25 µL of 0.9% NSS solution into each row H wells and each column 12 wells
- Dispense 50 µL of the final inoculum to each of the 96 wells

- Incubate the 96-microwell plate for 18-20 hours at 35 $^{\circ}$C
- Read and interpret the results
  - After these isolates were incubated, read the MICs value of each antibiotic at the first well that precipitate did not present
  - Calculate FICI values

$$FICI = \frac{MIC\ A_{\text{combination A and B}}}{MIC\ A_{\text{alone}}} + \frac{MIC\ B_{\text{combination A and B}}}{MIC\ B_{\text{alone}}}$$

  - Interpret the results following FICI values
    - FICI values ≤ 0.5 are classified as synergy
    - FICI values > 0.5 to 1 are classified as additive
    - FICI values > 1 to 4 are classified as indifferent
    - FICI values > 4 are classified as antagonism

	Row (Drug A)		1	2	3	4	5	6	7	8	9	10	11	12
Gentamicin MICs (µg/dl)	16	A												
	8	B												
	4	C												
	2	D												
	1	E												
	0.5	F												
	0.25	G												
	control	H												
			1,024	512	256	128	64	32	16	8	4	2	1	control
			Column (Drug B): Fosfomycin MICs (µg/dl)											

Figure 7 Checkerboard synergy testing for gentamicin-fosfomycin combination

2.2) Gentamicin-tigecycline

All CRE clinical isolates (n = 49) were included in synergistic testing.

- Desired range of two-fold serial dilutions (0.0625 to 8 µg/mL for gentamicin and 0.125 to 8 µg/mL for tigecycline)
- Prepare a serial of two-fold dilution of gentamicin
  - Use the micropipette to dispense 9.6 µL of the amikacin stock solution to the first and second channels of multichannel reservoirs
  - Use the micropipette to dispense 15 mL of the sterile water to all the channels across a row of multichannel reservoirs
  - Gently mix the solution in the first and second channels
  - Use the micropipette to continually carry out 15 mL of the gentamicin solution from the second channel to the next channels

- Discard 15 mL at the last channels of multichannel reservoirs

- Prepare a serial of two-fold dilution of tigecycline
  - Use the micropipette to dispense 32 µL of the tigecycline stock solution to the first and second channels of multichannel reservoirs
  - Use the micropipette to dispense 25 mL of the sterile water to all the channels across a row of multichannel reservoirs
  - Gently mix the solution in the first and second channels
  - Use the micropipette to continually carry out 25 mL of the tigecycline solution from the second channel to the next channels
  - Discard 25 mL at the last channels of multichannel reservoirs

- Prepare the final inoculum in each channel of multichannel reservoirs
  - Use the micropipette to mix 0.5 McFarland bacterial suspension 15 µL with cation-adjust MHB II 15 mL in a ratio of 1:1000

- Dispense the solution to 96 microwell plate
  - Dispense 25 µL of two-fold serial tigecycline solution across each well from row A to row G
  - Dispense 25 µL of two-fold serial gentamicin solution across each well from column 1 to column 11
  - Dispense 25 µL of 0.9% NSS solution into each row H wells and each column 12 wells

- Dispense 50 µL of the final inoculum to each of the 96 wells

- Incubate the 96-microwell plate for 18-20 hours at 35 $^{\circ}$C

- Read and interpret the results

- After these isolates were incubated, read the MICs value of each antibiotic at the first well that precipitate did not present
- Calculate FICI values

$$FICI = \frac{MIC\ A_{\text{combination A and B}}}{MIC\ A_{\text{alone}}} + \frac{MIC\ B_{\text{combination A and B}}}{MIC\ B_{\text{alone}}}$$

- Interpret the results following FICI values
  - FICI values ≤ 0.5 are classified as synergy
  - FICI values > 0.5 to 1 are classified as additive
  - FICI values > 1 to 4 are classified as indifferent
  - FICI values > 4 are classified as antagonism

	Row (Drug A)		1	2	3	4	5	6	7	8	9	10	11	12
Tigecycline MICs (µg/dl)	8	A												
	4	B												
	2	C												
	1	D												
	0.5	E												
	0.25	F												
	0.125	G												
	control	H												
			8	4	2	1	0.5	0.25	0.125	0.0625	0.03125	0.01562	0.0078	control
			Column (Drug B): Gentamicin MICs (µg/dl)											

Figure 8 Checkerboard synergy testing for gentamicin-tigecycline combination

3) Colistin-based regimens

For colistin-based regimens consisted of colistin combined with fosfomycin, gentamicin, amikacin, and fosfomycin

3.1) Colistin-fosfomycin

Nine CRE clinical isolates (n = 9) which had colistin MICs of ≤ 16 μg/mL and fosfomycin MICs of 12-256 μg/mL were included in synergistic testing.

- Desired range of two-fold serial dilutions (1 to 64 μg/mL for colistin and 1 to 1,024 μg/mL for fosfomycin)
- Prepare a serial of two-fold dilution of colistin
  - Use the micropipette to dispense 242 μL of the colistin stock solution to the first and second channels of multichannel reservoirs
  - Use the micropipette to dispense 6 mL of the sterile water to all the channels across a row of multichannel reservoirs
  - Gently mix the solution in the first and second channels
  - Use the micropipette to continually carry out 6 mL of the colistin solution from the second channel to the next channels
  - Discard 6 mL at the last channels of multichannel reservoirs
- Prepare a serial of two-fold dilution of fosfomycin
  - Use the micropipette to dispense 1.31 mL of the fosfomycin stock solution to the first and second channels of multichannel reservoirs

- Use the micropipette to dispense 16 mL of the sterile water to all the channels across a row of multichannel reservoirs
- Gently mix the solution in the first and second channels
- Use the micropipette to continually carry out 10 mL of the fosfomycin solution from the second channel to the next channels
- Discard 10 mL at the last channels of multichannel reservoirs

- Prepare the final inoculum in each channel of multichannel reservoirs
  - Use the micropipette to mix 0.5 McFarland bacterial suspension 21 µL with cation-adjust MHB II 21 mL in a ratio of 1:1000
- Dispense the solution to 96 microwell plate
  - Dispense 25 µL of two-fold serial colistin solution across each well from row A to row G
  - Dispense 25 µL of two-fold serial fosfomycin solution across each well from column 1 to column 11
  - Dispense 25 µL of 0.9% NSS solution into each row H wells and each column 12 wells
  - Dispense 50 µL of the final inoculum to each of the 96 wells
- Incubate the 96-microwell plate for 18-20 hours at 35 ^{O}C
- Read and interpret the results

- After these isolates were incubated, read the MICs value of each antibiotic at the first well that precipitate did not present
- Calculate FICI values

$$FICI = \frac{MIC\ A_{\text{combination A and B}}}{MIC\ A_{\text{alone}}} + \frac{MIC\ B_{\text{combination A and B}}}{MIC\ B_{\text{alone}}}$$

- Interpret the results following FICI values
  - FICI values ≤ 0.5 are classified as synergy
  - FICI values > 0.5 to 1 are classified as additive
  - FICI values > 1 to 4 are classified as indifferent
  - FICI values > 4 are classified as antagonism

	Row (Drug A)		1	2	3	4	5	6	7	8	9	10	11	12
Colistin MICs (µg/mL)	64	A												
	32	B												
	16	C												
	8	D												
	4	E												
	2	F												
	1	G												
	control	H												
			1,024	512	256	128	64	32	16	8	4	2	1	control
			Column (Drug B): Fosfomycin MICs (µg/mL)											

Figure 9 Checkerboard synergy testing for colistin-fosfomycin combination

3.2) Colistin-amikacin

Twenty-four CRE clinical isolates (n = 24) which had colistin MICs of ≤ 16 µg/mL were included in synergistic testing.

- Desired range of two-fold serial dilutions (0.0625 to 64 µg/mL for colistin and 0.5 to 32 µg/mL for amikacin)

- Prepare a serial of two-fold dilution of colistin
    - Use the micropipette to dispense 291 µL of the colistin stock solution to the first and second channels of multichannel reservoirs
    - Use the micropipette to dispense 6 mL of the sterile water to all the channels across a row of multichannel reservoirs
    - Gently mix the solution in the first and second channels
    - Use the micropipette to continually carry out 6 mL of the colistin solution from the second channel to the next channels
    - Discard 6 mL at the last channels of multichannel reservoirs
- Prepare a serial of two-fold dilution of amikacin
    - Use the micropipette to dispense 82 µL of the amikacin stock solution to the first and second channels of multichannel reservoirs
    - Use the micropipette to dispense 8 mL of the sterile water to all the channels across a row of multichannel reservoirs
    - Gently mix the solution in the first and second channels
    - Use the micropipette to continually carry out 8 mL of the amikacin solution from the second channel to the next channels
    - Discard 8 mL at the last channels of multichannel reservoirs
- Prepare the final inoculum in each channel of

multichannel reservoirs

- Use the micropipette to mix 0.5 McFarland bacterial suspension 15 µL with cation-adjust MHB II 15 mL in a ratio of 1:1000

- Dispense the solution to 96 microwell plate

- Dispense 25 µL of two-fold serial amikacin solution across each well from row A to row G
- Dispense 25 µL of two-fold serial colistin solution across each well from column 1 to column 11
- Dispense 25 µL of 0.9% NSS solution into each row H wells and each column 12 wells
- Dispense 50 µL of the final inoculum to each of the 96 wells

- Incubate the 96-microwell plate for 18-20 hours at 35 $^{\circ}$C

- Read and interpret the results

- After these isolates were incubated, read the MICs value of each antibiotic at the first well that precipitate did not present
- Calculate FICI values

$$FICI = \frac{MIC\ A_{\text{combination A and B}}}{MIC\ A_{\text{alone}}} + \frac{MIC\ B_{\text{combination A and B}}}{MIC\ B_{\text{alone}}}$$

- Interpret the results following FICI values
  - FICI values ≤ 0.5 are classified as synergy
  - FICI values > 0.5 to 1 are classified as additive
  - FICI values > 1 to 4 are classified as indifferent

- FICI values > 4 are classified as antagonism

	Row (Drug A)		1	2	3	4	5	6	7	8	9	10	11	12
Amikacin MICs (µg/mL)	32	A												
	16	B												
	8	C												
	4	D												
	2	E												
	1	F												
	0.5	G												
	control	H												
			64	32	16	8	4	2	1	0.5	0.25	0.125	0.0625	control
			Column (Drug B): Colistin MICs (µg/mL)											

Figure 10 Checkerboard synergy testing for colistin-amikacin combination

3.3) Colistin-gentamicin

Twenty-four CRE clinical isolates (n = 24) which had colistin MICs of ≤ 16 µg/mL were included in synergistic testing.

- Desired range of two-fold serial dilutions (0.0625 to 64 µg/mL for colistin and 0.25 to 16 µg/mL for gentamicin)

- Prepare a serial of two-fold dilution of colistin

- Use the micropipette to dispense 291 µL of the colistin stock solution to the first and second channels of multichannel reservoirs
- Use the micropipette to dispense 6 mL of the sterile water to all the channels across a row of multichannel reservoirs
- Gently mix the solution in the first and second channels
- Use the micropipette to continually carry out 6 mL of the colistin solution from the second channel to the next channels

- Discard 6 mL at the last channels of multichannel reservoirs

- Prepare a serial of two-fold dilution of gentamicin
  - Use the micropipette to dispense 10.24 µL of the gentamicin stock solution to the first and second channels of multichannel reservoirs
  - Use the micropipette to dispense 8 mL of the sterile water to all the channels across a row of multichannel reservoirs
  - Gently mix the solution in the first and second channels
  - Use the micropipette to continually carry out 8 mL of the gentamicin solution from the second channel to the next channels
  - Discard 8 mL at the last channels of multichannel reservoirs

- Prepare the final inoculum in each channel of multichannel reservoirs
  - Use the micropipette to mix 0.5 McFarland bacterial suspension 15 µL with cation-adjust MHB II 15 mL in a ratio of 1:1000

- Dispense the solution to 96 microwell plate
  - Dispense 25 µL of two-fold serial gentamicin solution across each well from row A to row G
  - Dispense 25 µL of two-fold serial colistin solution across each well from column 1 to column 11
  - Dispense 25 µL of 0.9% NSS solution into each row H wells and each column 12 wells

- Dispense 50 µL of the final inoculum to each of the 96 wells

- Incubate the 96-microwell plate for 18-20 hours at 35 ^{O}C

- Read and interpret the results

  - After these isolates were incubated, read the MICs value of each antibiotic at the first well that precipitate did not present
  - Calculate FICI values

$$FICI = \frac{MIC\ A_{\text{combination A and B}}}{MIC\ A_{\text{alone}}} + \frac{MIC\ B_{\text{combination A and B}}}{MIC\ B_{\text{alone}}}$$

  - Interpret the results following FICI values
    - FICI values ≤ 0.5 are classified as synergy
    - FICI values > 0.5 to 1 are classified as additive
    - FICI values > 1 to 4 are classified as indifferent
    - FICI values > 4 are classified as antagonism

	Row (Drug A)		1	2	3	4	5	6	7	8	9	10	11	12
Gentamicin MICs (µg/mL)	16	A												
	8	B												
	4	C												
	2	D												
	1	E												
	0.5	F												
	0.25	G												
	control	H												
			64	32	16	8	4	2	1	0.5	0.25	0.125	0.0625	control
			Column (Drug B): Colistin MICs (µg/mL)											

Figure 11 Checkerboard synergy testing for colistin-gentamicin combination

3.4) Colistin-tigecycline

Twenty-four CRE clinical isolates (n = 24) which had colistin MICs of ≤ 16 µg/mL were included in synergistic testing.

- Desired range of two-fold serial dilutions (0.0625 to 64 µg/mL for colistin and 0.03125 to 2 µg/mL for tigecycline)
- Prepare a serial of two-fold dilution of colistin
    - Use the micropipette to dispense 291 µL of the colistin stock solution to the first and second channels of multichannel reservoirs
    - Use the micropipette to dispense 6 mL of the sterile water to all the channels across a row of multichannel reservoirs
    - Gently mix the solution in the first and second channels
    - Use the micropipette to continually carry out 6 mL of the colistin solution from the second channel to the next channels
    - Discard 6 mL at the last channels of multichannel reservoirs
- Prepare a serial of two-fold dilution of tigecycline
    - Use the micropipette to dispense 5.12 µL of the tigecycline stock solution to the first and second channels of multichannel reservoirs
    - Use the micropipette to dispense 8 mL of the sterile water to all the channels across a row of multichannel reservoirs
    - Gently mix the solution in the first and second channels

- Use the micropipette to continually carry out 8 mL of the tigecycline solution from the second channel to the next channels
- Discard 8 mL at the last channels of multichannel reservoirs

- Prepare the final inoculum in each channel of multichannel reservoirs
  - Use the micropipette to mix 0.5 McFarland bacterial suspension 15 µL with cation-adjust MHB II 15 mL in a ratio of 1:1000
- Dispense the solution to 96 microwell plate
  - Dispense 25 µL of two-fold serial tigecycline solution across each well from row A to row G
  - Dispense 25 µL of two-fold serial colistin solution across each well from column 1 to column 11
  - Dispense 25 µL of 0.9% NSS solution into each row H wells and each column 12 wells
  - Dispense 50 µL of the final inoculum to each of the 96 wells
- Incubate the 96-microwell plate for 18-20 hours at 35 $^{\circ}$C
- Read and interpret the results
  - After these isolates were incubated, read the MICs value of each antibiotic at the first well that precipitate did not present
  - Calculate FICI values

$$FICI = \frac{MIC\ A_{\text{combination A and B}}}{MIC\ A_{\text{alone}}} + \frac{MIC\ B_{\text{combination A and B}}}{MIC\ B_{\text{alone}}}$$

- Interpret the results following FICI values
  - FICI values ≤ 0.5 are classified as synergy
  - FICI values > 0.5 to 1 are classified as additive
  - FICI values > 1 to 4 are classified as indifferent
  - FICI values > 4 are classified as antagonism

	Row (Drug A)		1	2	3	4	5	6	7	8	9	10	11	12
Tigecycline MICs (µg/mL)	2	A												
	1	B												
	0.5	C												
	0.25	D												
	0.125	E												
	0.0625	F												
	0.03125	G												
	control	H												
			64	32	16	8	4	2	1	0.5	0.25	0.125	0.0625	control
			Column (Drug B): Colistin MICs (µg/mL)											

Figure 12 Checkerboard synergy testing for colistin-tigecycline combination

4) Fosfomycin-based regimens

For fosfomycin-based regimens consisted of fosfomycin combined with tigecycline. Twenty-one CRE clinical isolates, including eighteen CRE clinical isolates (n = 18) for fosfomycin MICs of 12-256 µg/mL and three CRE clinical isolates (n = 3) for fosfomycin MICs of >1,024 µg/mL, were included to synergistic testing.

- Desired range of two-fold serial dilutions (0.125 to 8 µg/mL for tigecycline and 1 to 1,024 µg/mL for fosfomycin)
- Prepare a serial of two-fold dilution of tigecycline
  - Use the micropipette to dispense 17.92 µL of the tigecycline stock solution to the first and second channels of multichannel reservoirs

- Use the micropipette to dispense 7 mL of the sterile water to all the channels across a row of multichannel reservoirs
- Gently mix the solution in the first and second channels
- Use the micropipette to continually carry out 7 mL of the tigecycline solution from the second channel to the next channels
- Discard 7 mL at the last channels of multichannel reservoirs

- Prepare a serial of two-fold dilution of fosfomycin
  - Use the micropipette to dispense 1.31 mL of the fosfomycin stock solution to the first and second channels of multichannel reservoirs
  - Use the micropipette to dispense 16 mL of the sterile water to all the channels across a row of multichannel reservoirs
  - Gently mix the solution in the first and second channels
  - Use the micropipette to continually carry out 16 mL of the fosfomycin solution from the second channel to the next channels
  - Discard 16 mL at the last channels of multichannel reservoirs

- Prepare the final inoculum in each channel of multichannel reservoirs
  - Use the micropipette to mix 0.5 McFarland bacterial suspension 21 µL with cation-adjust MHB II 21 mL in a ratio of 1:1000

- Dispense the solution to 96 microwell plate
    - Dispense 25 µL of two-fold serial tigecycline solution across each well from row A to row G
    - Dispense 25 µL of two-fold serial fosfomycin solution across each well from column 1 to column 11
    - Dispense 25 µL of 0.9% NSS solution into each row H wells and each column 12 wells
    - Dispense 50 µL of the final inoculum to each of the 96 wells
- Incubate the 96-microwell plate for 18-20 hours at 35 $^{\circ}$C
- Read and interpret the results
    - After these isolates were incubated, read the MICs value of each antibiotic at the first well that precipitate did not present
    - Calculate FICI values

$$FICI = \frac{MIC\ A_{\text{combination A and B}}}{MIC\ A_{\text{alone}}} + \frac{MIC\ B_{\text{combination A and B}}}{MIC\ B_{\text{alone}}}$$

    - Interpret the results following FICI values
        - FICI values ≤ 0.5 are classified as synergy
        - FICI values > 0.5 to 1 are classified as additive
        - FICI values > 1 to 4 are classified as indifferent
        - FICI values > 4 are classified as antagonism

	Row (Drug A)		1	2	3	4	5	6	7	8	9	10	11	12
Tigecycline MICs (µg/mL)	8	A												
	4	B												
	2	C												
	1	D												
	0.5	E												
	0.25	F												
	0.125	G												
	control	H												
			1,024	512	256	128	64	32	16	8	4	2	1	control
			Column (Drug B): Fosfomycin MICs (µg/mL)											

Figure 13 Checkerboard synergy testing for tigecycline-fosfomycin combination

5) Triple-based regimens

For triple-based regimens consisted of two antibiotic regimens, including fosfomycin-tigecycline plus amikacin and fosfomycin-tigecycline plus gentamicin. Six CRE clinical isolates (n = 6) being high antibiotic resistance were included in synergistic testing.

5.1) Fosfomycin-tigecycline-amikacin

Three isolates (n = 3) included colistin MICs > 2 µg/mL, fosfomycin MICs > 32 µg/mL, tigecycline MICs > 0.5 µg/mL, amikacin MICs > 16 µg/mL, and gentamicin MICs ≤ 4 µg/mL.

- Desired range of two-fold serial dilutions (0.125 to 8 µg/mL for tigecycline, 1 to 1,024 µg/mL for fosfomycin and amikacin 4 µg/mL)
- Prepare a serial of two-fold dilution of tigecycline
    - Use the micropipette to dispense 11.52 µL of the tigecycline stock solution to the first and second channels of multichannel reservoirs

- Use the micropipette to dispense 4.5 mL of the sterile water to all the channels across a row of multichannel reservoirs
- Gently mix the solution in the first and second channels
- Use the micropipette to continually carry out 4.5 mL of the tigecycline solution from the second channel to the next channels
- Discard 4.5 mL at the last channels of multichannel reservoirs

- Prepare a serial of two-fold dilution of fosfomycin
  - Use the micropipette to dispense 286.72 µL of the fosfomycin stock solution to the first and second channels of multichannel reservoirs
  - Use the micropipette to dispense 3.5 mL of the sterile water to all the channels across a row of multichannel reservoirs
  - Gently mix the solution in the first and second channels
  - Use the micropipette to continually carry out 3.5 mL of the fosfomycin solution from the second channel to the next channels
  - Discard 3.5 mL at the last channels of multichannel reservoirs
- Prepare amikacin solution
  - Use the micropipette to dispense 23.04 µL of the amikacin stock solution to multichannel reservoirs

- Use the micropipette to dispense 18 mL of the sterile water to all the channels across a row of multichannel reservoirs
- Gently mix the solution in the first and second channels

- Prepare the final inoculum in each channel of multichannel reservoirs
  - Use the micropipette to mix 0.5 McFarland bacterial suspension 5 µL with cation-adjust MHB II 5 mL in a ratio of 1:1000
  - Add G-6-P 0.4 µL to each of the multichannel reservoirs
- Dispense the solution to 96 microwell plate
  - Dispense 25 µL of two-fold serial tigecycline solution across each well from row A to row H
  - Dispense 25 µL of two-fold serial fosfomycin solution across each well from column 1 to column 12
  - Dispense 25 µL of amikacin solution across each of well, except column 12 and row H
  - Dispense 50 µL of 0.9% NSS solution into each row H wells and each column 12 wells
  - Dispense 50 µL of the final inoculum to each of the 96 wells
- Incubate the 96-microwell plate for 18-20 hours at 35 $^{\circ}$C
- Read and interpret the results

- After these isolates were incubated, read the MICs value of each antibiotic at the first well that precipitate did not present
- Calculate FICI values

$$FICI = \frac{MIC\ A_{\text{combination A and B}}}{MIC\ A_{\text{alone}}} + \frac{MIC\ B_{\text{combination A and B}}}{MIC\ B_{\text{alone}}}$$

- Interpret the results following FICI values
  - FICI values ≤ 0.5 are classified as synergy
  - FICI values > 0.5 to 1 are classified as additive
  - FICI values > 1 to 4 are classified as indifferent
  - FICI values > 4 are classified as antagonism

	Row (Drug A)		1	2	3	4	5	6	7	8	9	10	11	12
Tigecycline MICs (µg/mL)	8	A												
	4	B												
	2	C												
	1	D												
	0.5	E												
	0.25	F												
	0.125	G												
	control	H												
Column 1-11 and row A-H: add **amikacin 4 µg/mL**			1,024	512	256	128	64	32	16	8	4	2	1	control
			Column (Drug B): Fosfomycin MICs (µg/mL)											

Figure 14 Checkerboard synergy testing
for tigecycline-fosfomycin-amikacin combination

5.2) Fosfomycin-tigecycline-gentamicin

Three isolates (n = 3) included colistin MICs > 2 µg/mL, fosfomycin MICs > 32 µg/mL, tigecycline MICs > 0.5 µg/mL, amikacin MICs ≤ 16 µg/mL and gentamicin MICs > 4 µg/mL.

- Prepare a serial of two-fold dilution of tigecycline
    - Use the micropipette to dispense 11.52 µL of the tigecycline stock solution to the first and second channels of multichannel reservoirs
    - Use the micropipette to dispense 4.5 mL of the sterile water to all the channels across a row of multichannel reservoirs
    - Gently mix the solution in the first and second channels
    - Use the micropipette to continually carry out 4.5 mL of the tigecycline solution from the second channel to the next channels
    - Discard 4.5 mL at the last channels of multichannel reservoirs
- Prepare a serial of two-fold dilution of fosfomycin
    - Use the micropipette to dispense 286.72 µL of the fosfomycin stock solution to the first and second channels of multichannel reservoirs
    - Use the micropipette to dispense 3.5 mL of the sterile water to all the channels across a row of multichannel reservoirs
    - Gently mix the solution in the first and second channels

- Use the micropipette to continually carry out 3.5 mL of the fosfomycin solution from the second channel to the next channels
- Discard 3.5 mL at the last channels of multichannel reservoirs

- Prepare gentamicin solution
  - Use the micropipette to dispense 6.08 µL of the gentamicin stock solution (12,500 µg/mL) to multichannel reservoirs
  - Use the micropipette to dispense 19 mL of the sterile water to all the channels across a row of multichannel reservoirs
  - Gently mix the solution in the first and second channels
- Prepare the final inoculum in each channel of multichannel reservoirs
  - Use the micropipette to mix 0.5 McFarland bacterial suspension 5 µL with cation-adjust MHB II 5 mL in a ratio of 1:1000
  - Add G-6-P 0.4 µL to each of the multichannel reservoirs
- Dispense the solution to 96 microwell plate
  - Dispense 25 µL of two-fold serial tigecycline solution across each well from row A to row H
  - Dispense 25 µL of two-fold serial fosfomycin solution across each well from column 1 to column 12
  - Dispense 25 µL of amikacin solution across each of well, except column 12 and row H

- Dispense 25 µL of 0.9% NSS solution into each row H wells and each column 12 wells
- Dispense 25 µL of the final inoculum to each of the 96 wells

- Incubate the 96-microwell plate for 18-20 hours at 35 $^{\circ}C$
- Read and interpret the results
  - After these isolates were incubated, read the MICs value of each antibiotic at the first well that precipitate did not present
  - Calculate FICI values

$$FICI = \frac{MIC\ A_{\text{combination A and B}}}{MIC\ A_{\text{alone}}} + \frac{MIC\ B_{\text{combination A and B}}}{MIC\ B_{\text{alone}}}$$

  - Interpret the results following FICI values
    - FICI values ≤ 0.5 are classified as synergy
    - FICI values > 0.5 to 1 are classified as additive
    - FICI values > 1 to 4 are classified as indifferent
    - FICI values > 4 are classified as antagonism

	Row (Drug A)		1	2	3	4	5	6	7	8	9	10	11	12
Tigecycline MICs (µg/mL)	8	A												
	4	B												
	2	C												
	1	D												
	0.5	E												
	0.25	F												
	0.125	G												
	control	H												
Column 1-11 and row A-H: add **gentamicin 1 µg/mL**			1,024	512	256	128	64	32	16	8	4	2	1	control
			Column (Drug B): Fosfomycin MICs (µg/mL)											

Figure 15 Checkerboard synergy testing
for tigecycline-fosfomycin-gentamicin combination

3.4.1.3 Genotyping testing

3.4.1.3.1 DNA Extraction

Before doing any PCR test, the CRE clinical isolates from Pramongkutklao hospital are included to extract DNA (GeneJET™ Genomic DNA Purification Kit, Thermo Fisher Scientific, Co., LTD). The procedure is following to the manufacturer's instruments.

1) Lysis the CRE clinical isolates

- Add 180 µL of digestion solution to Eppendorf 2 mL

- Using a sterile loop, harvest up to 1 full loop of the colony of CRE isolates on the surface of blood agar and suspend thoroughly by vortex

- Add 20 µL of proteinase K solution and mix thoroughly by vortex to obtain a uniform suspension.

- Incubate the samples at 56 °C with a water bath for less than 30 minutes (shake them occasionally) until the cells are completely lysed. The color of the samples would change from white to light white.

- Add 20 µL of RNase A Solution and mix thoroughly by vortex

- Incubate the samples for 10 min at room temperature

- Add 200 µL of lysis solution to the sample and mix thoroughly by vortex for about 15 s until a homogeneous mixture is obtained.

- Add 400 µL of 50% ethanol and mix by vortex

2) Purification of the lysate DNA

- Transfer the lysate samples to a GeneJET Genomic DNA Purification Column inserted in a collection tube.

- Centrifuge the column in a collection tube for 1 min at 6,000 × g, discard the flow-through solution and place the purification column back into the collection tube.

- Add 500 µl of wash buffer I (with ethanol added), centrifuge for 1 min at 8,000 × g, discard the flow-through, and place the purification column back into the collection tube.

- Add 500 µl of wash buffer II (with ethanol added), centrifuge for 3 min at 12,000 × g), discard the flow-through, and the collection tube

- Place the purification column back into the Eppendorf 2 mL

- Add 60 of elution buffer to the center of the GeneJET Genomic DNA Purification Column membrane to elute genomic DNA, incubate for 2-5 min at room temperature, centrifuge for 1 min at 8,000 × g, and first collect the purified DNA in Eppendorf

- Add 75 of elution buffer to the center of the GeneJET Genomic DNA Purification Column membrane to elute genomic DNA, incubate for 2-5 min at room temperature, centrifuge for 2 min at 12,000 × g, and second collect the purified DNA in Eppendorf

- Label each Eppendorf

- Discard the purification column.

- Store at -20 °C

3.4.1.3.2 Amplification

Polymerase chain reaction (PCR) is an amplification method to generate multiple copies of a DNA sequence. For this study, Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) and multiplex PCR are used to analyze bacterial diversity and detect *mcr-1* genes and carbapenemase genes.

1) Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR)

Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) is a method for analyzing bacterial diversity. The procedures consist of three steps.

1.1) Select primer [100]

Universal primers are used for ERIC-PCR, including

- Primer ERIC-F: 5'-ATGTAAGCTCCTGGGGATTCAC-3',
- Primer ERIC-R: 5'-AAGTAAGTGACTGGGGTGAGCG-3'

1.2) Prepare a master mix

Table 5 showed ERIC master mix for a PCR reaction (1X reaction = 1 sample). Calculate a total volume of ERIC master mix needed to multiple amounts of ERIC master mix per reaction by the number of DNA samples.

Table 5 ERIC master mix (1X reaction)

No	Reagent	Volume (µl)
1	MQ water	5.7
2	2X PCR master mix	7.5
3	20X ERIC-F (x)	0.4
4	20X ERIC-R (x)	0.4
5	DNA template	1
	Total volume	**15**

1.3) Incubate reactions in a thermal cycler [100]

Amplification was performed overnight using a thermal cycler as follows: one cycle of 2 min at 95°C (initial denaturation), followed by 35 cycles of 50 seconds at 95°C (denature), 30 seconds at 46°C (anneal), and 30 seconds at 49°C (elongation) and 3 min at 72°C (final elongation). The amplification ended with one cycle of 10 min at 72°C.

1.4) Analyze and Interpret results [100]

- The amplified products of ERIC-PCR and a standard 1 kb ladder maker as a reference are analyzed by 1% electrophoresis agarose gel in 0.5 × Tris/Borate/EDTA (TBE)

- Stained with ethidium bromide

- Visualize DNA by using UV transilluminator

- Read the results by identifying and grouping the positions of bands (amplicon sizes) between the PCR products and the PCR reference

- Interpret the results: if positions of band patterns are different in more than three bands, it indicates that different strains

- Cluster the CRE isolates following the results into the similarity or difference groups

2) Multiplex- polymerase chain reaction (Multiplex-PCR)

2.1) Select primers

Primer sequences used for multiplex PCR are selected from studies conducted by Poirel et al in 2011 [101]. Primers used to identify carbapenemase genes and *mcr-1* gene are shown in Table 6.

Table 6 Primers used to identify carbapenemase genes

Targeted Gene	Primer sequence (5' to 3' Direction)	Amplicon Size, bp
bla_{KPC}	F-5′-CGTCTAGTTCTGCTGTCTTG-3′ R- 5′-CTTGTCATCCTTGTTAGGCG-3′	798
bla_{NDM}	F- 5′-GGTTTGGCGATCTGGTTTTC-3′ R- 5′-CGGAATGGCTCATCACGATC-3′	621
bla_{IMP}	F- 5′-GGAATAGAGTGGCTTAAYTCTC-3′ R- 5′-GGTTTAAYAAAACAACCA C-3’	232
bla_{VIM}	F- 5′-GATGGTGTTTGGTCGCATA-3′ F- 5′-CGAATGCGCAGCACCAG-3’	390
bla_{OXA-48}	F- 5′-GCGTGGTTAAGGATGAACAC-3′ R- 5′-CATCAAGTTCAACCCAACCG-3	438
mcr-1	F- 5’-CGG TCA GTC CGT TTG TTC-3’ R- 5’-CTT GGT CGG TCT GTA GGG-3’	205

Bacterial strains for used as positive control [102]

- KPC-3 positive *K. pneumoniae* (NCTC 13438)
- NDM positive *K. pneumoniae* (NCTC 13443)
- IMP-type positive *E. coli* (NCTC 13476)
- VIM-10 positive *Pseudomonas aeruginosa* (NCTC 3437)
- OXA-48 positive *K. pneumoniae* (NCTC 13442)

Negative control: No template control (PCR master mix only)

2.2) Prepare master mix [103]

Table 7 and 8 showed multiplex-PCR master mix for a PCR reaction (1X reaction = 1 sample). Calculate a total volume of multiplex-PCR master mix needed to multiple amounts of multiplex-PCR master mix per reaction by the number of DNA samples.

Table 7 Multiplex-PCR master mix for *mcr-1* gene (1X reaction)

No	**Reagent**	**Volume (µl)**
1	MQ water	5.7
2	2X PCR	7.5
3	20X *mcr-1*-F (x)	0.4
4	20X *mcr-1*-R (x)	0.4
5	DNA template	1
	Total volume	**15**

Table 8 Multiplex-PCR master mix for carbapenemase gene (1X reaction)

No	**Reagent**	**Volume (µl)**
1	MQ water	3.5
2	PCR master mix	7.5
3	20 µM-IMP (2x)	0.4
4	20 µM-VIM (2x)	0.4
5	20 µM-OXA-48 (2x)	0.4
6	20 µM-NDM (2x)	0.4
7	20 µM-KPC (2x)	0.4
8	DNA template	2
	Total volume	**15**

2.3) Incubate reactions in a thermal cycler [103]

- The *mcr-1* PCR conditions for amplification were as follows: one cycle of 3 min at 94°C (initial denaturation), followed by 35 cycles of 30 seconds at 94°C (denature), 35 seconds at 53°C (anneal), and 45 seconds at 72°C (elongation); and a final extension step for 5 min at 72 °C (final elongation).

- The multiplex PCR conditions for amplification were as follows: a cycle of preincubation for 3 min at 94 °C (initial denaturation), 35 cycles of 30 seconds at 94 °C (denature), 35 seconds at 57 °C (anneal), and 45 seconds at 72 °C (extension); and a final extension step for 5 min at 72 °C (final extension) [104].

2.4) Analyze and Interpret results [100]

- The amplified products of multiplex-PCR (DNA fragments) and control are analyzed by 1 % electrophoresis agarose gel in 0.5 × Tris/Borate/EDTA (TBE)

- Stained with ethidium bromide

- Visualize DNA by using a UV transilluminator equipped with a camera

- Read the results by identifying the positions of bands (amplicon sizes) between the PCR products and the PCR reference

- Interpret the results: if positions of band patterns are similar, it indicates that similar carbapenemase genes or *mcr-1* genes

3.4.2 PK/PD study

All pharmacokinetic parameters of critically ill patients were obtained from previous studies. The relationship between drug concentration levels and time was generated using a one-compartment model for meropenem, gentamicin, colistin, and ceftazidime-avibactam, or two-compartment model for imipenem, amikacin, tigecycline, CMS and fosfomycin with the linear pharmacokinetic behavior. For meropenem, amikacin, gentamicin, colistin, and fosfomycin, these antibiotics were simulated the plasma concentration-time using the equations containing CrCL as a covariate factor.

Two steps are developed to design the optimal combination regimens in the PK/PD study, including the selection of pharmacokinetic or pharmacodynamic parameters and Monte Carlo simulation.

3.4.2.1 Pharmacokinetic/pharmacodynamic (PK/PD) parameters

Pharmacokinetic and pharmacodynamic parameters should firstly be selected in order to design optimal antibiotic dosing regimens. This section describes the

population pharmacokinetic parameters, antibiotic PK/PD parameters, and antibiotic dosage regimens for simulation, based on antibiotic options for the CRE treatment.

1) Meropenem

1.1) Population pharmacokinetic parameters

The population pharmacokinetic model for meropenem was the one-compartment model. Pharmacokinetic data from Jaruratanasirikul S, et al in 2015 was retrieved [64]. The patients (n =9; 171 samples) are Thai critically ill who had APACHE II score 16-33, SOFA score 5-14, and CrCL > 50 mL/min. A final model was as follows:

$$- CL = TVCL \times e^{\eta_1}; TVCL = (\theta_1 + \theta_2) \times MDRD\ Cl_{cr}$$

$$- V = TVV \times e^{\eta_2}$$

A set of parameters was generated is shown in Table 9.

Table 9 A set of parameters of meropenem

PK parameters	Estimate	% RSE
CL (L/h)	7.82	22.1
V_d (L)	23.7	12.6

Abbreviations: V_d, volume of distribution; CL, total body clearance; RSE = Relative standard error; IIV = Interindividual variability

1.2) PK/PD index and target

PK/PD index and target for meropenem are 100% *f*T > MIC [62].

1.3) Antibiotic dosage regimens for simulation

Twenty-eight meropenem dosage regimens (n = 28) for simulation are loading dose 0-2 g, followed by 1-2 g infusion 0.5-3 hours every 6-8 hours. Both typical parameters and covariate parameters with final models were simulated. Furthermore, different PK profiles were simulated with patients having CrCL of < 10

mL/min, 10–25 mL/min, 26–50 mL/min, and 51–90 mL/min, respectively. The dosing regimens were simulated over a 24 h period.

2) Imipenem

2.1) Population pharmacokinetic parameters

The population pharmacokinetic model for imipenem was the two-compartment model. Pharmacokinetic data from Jaruratanasirikul S, et al in 2013 was retrieved [63]. The patients (n = 9) are Thai critically ill who were intubated, received mechanical ventilation, and CrCL > 60 mL/min. A set of parameters was generated shown in Table 10.

Table 10 A set of parameters of imipenem

PK parameters	Mean	S.D.
CL (L/h)	20.86	6.130
V_d (L)	23.59	5.070
V_d (L/kg)	0.368	0.123
K_{12} (/h)	2.819	1.448
K_{21} (/h)	5.598	5.283
K_e (/h)	0.901	0.283

Abbreviations: CL, total body clearance; V_d, volume of distribution;

k_{12} = intercompartmental transfer rate constant from central to peripheral compartment;

k_{21} = intercompartmental transfer rate constant from peripheral to central compartment;

k_e = elimination rate constant from central compartment

2.2) PK/PD index and target

PK/PD index and target for imipenem are 100% $fT > MIC$ [62].

2.3) Antibiotic dosage regimens for simulation

Twelve imipenem dosage regimens (n = 12) for simulation are loading dose 0-1 g, followed by 0.5-1 g infusion 2-3 hours every 6 hours. Only typical parameters are used to simulate. The dosing regimens were simulated over a 24 h period.

3) Amikacin

3.1) Population pharmacokinetic parameters

The population pharmacokinetic model for amikacin was the two-compartment model. Pharmacokinetic data from Delattre IK, et al in 2010 was retrieved [105]. Eighty-eight adult patients with sepsis or septic shock were enrolled in the study. They had APACHE II score of 20 (6-45), SOFA score of 8 (1-19), and CrCL 55.5 (12.3-408.3) mL/min. A final covariate model was as follows: $CL_{pop} = CL + CrCL^{\theta_{CL\text{-}CrCL}}$

A set of parameters was generated are shown in Table 11.

Table 11 A set of parameters of amikacin

PK parameters	Estimates	% RSE
CL (L/h)	0.77	28.4
V_c (L)	19.2	5.31
Q (L/h)	4.38	18.3
V_p (L)	9.38	7.15
$\theta_{CL\text{-}CrCL}$ (mL/min)	1.42	18.4

Abbreviations: CL, Clearance; V_c, Central volume of distribution; Q, intercompartmental clearance; V_p, Peripheral volume of distribution; RSE, Relative standard error; $\theta_{CL\text{-}CrCL}$, fractional change on CL resulting from CrCL; CrCL, creatinine clearance (estimated by the Cockcroft-Gault); Covariate model: $CL_{pop} = CL + CrCL^{\theta_{CL\text{-}CrCL}}$

3.2) PK/PD index and target

The effective PK/PD targets and indices of aminoglycosides were C_{max}/MIC > 8, indicative of good clinical outcomes [106]. The safe PK/PD targets of aminoglycosides were $AUC_{0\text{-}24}$ (mg × h/L) > 700 which occurred nephrotoxicity rate of more than 10% [107].

3.3) Antibiotic dosage regimens for simulation

Twenty-nine dosage regimens (n = 29) for simulation are loading dose 25-30 mg/kg, followed by 15-20 mg/kg q 24 hours. Both typical parameters and covariate parameters with final models were simulated. Furthermore, different PK profiles were simulated with patients having CrCL of < 10 mL/min, 10–25 mL/min, 26–50 mL/min, 51–90 mL/min, and 91-130 mL/min, respectively. The dosing regimens were simulated over a 24 h period.

4) Gentamicin

4.1) Population pharmacokinetic parameters

The population pharmacokinetic model for gentamicin was the one-compartment model. Pharmacokinetic data from Rea RS, et al in 2008 were retrieved. The patients were ICU patients (n = 102) with GFR 48.1 ± 26.5 (mL/min) [108]. A final covariate model was as follows: $CL = \frac{3.14 \times \mathbf{CrCL}^{1.2}}{54.8^{1.2} + \mathbf{CrCL}^{1.2}}$, where CrCL, creatinine clearance (estimated by glomerular filtration rate (GFR):

$GFR\ (mL/min) = 186.3 \times Cr^{-1.154} \times Age^{-0.203} \times (1.212 \text{ if black}) \times (0.742 \text{ if female})$

where Cr is serum creatinine level (μg/mL)

A set of parameters was generated shown in Table 12.

Table 12 A set of parameters of gentamicin

PK parameters	Estimate	% CV
CL (L/h)	3.14	83.7
V(L)	53.0	64.4

Abbreviations: CL, Clearance; $V_{C,}$ Central volume of distribution; Q, intercompartmental clearance; $V_{p,}$ Peripheral volume of distribution; CV, Coefficient of variation;

Covariate model: $CL = \frac{3.14 \times \mathbf{CrCL}^{1.2}}{54.8^{1.2} + \mathbf{CrCL}^{1.2}}$;

CrCL, creatinine clearance (estimated by glomerular filtration rate (GFR):

$GFR\ (mL/min) = 186.3 \times Cr^{-1.154} \times Age^{-0.203} \times (1.212 \text{ if black}) \times (0.742 \text{ if female})$

where Cr is serum creatinine level (μg/mL)

4.2) PK/PD index and target

The effective PK/PD targets and indices of aminoglycosides were C_{max}/MIC > 8, indicative of good clinical outcomes [106]. The safe PK/PD targets of aminoglycosides were AUC_{0-24} (mg × h/L) > 700 which occurred nephrotoxicity rate of more than 10% [107].

4.3) Antibiotic dosage regimens for simulation

Thirty-one dosage regimens (n = 31) for simulation are loading dose 7-8 mg/kg, followed by 5-7 mg/kg q 24 hours. Both typical parameters and covariate parameters with final models were simulated. Furthermore, different PK profiles were simulated with patients having CrCL of < 10 mL/min, 10–25 mL/min, 26–50 mL/min, 51–90 mL/min, and 91-130 mL/min, respectively. The dosing regimens were simulated over a 24 h period.

5) Tigecycline

5.1) Population pharmacokinetic parameters

The population pharmacokinetic model for tigecycline was the two-compartment model. Pharmacokinetic data from Borsuk-De Moor A, et al in 2018 was retrieved. The patients are ICU patients with sepsis or septic shock in Poland (n = 37; SOFA = 13 (2-21)) [109]. A set of parameters was generated as shown in Table 13.

Table 13 A set of parameters of tigecycline

PK parameters	Estimate	% RSE
CL (L/h)	22.1	3.16
V_C (L)	162.0	5.3
Q (L/h)	69.4	32.6
V_p (L)	87.9	8.67

Abbreviations: CL, Clearance; V_C, Central volume of distribution;
Q, intercompartmental clearance; V_p, Peripheral volume of distribution;
RSE, Relative standard error

5.2) PK/PD index and target

PK/PD index and target for tigecycline are $fAUC_{0-24}$/MIC > 0.9 [73].

5.3) Antibiotic dosage regimens for simulation

Six dosage regimens (n = 6) for simulation are loading doses of 200-400 mg, followed by 100-200 mg every 12 hours or 100-200 mg every 24 hours. Only typical parameters were simulated. The tigecycline unbound fraction of 80% was used. The dosing regimens were simulated over a 24 h period.

6) Colistimethate sodium (CMS) and colistin

6.1) Population pharmacokinetic parameters

The population pharmacokinetic model for colistimethate sodium (CMS) was two-compartment; colistin was the one-compartment model [110]. Pharmacokinetic data from Nation R.L, et al. was retrieved [111]. The patients (n = 214) had APACHE II score (median 21; range 4-43), whereas Cl_{cr} 39.8 mL/min (range 0-314 mL/min). A set of parameters was generated are shown in Table 14.

6.2) PK/PD index and target

PK/PD index and target for colistin are *f*AUC/MIC ≥ 25 [112].

6.3) Antibiotic dosage regimens for simulation

Twenty-one colistin dosage regimens (n = 21) for simulation are loading dose 300 mg, followed by 100-150 mg every 12 hours or 150-180 mg every 24 hours. Only covariate parameters with final models were simulated. Furthermore, different PK profiles were simulated with patients having creatinine clearance of < 10 mL/min, 10–25 mL/min, 26–50 mL/min, 51–90 mL/min, and 91-130 mL/min, respectively. The dosing regimens were simulated over a 24 h period.

Table 14 A set of parameters of colistimethate sodium (CMS) and colistin

Category	Parameter	Estimate	%SE	%IIV
CMS	CLD_1 (L/h)	12.9	-	40.4
	V_1 (L)	16.1	-	70.9
	V_2 (L)	9.57	10.5	80.1
	CLR_{CMS} (L/h/CrCL)	0.0340	6.85	75.2
	$CLNR_{CMS}$ (L/h)	2.52	3.71	39.8
Colistin	V_3/fm (L)	57.2	5.13	43.5
	CLT_C/fm (L/h)	3.59	-	37.9
	CLR_C/fm (L/h/CrCL)	0.00834	27.7	-
	$CLNR_C$/fm (L/h)	3.11	4.38	-

Abbreviations: CLD_1 = Distributional clearance between the central and peripheral compartments for CMS; V1 = Central volume for CMS; V2 = Peripheral volume for CMS; CLR_{CMS} = Renal clearance of CMS, $CLNR_{CMS}$ = Non-renal clearance of CMS; V_3 = Volume of distribution of formed colistin; CLT_c = Total clearance of colistin; CLR_C = Renal clearance of colistin; $CLNR_C$ = Non-renal clearance of colistin; %SE = The standard error of the estimates; %IIV = The inter-individual variability in the population; CrCL = creatinine clearance

7) Fosfomycin

7.1) Population pharmacokinetic parameters

The population pharmacokinetic model for fosfomycin was the two-compartment model. Pharmacokinetic data from Parker S. L, et al in 2015 was retrieved [113]. The patients were ICU/critically ill patients (n = 10; APACHE II score = 11.5 (8.8 - 16.5); SOFA score on ICU admission = 7 (6 to 10) in Greece. A final model is represented as follows:

$$TV_{CL} = \theta_{1\text{-}6} \times CL_{CR}/90$$

where TV_{CL} = typical value of CL

CL_{CR} = creatinine clearance

$\theta_{1\text{-}6}$ = typical value of fosfomycin CL in the population, with each sampling day defined as an individual occasion (θ), from days 1 (θ_1), 2 (θ_2), 3 (θ_3), 4 (θ_4), 5 (θ_5), 6 (θ_6), 7 (θ_7)

A set of parameters was generated are showed in Table 15 [113].

Table 15 A set of parameters of fosfomycin

PK parameters	Estimate	% CV
CL (L/h)	2.06	91.9
V_C (L)	26.5	39.0
Q (L/h)	19.8	-
V_p (L)	22.3	-

Abbreviations: CL, Clearance; V_C, Central volume of distribution; Q, intercompartmental clearance; V_p, Peripheral volume of distribution; %CV, coefficient of variation

7.2) PK/PD index and target

PK/PD index and target for fosfomycin are *f*AUC_{0-24}h/MIC = 21.5 [114].

7.3) Antibiotic dosage regimens for simulation

Twenty fosfomycin dosage regimens (n = 20) for simulation are loading doses of 4-8 g, followed by 2-6 g every infusion 1-2 hours every 4-6 hours. Both typical parameters and covariate parameters with final models were simulated. Furthermore, different PK profiles were simulated with patients having CrCL < 10 mL/min, 10–25 mL/min, 26–50 mL/min, and 51–90 mL/min, respectively. The dosing regimens were simulated over a 24 h period.

8) Ceftazidime-avibactam

8.1) Population pharmacokinetic parameters

The population pharmacokinetic model for ceftazidime-avibactam was the one-compartment model. Pharmacokinetic data from Stein GE, et al in 2019 was retrieved [84]. The patients were ICU and critically ill patients (n = 10; APACHE II score = 21 (11-33)) in Michigan, USA. A set of parameters of ceftazidime-avibactam were presented in Table 16.

Table 16 A set of parameters of ceftazidime-avibactam

Antibiotics	PK parameters	Mean	SD
Ceftazidime	CL (L/h)	6.14	3.80
	V (L)	34.78	10.49
Avibactam	CL (L/h)	11.09	6.78
	V (L)	50.81	14.32

Abbreviations: CL, Clearance; V, Volume of distribution; SD, standard deviation

8.2) PK/PD index and target

PK/PD index and target for ceftazidime-avibactam are 100% *f*T>MIC for ceftazidime and 100%*f*T > 1 μg/mL for avibactam [84].

8.3) Antibiotic dosage regimens for simulation

Eight ceftazidime-avibactam dosage regimens (n = 8) for simulation are 2.5 g every 6 or 8 hours. Only typical parameters were simulated. The dosing regimens were simulated over a 24 h period.

3.4.2.2 Monte Carlo simulation

3.4.2.2.1 Calculate antibiotic concentrations in plasma [115]

The values from population pharmacokinetic and antibiotic parameters are substituted into mathematic equations to calculate antibiotic concentrations in plasma at any time point from the start of infusion to the achievement in steady-state. The equations are divided into two groups, based on the number of compartment models.

1.1) IV infusion for one-compartment model

IV infusion for the one-compartment model is used to calculate antibiotic concentrations in plasma of meropenem, amikacin, gentamicin, and colistin.

Formula:

$$C(t) = \frac{D}{T_{inf}} \times \frac{1}{kV_d} \times \left(1-e^{-k\,(t-t_D)}\right) \quad ;\text{if } t-t_D \le T_{inf}$$

$$C(t) = \frac{D}{T_{inf}} \times \frac{1}{kV_d} \times \left(1-e^{-kT_{inf}}\right) \times e^{-k\,(t-t_D-T_{inf})} \quad ;\text{if } t-t_D > T_{inf}$$

where C (t) = plasma concentration during IV infusion at any time (t)

D = antibiotic dose (g)

t = at time t after dose D given at time t_D

T_{inf} = infusion time (duration of infusion)

V_d = volume of distribution

K = first order elimination

1.2) IV infusion for two-compartment model

IV infusion for two-compartment model is used to calculate antibiotic concentrations in plasma of imipenem, tigecycline, CMS, and fosfomycin.

Formula:

$$C(t) = \frac{D}{T_{inf}} \times \left[\frac{A}{\alpha} \times \left(1-e^{-\alpha\,(t-t_D)}\right) + \frac{B}{\beta} \times \left(1-e^{-\beta\,(t-t_D)}\right)\right] \quad ;\text{if } t-t_D \le T_{inf}$$

$$C(t) = \frac{D}{T_{inf}} \times \left[\begin{array}{l} \frac{A}{\alpha} \times \left(1-e^{-\alpha T_{inf}}\right) \times e^{-\alpha\,(t-t_D-T_{inf})} \\ + \frac{B}{\beta} \times \left(1-e^{-\beta T_{inf}}\right) \times e^{-\beta\,(t-t_D-T_{inf})} \end{array}\right] \quad ;\text{if } t-t_D > T_{inf}$$

where C (t) = plasma concentration during IV infusion at any time (t)

D = antibiotic dose (g)

t = at time t after dose D given at time t_D

T_{inf} = infusion time (duration of infusion)

V_1 = volume of distribution of central compartment

V_2 = volume of distribution of peripheral compartment

K_{12} = first order elimination from central compartment to peripheral compartment

K_{21} = first order elimination from peripheral compartment to central compartment

CL = the central compartmental clearance

Q = the inter-compartmental clearance

α, β = parameterized in micro-constants

$$A = \frac{1}{V_1} \times \frac{\alpha - k_{21}}{\alpha - \beta} = \frac{1}{V_1} \times \frac{\alpha - \frac{Q}{V_2}}{\alpha - \beta}$$

$$B = \frac{1}{V_1} \times \frac{\beta - k_{21}}{\beta - \alpha} = \frac{1}{V_1} \times \frac{\beta - \frac{Q}{V_2}}{\beta - \alpha}$$

$$\alpha = \frac{k_{21} \times k}{\beta} = \frac{\frac{Q}{V_2} \times \frac{CL}{V_1}}{\beta}$$

$$\beta = \frac{1}{2}\left[k_{12} + k_{21} + k - \sqrt{(k_{12} + k_{21} + k)^2 - 4k_{21}k}\right]$$

$$= \frac{1}{2}\left[\frac{Q}{V_1} + \frac{Q}{V_2} + \frac{CL}{V_1} - \sqrt{\left(\frac{Q}{V_1} + \frac{Q}{V_2} + \frac{CL}{V_1}\right)^2 - 4\frac{Q}{V_2}\frac{CL}{V_1}}\right]$$

3.4.2.2.2 Generate data by using Monte Carlo simulation

All parameters of each antibiotic and the results of calculated antibiotic concentrations will be added to Microsoft Excel. The lognormal distribution is used to generate random sample data for Monte Carlo simulation (Oracle Crystal Ball program).

3.4.2.2.3 Perform and calculate the results

To calculate PK/PD target and index using the 10,000 virtual plasma concentration and time. The trapezoidal rule was used to calculate AUC;

*f*AUC was calculated and divided by the given MIC to estimate the desired PK/PD value.

After calculating the data following to desired PK/PD target and index, the Probability of Target Attainment (PTA) was calculated. Then, calculate the proportion or the percentage of Cumulative fraction of response (CFR) from PTA following the below equation [116].

$$CFR = \sum_{i=1}^{n} PTA_i \times F_i$$

where PTA_i = PTA of each MICs

F_i = the fraction of the population of CRE strains at each MICs

3.4.3 Clinical study

3.4.3.1 Design of the optimal antibiotic combination regimens

After investigated the in vitro study to select the optimal antibiotic options and simulated the PK/PD of antibiotics to select the optimal antibiotic dosing regimens, the optimal antibiotic combination regimens for the treatment of BSIs are designed and are approved by ID physicians, ID pharmacists and a researcher. The details of the optimal antibiotic combination regimens revealed in appendix i.

3.4.3.2 Recruited participants, data collection and intervention

For the clinical study, all recruited participants were divided into two groups – control groups and intervention groups. The research procedure could be done by the groups of participants as follows:

3.4.3.2.1 Control groups

Retrospective study with chart review via electronic medical record can be used to collect patients' data. The procedure consisted of as follows:

- Requested the CEO of the hospital for collecting patients' data
- Recruited participants infected CRE, especially carbapenem-resistant *K. pneumoniae*, *E.Coli* and

Enterobacter cloacae, from 1 January 2018 to 30 September 2020

- Screened participants using inclusion criteria as follows: adult BSI patients (≥18 years) with the positive CRE hemoculture during the period.
- Excluded participants using exclusion criteria as follows: pregnancy or breastfeeding, insufficient or incomplete data, duplicated data, and not receiving antibiotics for the empirical therapy for at least 2 days followed by the documented therapy for the CRE treatment.
- Data of eligible participants would be reviewed and collected by the electronic medical record.
- Collected the participant data and their clinical outcomes
- Analyzed and concluded the results

3.4.3.2.2 Intervention groups

A prospective study with a quasi-experimental design can be used to collect patients' data. The procedure consisted of as follows:

- Requested the CEO of the hospital for collecting patients' data
- To generate the suggested recommendation based on infection disease
- Recruited participants infected CRE, especially carbapenem-resistant *K. pneumoniae*, *E.Coli* and *Enterobacter cloacae*, from 1 October 2020 to 30 September 2022
- Screened participants using inclusion criteria as follows: adult BSI patients (≥18 years) with the positive CRE hemoculture during the period (routine blood culture collection)

- Contact participants or their authorized relatives to provide informed consent for research. Investigators communicated the objective of the study, the procedure for collecting specimens, and the risks and benefits before obtaining informed consent.
- The rest of the routine blood culture collections are cultured using DKMGN plates. Next, the antimicrobial susceptibility testing results are reported to physicians.
- The physicians decided on the treatment infection and whether the suggested recommendation.
- Data of eligible participants would be reviewed and collected by the medical record.
- Followed up and collected the clinical outcomes of the participant until finished a course of antibiotics for the CRE treatment
- Analyzed and concluded the results

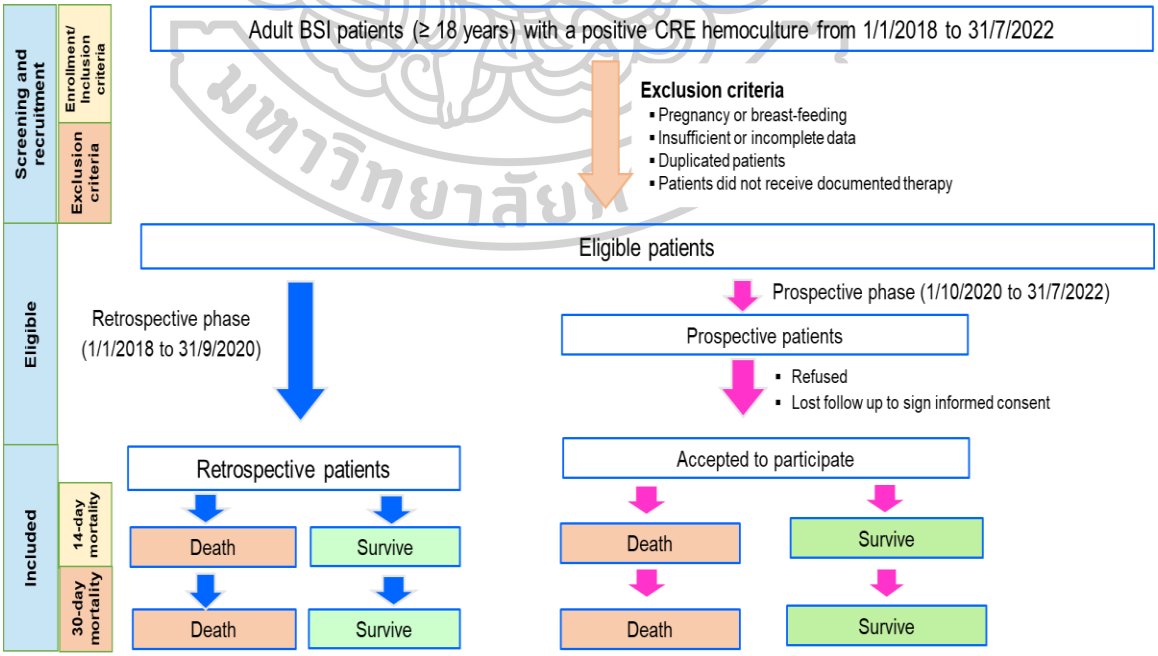

Figure 16 Clinical study procedure

3.5 Definition of the study

- **Carbapenem Resistance Enterobacteriaceae (CRE)** is defined as any Enterobacteriaceae which are resistant to any carbapenem (not including intermediate) MIC of meropenem, imipenem, doripenem ≥ 4 μg/mL or MIC ertapenem ≥ 2 μg/mL [15]
- **Sterile site** is defined as the specimens from blood, cerebrospinal fluid (CSF), pleural fluid, peritoneal fluid, pericardial fluid, bone, joint fluid, and internal body sites
- **Non-sterile sites with clinical symptoms** are defined as non-sterile sites with clinical symptoms followed by CDC/NHSN Surveillance Definitions for Specific Types of Infections [90]
- **Monotherapy** is defined as any regimens that contain one antibiotic agent for the CRE treatment, including meropenem, imipenem, amikacin, gentamicin, tigecycline, colistin, and fosfomycin (7 antibiotic agents)
- **Combination regimens** are defined as any regimens that contain two antibiotic agents for the CRE treatment, including colistin-based regimens aminoglycoside-based regimens, fosfomycin-based regimens
- **Colistin-based regimens** are defined as colistin plus other antibiotics, including colistin-carbapenems, colistin-aminoglycosides, colistin-tigecycline, colistin-fosfomycin
- **Aminoglycoside-based regimens** are defined as amikacin-based regimens and gentamicin-based regimens.
- **Amikacin-based regimens** are defined as amikacin plus other antibiotics, including amikacin-carbapenems, amikacin-tigecycline, amikacin-fosfomycin
- **Gentamicin-based regimens** are defined as gentamicin plus other antibiotics, including gentamicin-carbapenems, gentamicin-tigecycline, and gentamicin-fosfomycin.
- **Fosfomycin-based regimens** are defined as fosfomycin plus other antibiotics including fosfomycin-carbapenems or fosfomycin-tigecycline.

- **Triple based regimens** are defined as a combination of three antibiotics for the treatment of CRE
- **Probability of target attainment (PTA)** is defined as the probability that at least a specific value of a pharmacodynamic index (*f*T>MIC, C_{max}: MIC, *f*AUC/MIC) is achieved at a certain concentration (mostly, Minimum Inhibitory concentration) in Monte Carlo simulations
- **Cumulative fraction of response (CFR)** is defined as the expected population probability of target attainment for a specific drug dose (PTA) and a specific population of microorganisms (which the MIC category ranked from lowest to highest MIC value of a population of microorganisms) [116].
- **Optimal combination regimens** are defined as the dosing regimens of each antibiotic MIC or the MIC values from the synergistic study reached greater than 90% of PTA for documented therapy and greater than 90% of CFR for empirical therapy. The protocol of the optimal combination regimens was designed based on in vitro study of the clinical CRE isolates of Phramongkutklao hospital.
- **Multi-drug resistant (MDR) pathogen** is defined as an isolated CRE that is non-susceptible to at least 1 agent in greater than 3 classes of antimicrobials [117].
- **Extensively drug-resistant (XDR)** is defined as an isolated CRE that is non-susceptible to at least 1 agent in all, excepting fewer than 2 classes of antimicrobials [117].
- **Pan drug-resistant (PDR) bacteria** are defined as isolated CRE that are non-susceptible to all agents in all classes of antimicrobials [117].
- **Appropriate documented antibiotic therapy** is defined as receiving antibiotic regimens that are coverage activities against the suspected pathogens within 48 hours after the diagnosis of BSI.
- **CRE infections** are defined as any infections caused by CRE reported microbial laboratory reports and diagnosed by ID physicians at Phramongkutklao hospital
- **Systemic Inflammatory Response Syndrome (SIRS)** are 2 or more of four following conditions: temperature >38°C or < 36°C; 2) heart rate > 90/min; 3) respiratory rate > 20/min or PaCO2 < 32 mm Hg (4.3 kPa); 4) White blood cell count > 12 000/mm^3 or < 4000/mm^3 [94]

- **Sepsis** is defined as a life-threatening syndrome that induced organ dysfunction caused by dysregulation of the host response to infection. The criteria of defined as sepsis in the study consist of 1) SIRS, 2) documented CRE infections and 3) at least 2 points of SOFA score [94]
- **Septic shock** is defined as a subset of sepsis that had abnormalities of circulation and metabolism leading to substantially increased mortality. The criteria defined as a septic shock in the study consist of 1) having sepsis 2) hypotension (mean arterial blood pressure of < 65 mmHg) that needs one or more vasopressor therapy to maintain blood circulation and 3) a serum lactate level > 2 mmol/L after adequate fluid resuscitation after adequate fluid resuscitation [94]
- **Vasopressor use** is defined as the use of vasopressor during receiving antibiotics for documented therapy.
- **Treatment outcomes** are defined as clinical outcomes, process outcomes, and microbiological outcomes.
- **Clinical outcomes** are defined as clinical improvement/failure, 14- and 30- day mortality, and length of stay.
- **Clinical improvement** is defined as the resolution of the signs and symptoms of the infection with no change or additional antibiotic agents to treatment at the end of the treatment course, except for de-escalation to narrow-spectrum antibiotics.
- **Clinical failure** is defined as the signs and symptoms of the infection being more serious with change or additional antibiotic agents to treatment, failure because the treating physicians judged clinical improvement to be insufficient. In addition, recurrence of the infection, development of superinfection, or an adverse drug effect constituted a failure [118]
- **14- day mortality** is defined as all-cause mortality within 14 days, measured from the day indicated as CRE infections (day 0).
- **30- day mortality** is defined as all-cause mortality within 30 days, measured from the day indicated as CRE infections (day 0).

- **Length of stay (LOS)** is defined as the duration of a hospitalization, measured from admitted to discharged day.
- **Process outcome** is defined as the following recommendations of optimal combination regimens which collect data from ID physicians.
- **Microbiological outcome** is defined as microbiological eradication or microbiological persistence after the treatment.

3.6 Data collection

3.6.1 *In vitro* study (detail in appendix section)

- Reported culture date
- Ward (if any)
- Bacteria species
- Source of specimens
- MICs of antibiotic agents for single antibiotic agents with SIR category and combined antibiotic agents
- CP-CRE genes from multiplex-PCR
- FICI values and types of synergistic activities from synergistic testing

3.6.2 PK/PD study

- % PTA of each antibiotic
- % CFR of each antibiotic

3.6.3 Clinical study (detail in appendix section)

3.6.3.1 Patient data

- Sex
- Age
- Admission date
- Baseline laboratory: serum creatinine (S_{cr}), alanine aminotransferase (ALT)
- Comorbidity: Charlson Comorbidity Index (CCI)
- Severity: Pitt bacteremia score and SIR criteria

3.6.3.2 Pathogen data

- Culture date: collected culture date

- Bacteria species
- Source of specimens
- MICs of each antibiotic with SIR category

3.6.3.3 Treatment data

- Antibiotic exposure (< 90 days before admission)
- Diagnosis date of CRE infections
- Type of infections
- Vasopressor use
- Mechanical use
- Sepsis
- Sepsis shock
- Appropriate antibiotic therapy
- Antibiotic combination regimens
- Antibiotic dosing regimens
- Duration of treatment

3.9.3.4 Treatment outcomes

- Clinical improvement/failure, 14-day clinical failure
- Mortality at 14 and 30 days
- Length of stays (days)
- Acceptance to the protocol or not (process outcomes)
- Microbiological eradication or persistence (microbiological outcomes)

3.7 Outcomes

3.7.1 *In vitro* study

- Frequency and percentage of CRE (only Phramongkutklao hospital)
- Frequency and percentage of bacteria species and source of specimens
- Frequency and percentage of antimicrobial susceptibility (SIR), MIC distribution

- Frequency and percentage of CP-CRE, types of carbapenemase genes (*bla_{NDM}*, *bla_{VIM}*, *bla_{IMP}*, *bla_{KPC}*, *bla_{OXA-48}*)
- MICs at 50 and 90 percentiles of single antibiotic agents
- Frequency and percentage of synergistic activity of each combination

3.7.2 PK/PD study

- PTA and CFR of single antibiotic dosing regimens

3.7.3 Clinical study

3.7.3.1 Patient data

- Frequency and percentage of sex, underlying disease
- Mean ± standard deviation or median (interquartile range) of age, Charlson Comorbidity Index (CCI) for comorbidity, Pitt bacteremia score and SIR criteria for severity, Scr, ALT

3.7.3.2 Pathogen data

- Frequency and percentage of antimicrobial susceptibility (SIR), type of bacteria

3.7.3.3 Treatment data

- Frequency and percentage of type of antibiotic exposure (< 90 days before admission)
- Frequency and percentage of vasopressor use, mechanical use, sepsis, septic shock
- Frequency and percentage of type of infections
- Frequency and percentage of appropriate antibiotic therapy
- Frequency and percentage of antibiotic combination regimens
- Mean ± standard deviation or median (interquartile range) of duration of treatment

3.7.3.4 Treatment outcomes

3.7.3.4.1 Clinical outcomes

- Frequency and percentage of clinical improvement/failure

- Frequency and percentage of 14- and 30- days of mortality
- Mean ± standard deviation or median (interquartile range) of the length of stay

3.7.3.4.2 Process outcomes

- Frequency and percentage of acceptance of the protocol

3.7.3.4.3 Microbiological outcomes

- Frequency and percentage of eradication or persistence

3.8 Statistics and analysis

3.8.1 Statistical and analysis methods

3.8.1.1 Descriptive statistics

Descriptive statistics will be used to describe the results. Categorical variables will be displayed as frequency and percentage, whereas continuous variables will be displayed as mean ± standard deviation (if normal distribution) or median ± interquartile range (if not normal distribution).

3.8.1.1.1 *In vitro* study

- Frequency and percentage of CRE (only Phramongkutklao hospital), bacteria species, antimicrobial susceptibility (SIR), MIC distribution, types of carbapenemase genes (bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{KPC}, bla_{OXA-48}), synergistic effects of each combination
- MICs at 50 and 90 percentiles of single antibiotic agents

3.8.1.1.2 PK/PD study

- PTA and CFR of each antibiotic dosing regimen

3.8.1.1.3 Clinical study

Categorical variables

- Patient data: frequency and percentage of sex, CCI ≥ 3, Pitt bacteremia score ≥ 4
- Pathogen data: antimicrobial susceptibility
- Treatment data: frequency and percentage of antibiotic exposure (< 90 days before admission), type of antibiotic combination regimens, type of infections, appropriate antibiotic therapy, vasopressor use, type of antibiotic treatment regimens, vasopressor use, mechanical use, sepsis, sepsis shock
- Treatment outcomes: frequency and percentage of 14- and 30- days of mortality, clinical improvement/failure, process outcomes, and microbiological outcomes

Continuous variables

- Patient data: mean ± S.D. or median (interquartile range) of age, Charlson Comorbidity Index (CCI) for comorbidity, SIR criteria and Pitt bacteremia score for severity, S_{cr} and ALT
- Treatment data: mean ± S.D. or median (interquartile range) for the duration of antibiotic treatment
- Treatment outcomes: mean ± S.D. or median (interquartile range) for a length of stay

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3.8.1.2 Inferential statistics

Inferential statistics are used to test normal distribution and compare the differences between retrospective and prospective groups as well as

survival and non-survival groups. Only continuous variables and normal distribution will be evaluated using the Shapiro-Wilk test

1) For comparisons categorical variables between two groups in the clinical study will be analyzed using the pearson X^2 test or fisher's exact test (if an expected value for each cell (E) is less than 5 and a frequency of the expected value (E < 5) below 20% of cells).

2) For comparisons continuous variables between two groups in the clinical study will be analyzed using the independence t-test (if normal distribution) or Wilcoxon-Mann Whitney test (if not normal distribution).

3) For 14-day and 30-day mortality, and 14-day clinical failure Kaplan–Meier was used to estimate the survival and failure curves, and the log-rank test was used to test the differences between the two groups.

4) Logistic regression is used to analyze risk factors for mortality.

All tests of inferential statistics will be used two-tailed, P-values ≤ 0.05 for statistical significance testing.

3.8.1.3 Programs for analysis

Software programs will be used for analysis as follows:

- Microsoft Excel for descriptive statistics and PK/PD study
- Statistical program for inferential statistics (Stata 14)
- Crystal ball program for Monte Carlo simulation in PK/PD study

3.9 Ethical approval

Ethical approval for this study was obtained from the Ethics Committee for Human Research of Silpakorn University, Nakhon Pathom, Thailand (Ethics number: REC 63.0429-033-1871 issued on 13 August 2020) and the ethics review committee of the Royal Thai Army Medical Department and Phramongkutklao Hospital, Bangkok, Thailand (Ethics number: Q011h/63 issued on 13 July 2020) .

CHAPTER IV
RESULTS

4.1 *In vitro* results

A total of 199 non-duplicated CRE clinical isolates from various specimens were collected from 12 multicenter hospitals – 49 isolates from a university hospital (hospital-level U) and 150 isolates from 4 regional hospitals (advanced hospitals – Hospital-level A) and 8 general hospitals (5 standard hospitals – hospital-level S and 3 middle hospital – Hospital-level M1).

4.1.1 Phenotypic results

4.1.1.1 Antimicrobial susceptibility results

Using CLSI and EUCAST breakpoint for susceptibility interpretations, the majority of CRE isolates showed non-susceptibility to studied antibiotics, except aminoglycosides. Antimicrobial susceptibility testing results were performed for 163 strains of CRKP (81.91%), 33 strains of CREC (16.58%), and 3 strains of CREclo (1.51%). The total antibiotic susceptible rates were 175 (87.94%) for amikacin, 141 (70.85%) for gentamicin, 112 (56.28%) for colistin, 95 (47.74%) for tigecycline, 59 (30.41%) for ceftazidime/avibactam, 20 (10.05%) for imipenem, 4 (8.16%) for fosfomycin, 15 (7.54%) for meropenem, 7 (3.52%) for aztreonam. The antibiotic susceptibility rates, MIC_{50}, MIC_{90}, and MIC distributions of CRE clinical isolates were presented in Table 17.

The MIC distributions of the studied antibiotics were distributed in high-level resistance at the university hospitals. In the comparison of the antibiotic susceptible rates between the university hospital (hospital-level U) and the non-university hospitals (Hospital-level A, S and M1), CRE clinical isolates of the university hospital were more non-susceptible to meropenem, imipenem, colistin, and tigecycline than other hospital levels. On the other hand, these non-university hospitals remained less susceptible to aminoglycoside and ceftazidime-avibactam than the university hospital. The antibiotic susceptibility rates, MIC_{50}, MIC_{90}, and MIC distributions of CRE clinical isolates divided by hospital levels were presented in Table 18 and Figure 17-25 .

Table 17 The antibiotic susceptibility, MIC_{50}, and MIC_{90} of total isolates

Antibiotics	Total isolates (n)	MIC range	MIC_{50}	MIC_{90}	N (%S) [$]
MEM	199	<=0.125->16	>16	>16	15 (7.54)
IMP	199	<=0.5->16	16	>16	20 (10.05)
AMK	199	<=4->32	8	32	175 (87.94)
GEN	199	<=0.5->8	1	>8	141 (70.85)
COL	199	0.125->8	2	>8	112 (56.28)
TGC	199	<=0.25-4	1	2	95 (47.74)
FOS	49	12->1,024	>1,024	>1,024	4 (8.16)
ATM	199	<=0.5->32	>32	>32	7 (3.52)
CZA	194	<=0.5/4->16/4	>16/4	>16/4	59 (30.41)

Abbreviations: MEM, Meropenem; IMP, Imipenem; AMK, Amikacin; GEN, Gentamicin; COL, Colistin; TGC, Tigecycline; FOS, Fosfomycin; ATM, Aztreonam; CZA, Ceftazidime-avibactam

[$] Using the intermediate breakpoint following CLSI 2021

Table 18 The antibiotic susceptibility, MIC_{50} and MIC_{90} of total isolates divided by hospital levels

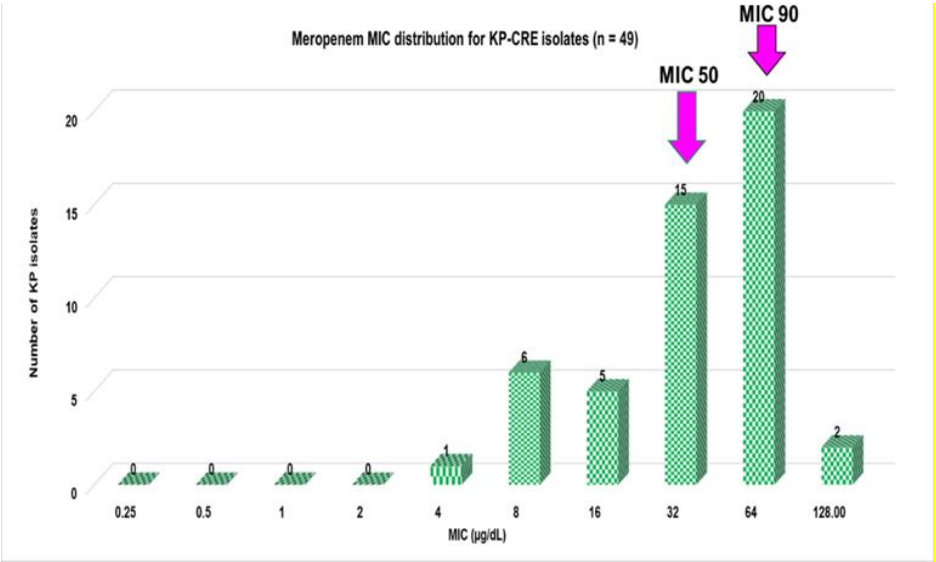
Antibiotics	MIC 50				MIC 90				%S[$,*]			
	U	A	S	M1	U	A	S	M1	U	A	S	M1
MEM	32	>16	>16	>16	64	>16	>16	>16	0	13.89	5.56	8.33
IMP	16	16	16	16	32	>16	>16	>16	2.04	16.67	5.56	8.33
AMK	4	8	8	8	16	>32	16	>32	91.84	80.56	98.15	79.17
GEN	0.25	1	1	2	4	>8	>8	>8	89.80	63.89	70.37	54.17
COL	32	1	1	1	≥ 128	>8	>8	4	18.37	63.89	70.37	79.17
TGC	1	≤0.25	0.5	0.5	2	4	2	2	20.41	51.39	57.41	70.83
FOS	>1,024	-	-	-	>1,024	-	-	-	18.37	-	-	-
ATM	>128	>32	>32	>32	>128	>32	>32	>32	2.04	5.56	1.85	4.17
CZA	2	>16/4	>16/4	>16/4	>16/4	>16/4	>16/4	>16/4	55.10	36.11	11.11	20.83

Abbreviations: MEM, Meropenem; IMP, Imipenem; AMK, Amikacin; GEN, Gentamicin; COL, Colistin; TGC, Tigecycline; FOS, Fosfomycin; ATM, Aztreonam; CZA, Ceftazidime-avibactam

[$] Using the intermediate breakpoint following CLSI 2021

* Total isolates in each hospital levels: n = 49 for hospital-level U ; n = 65 for Hospital-level A; n = 37 for Hospital-level S; n = 12 for Hospital-level M1

(a) University hospital (Pramongkutklao hospital)



(b) Non-university hospital (western hospitals in health region V)

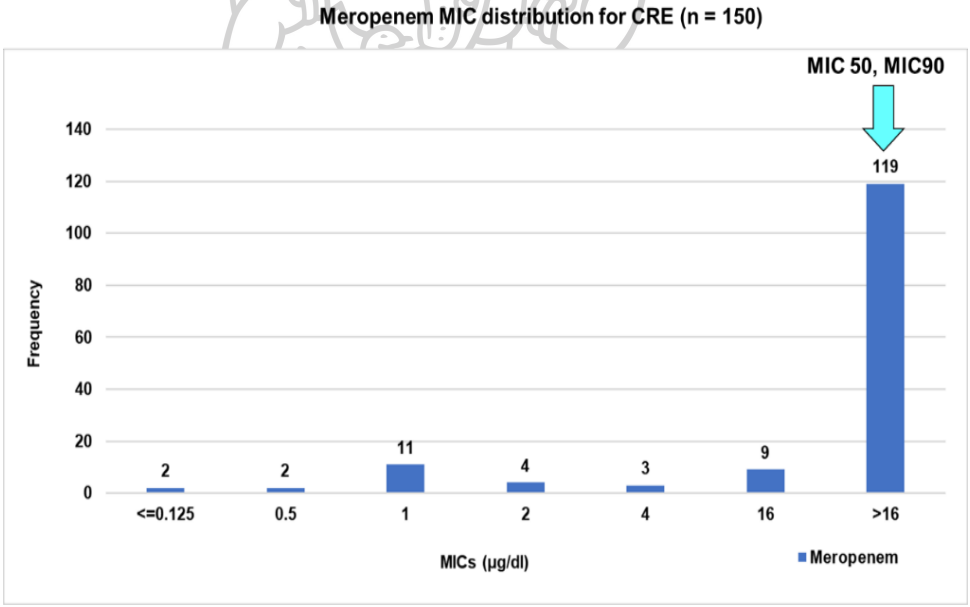
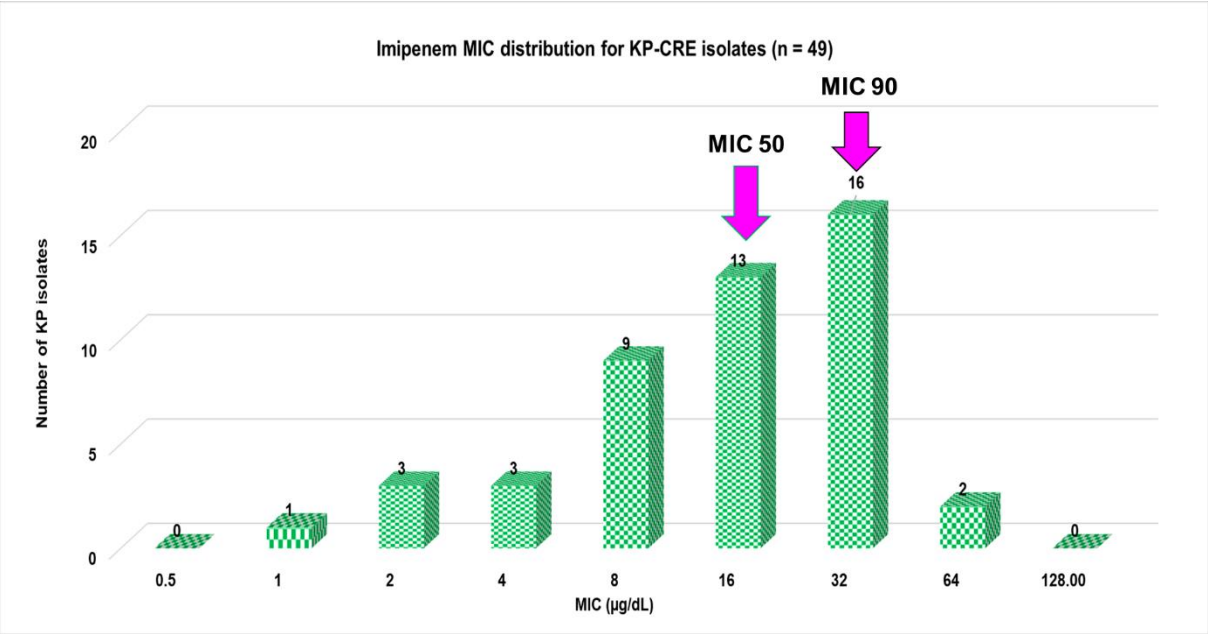


Figure 17 MIC distributions, MIC_{50} and MIC_{90} of meropenem divided by settings

(a) University hospital (Pramongkutklao hospital)



(b) Non-university hospital (western hospitals in health region V)

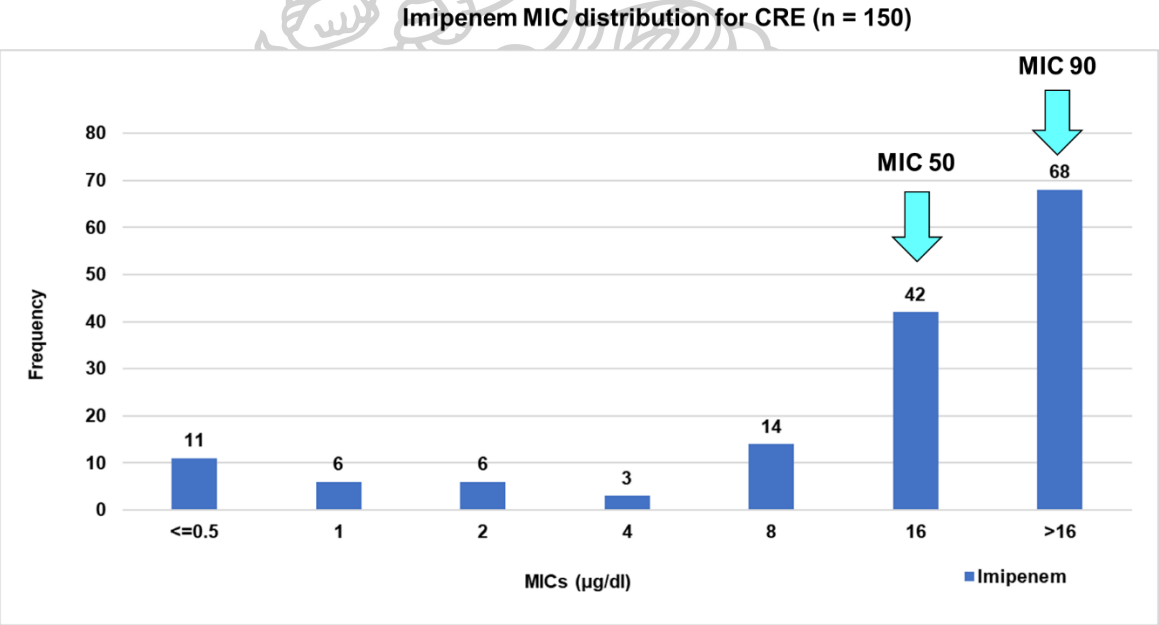
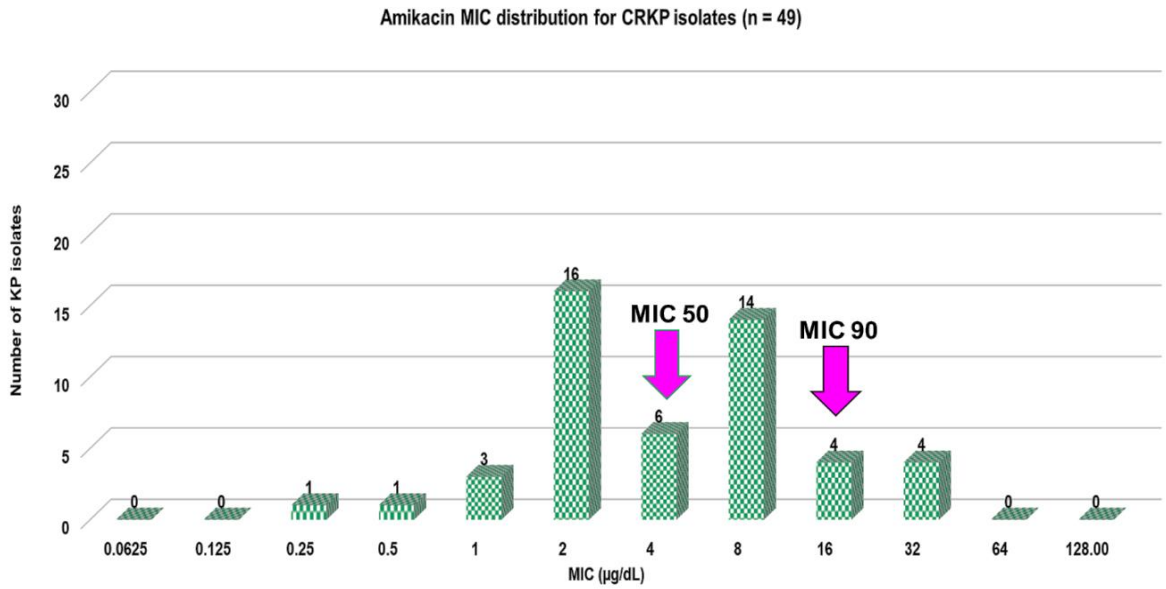


Figure 18 MIC distributions, MIC_{50} and MIC_{90} of imipenem divided by settings

(a) University hospital (Pramongkutklao hospital)



(b) Non-university hospital (western hospitals in health region V)

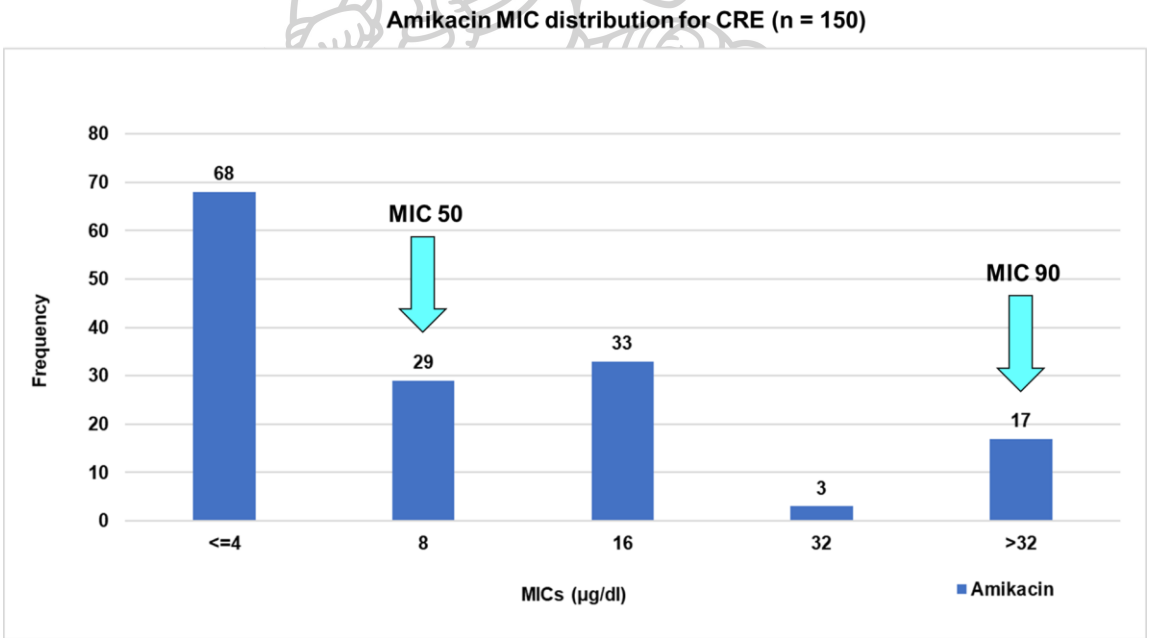
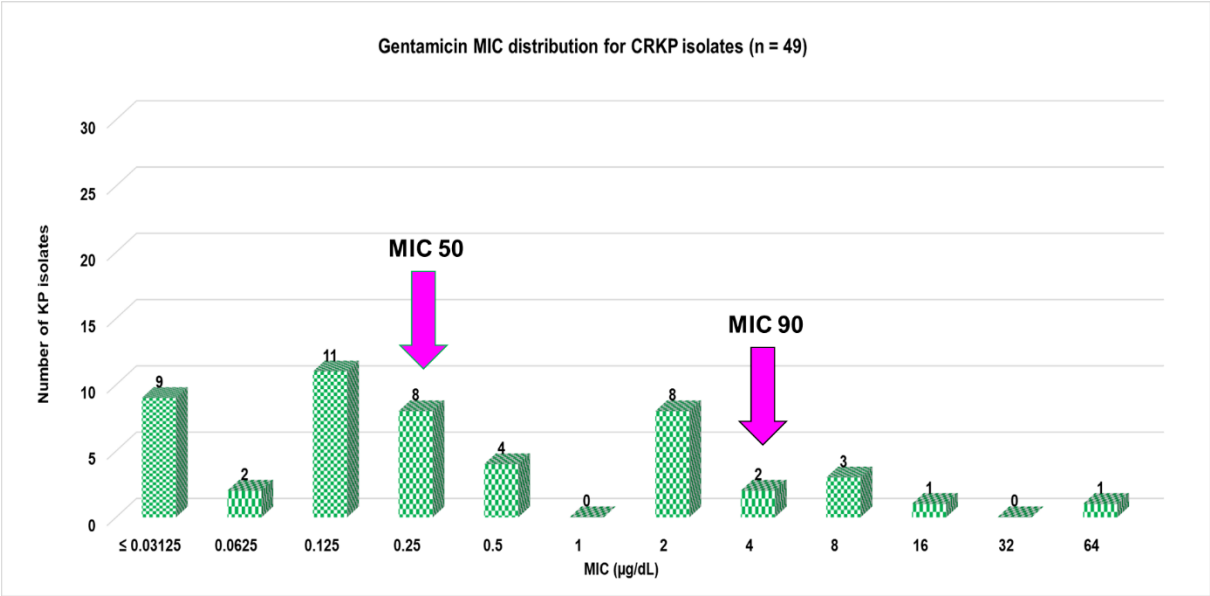


Figure 19 MIC distributions, MIC_{50} and MIC_{90} of amikacin divided by settings

(a) University hospital (Pramongkutklao hospital)



(b) Non-university hospital (western hospitals in health region V)

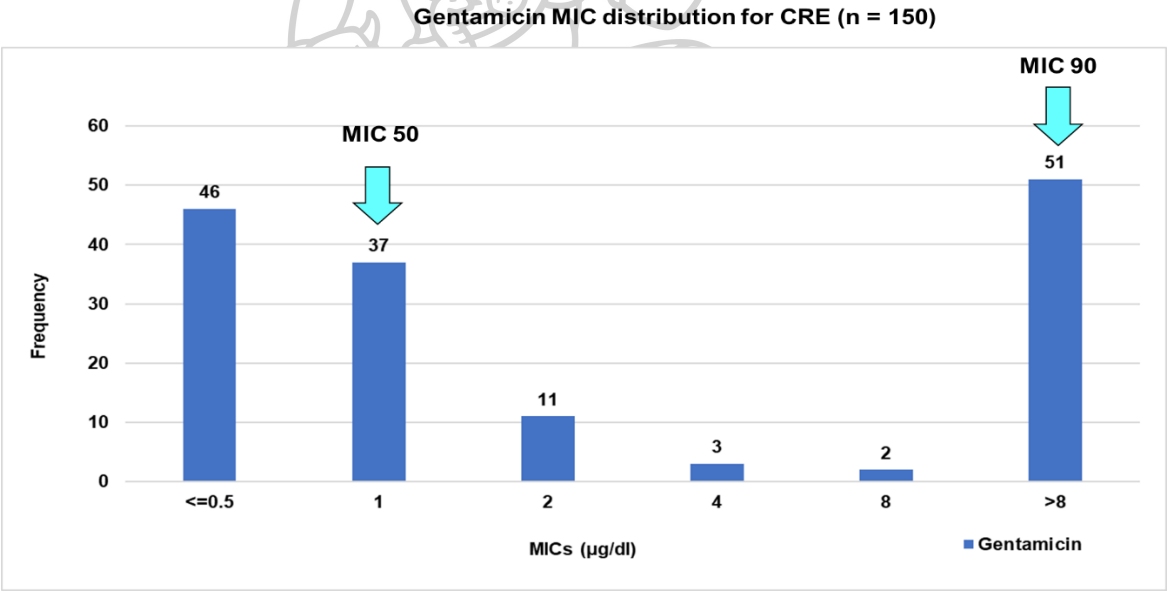
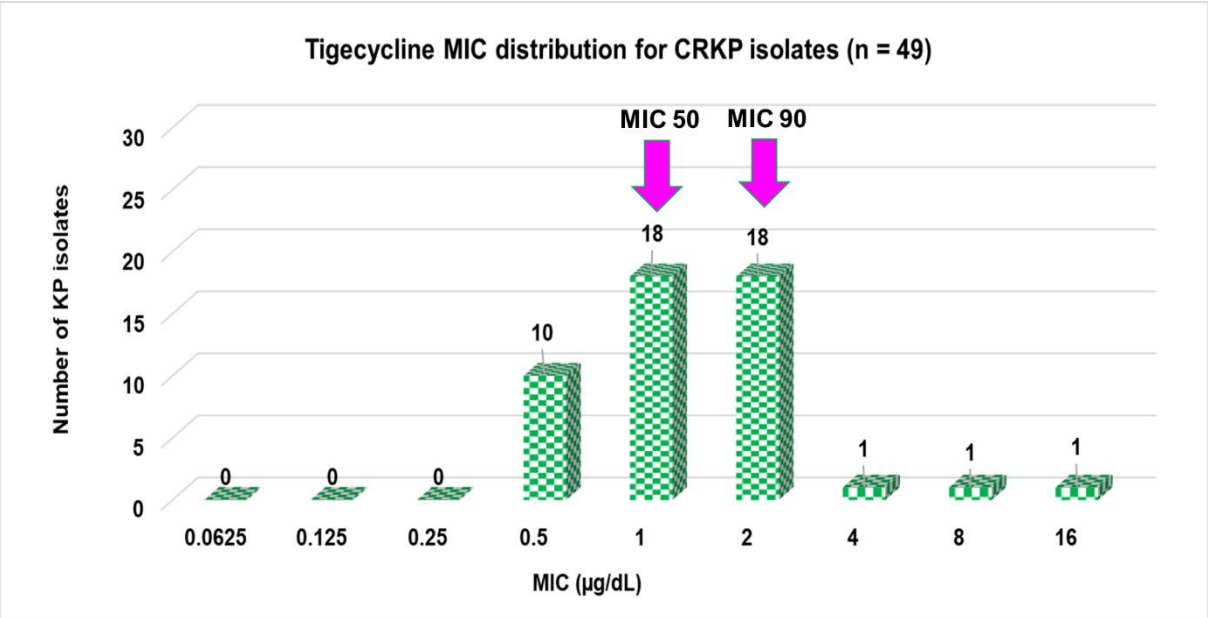


Figure 20 MIC distributions, MIC_{50} and MIC_{90} of gentamicin divided by settings

(a) University hospital (Pramongkutklao hospital)



(b) Non-university hospital (western hospitals in health region V)

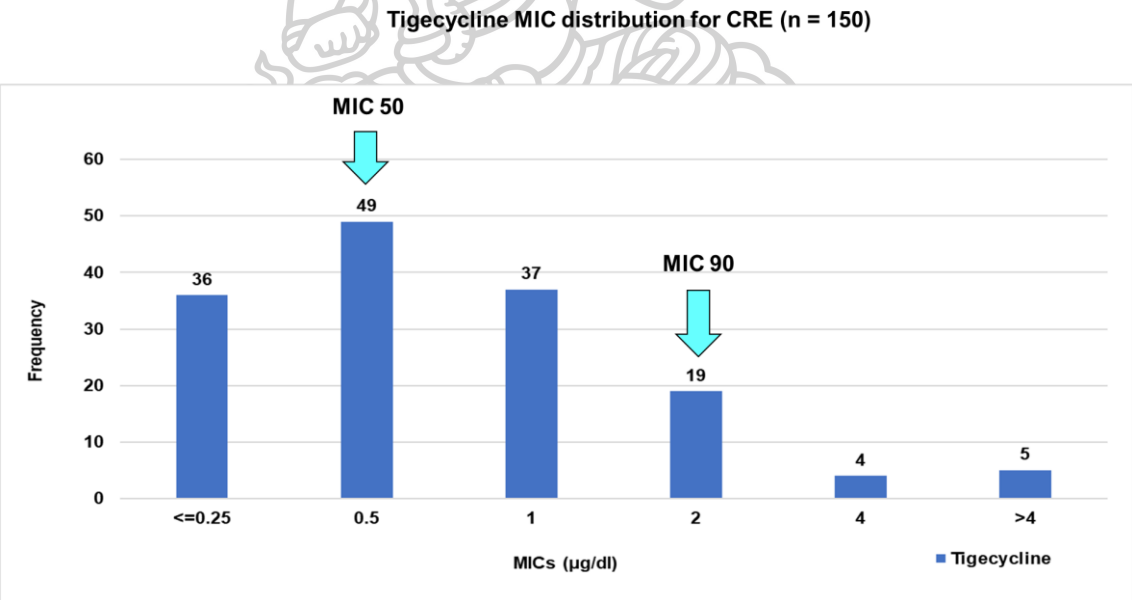
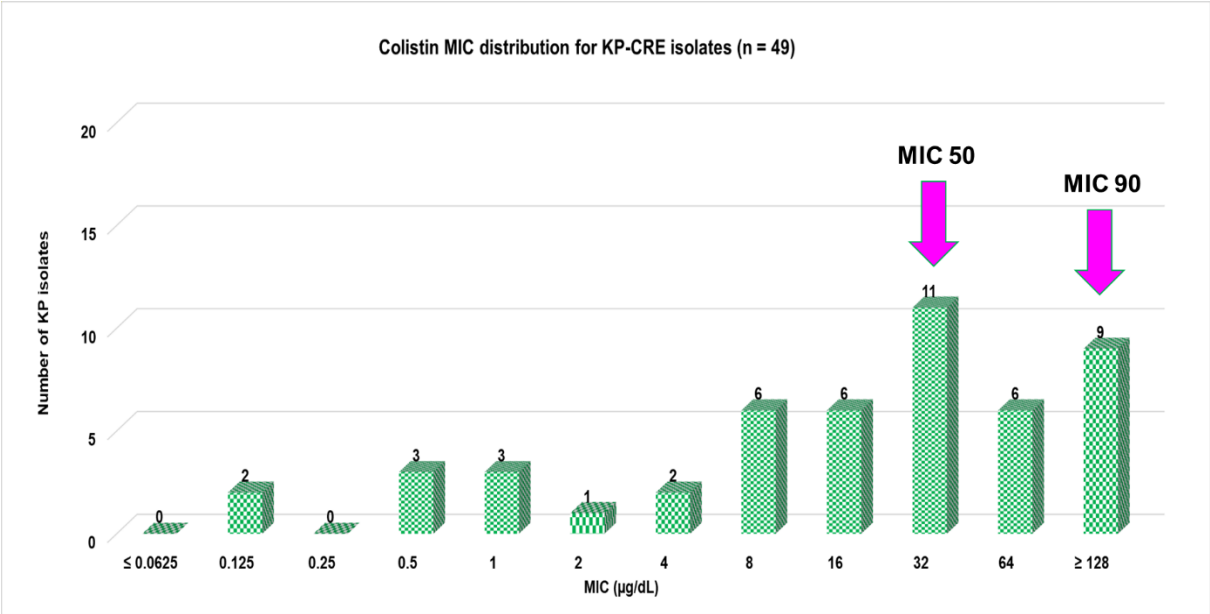


Figure 21 MIC distributions, MIC_{50} and MIC_{90} of tigecycline divided by settings

(a) University hospital (Pramongkutklao hospital)



(b) Non-university hospital (western hospitals in health region V)

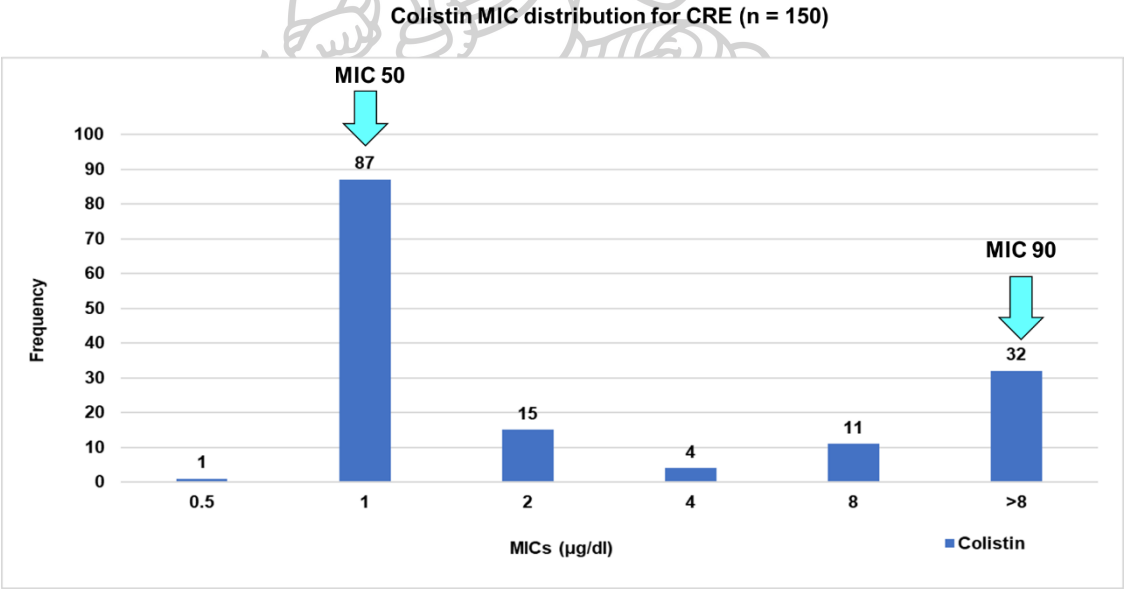


Figure 22 MIC distributions, MIC_{50} and MIC_{90} of colistin divided by settings

(a) University hospital (Pramongkutklao hospital)

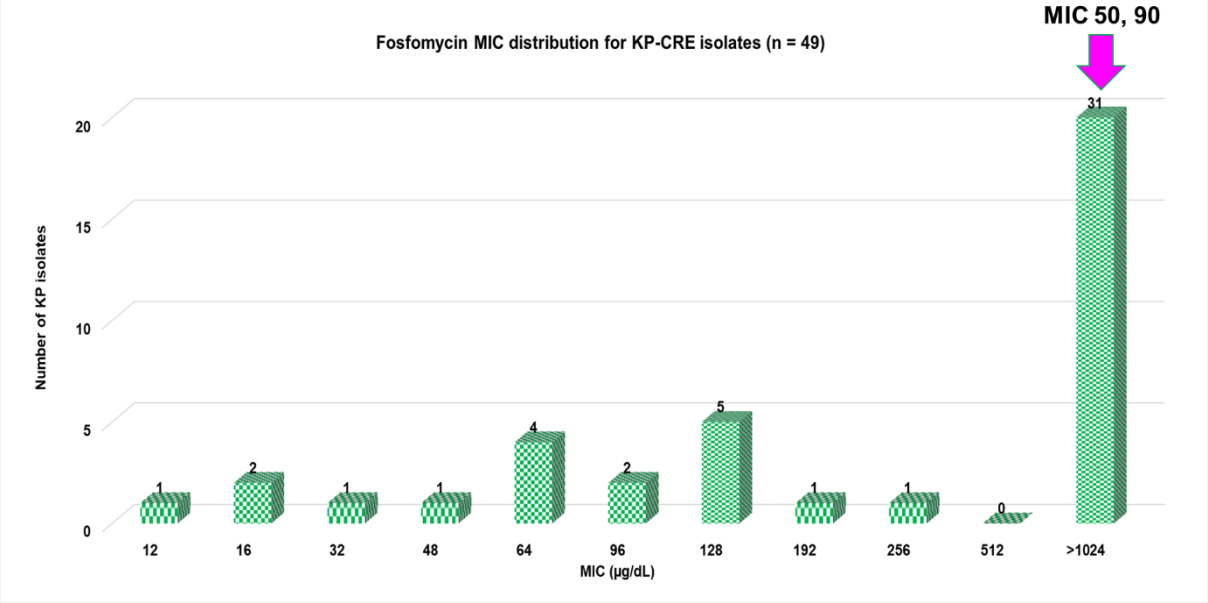
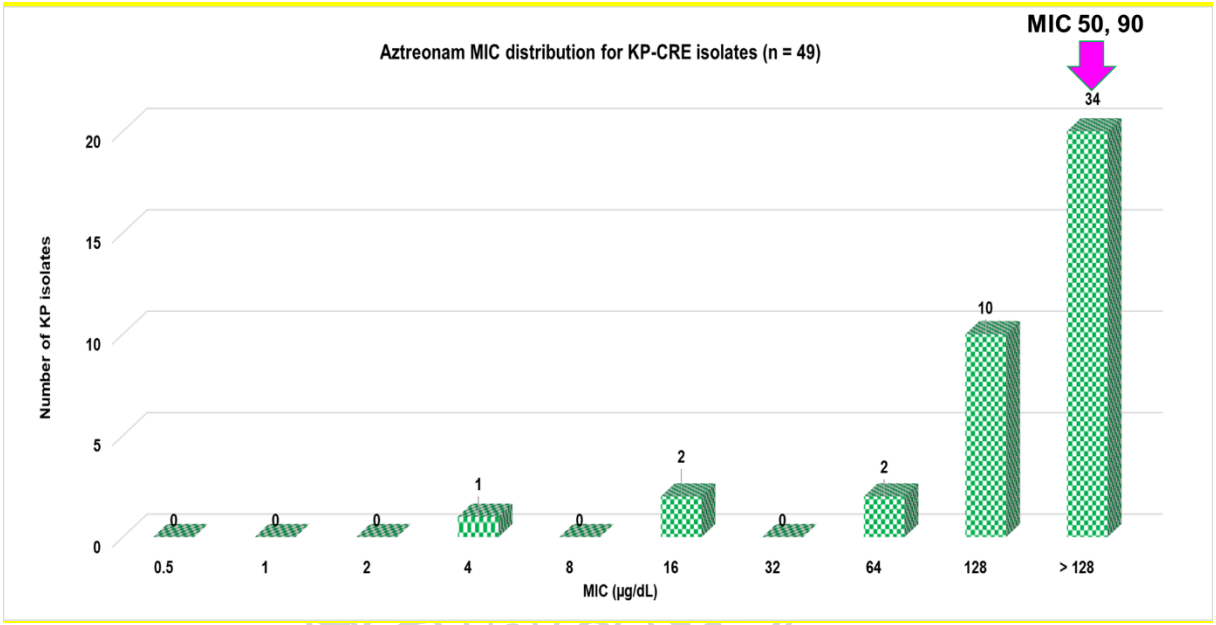


Figure 23 MIC distributions, MIC_{50} and MIC_{90} of fosfomycin

(a) University hospital (Pramongkutklao hospital)



(b) Non-university hospital (western hospitals in health region V)

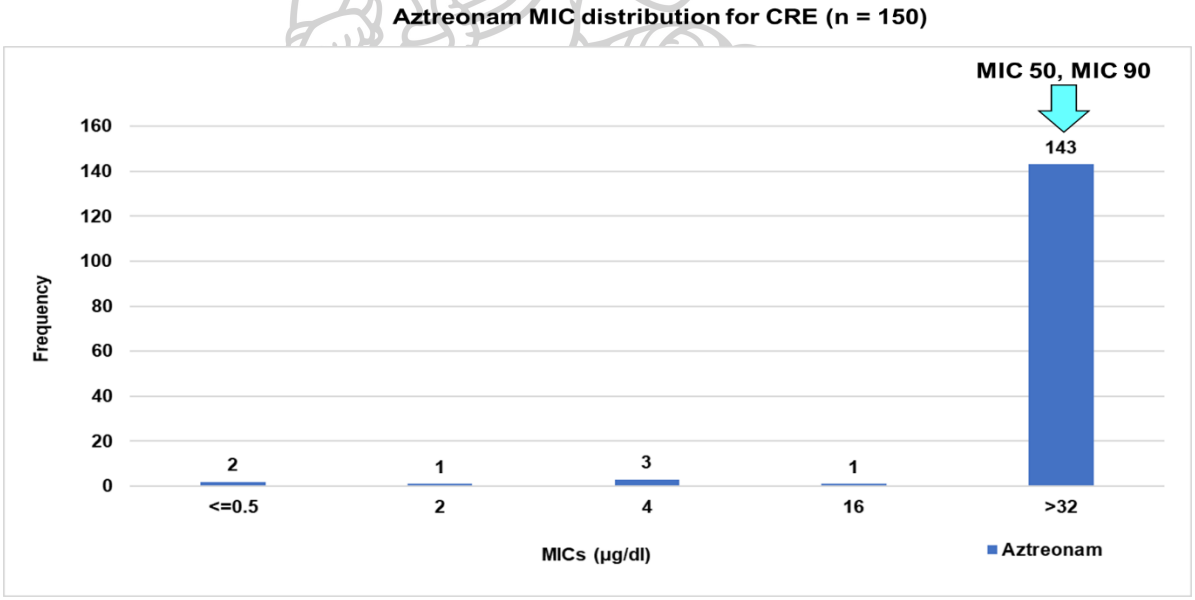
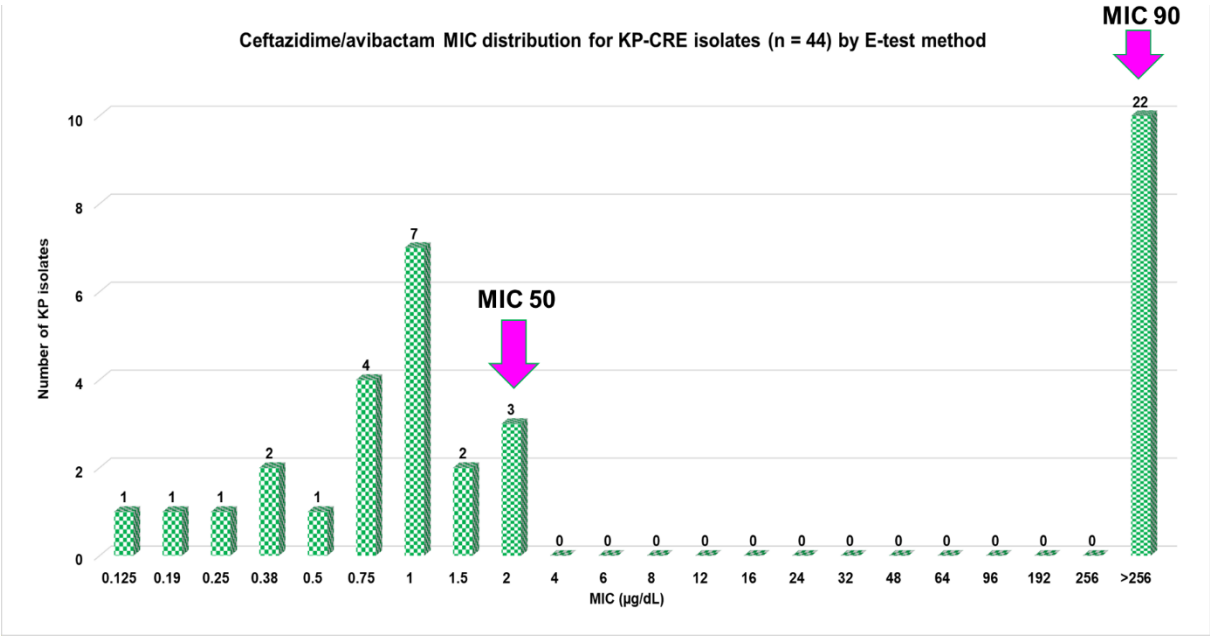


Figure 24 MIC distributions, MIC_{50} and MIC_{90} of aztreonam divided by settings

(a) University hospital (Pramongkutklao hospital)



(b) Non-university hospital (western hospitals in health region V)

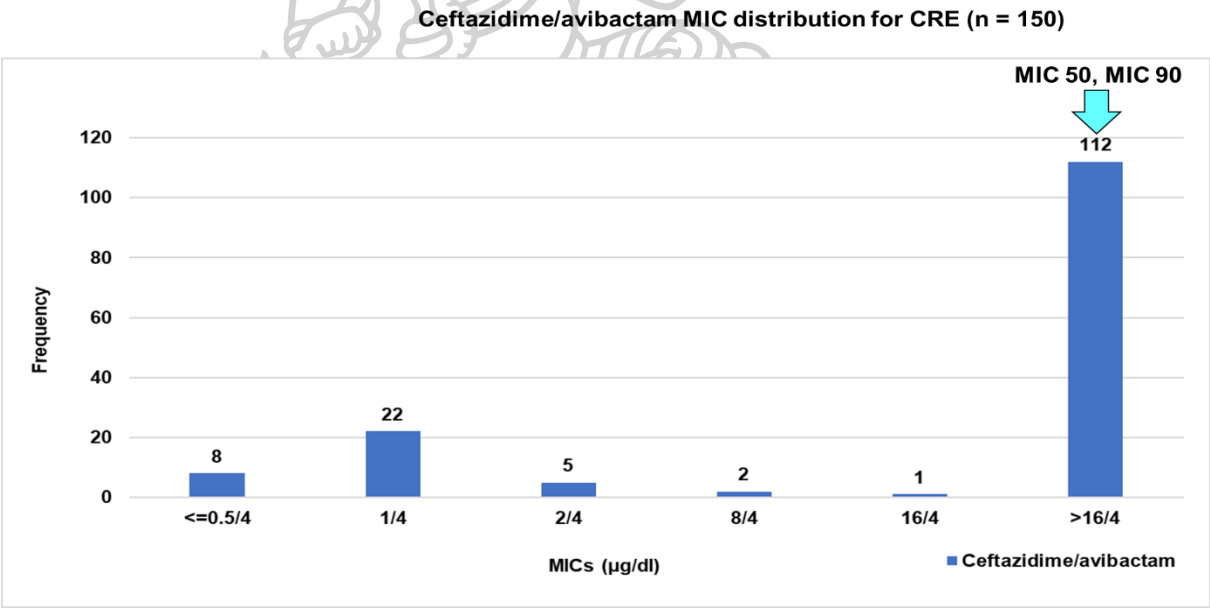


Figure 25 MIC distributions, MIC_{50} and MIC_{90} of ceftazidime-avibactam divided by settings

4.1.1.2 Antibiotic combination results

A total of CRE isolates (n = 49) from the university hospital were included to investigate synergistic effects with 11 antibiotic combinations divided into 5 combination antibiotic-based-regimens, including colistin-based regimens, amikacin-based regimens, gentamicin-based regimens, fosfomycin-based regimens, and triple antibiotic-based regimens.

The top five synergistic effects in antibiotic combinations as shown in 8 of 18 (44.44%) isolates for amikacin – fosfomycin, 7 of 21 (33.33%) isolates for fosfomycin – tigecycline, 2 of 9 (22.22%) isolates for colistin – fosfomycin, 3 of 18 (16.67%) isolates for gentamicin – fosfomycin and 1 of 6 (16.67%) isolates for fosfomycin-tigecycline-amikacin (16.67%).

The top five of additive effect in antibiotic combinations was shown in 37 of 49 (75.5%) isolates for amikacin-tigecycline, 34 of 49 (69.4%) for gentamicin-tigecycline, 15 of 23 (65.22%) isolates for gentamicin-colistin, 14 of 23 (60.87%) isolates for amikacin-colistin and 13 of 23 (56.52%) isolates for colistin-tigecycline, respectively.

Moreover, an indifferent effect was observed in triple combination regimens – 5 of 6 (83.33%) isolates for fosfomycin-tigecycline-gentamicin and 4 of 6 (66.66%) isolates for fosfomycin-tigecycline-amikacin, respectively.

None of any CRE isolates showed antagonism in the checkerboard assays. The synergistic testing results were shown in Table 19.

Table 19 Results of synergy from the checkerboard method for all carbapenem-resistant Klebsiella pneumoniae (CRKP) isolates (n = 49) divided by combination antibiotic based-regimens

Antibiotic combination based regimens	Antibiotic based regimens	Synergy	Additive	Indifference	Antagonism
Colistin-based regimens[$]	COL-FOS (n = 9)	2 (22.22%)	0 (0%)	7 (77.78%)	0 (0%)
	COL-GEN (n = 23)	4 (17.39%)	15 (65.22%)	4 (17.39%)	0 (0%)
	COL-AMI (n = 23)	2 (8.7%)	14 (60.87%)	7 (30.43%)	0 (0%)
	COL-TGC (n = 23)	4 (17.39%)	13 (56.52%)	6 (26.09%)	0 (0%)
Amikacin-based regimens[$]	AMI-FOS (n = 18)	8 (44.44%)	2 (11.12%)	8 (44.44%)	0 (0%)
	AMI-TGC (n = 49)	4 (8.16%)	37 (75.51%)	8 (16.33%)	0 (0%)
Gentamicin-based regimens[$]	GEN-FOS (n = 18)	3 (16.67%)	2 (11.11%)	13 (72.22%)	0 (0%)
	GEN-TGC (n = 49)	4 (8.16%)	34 (69.39%)	11 (22.45%)	0 (0%)
Fosfomycin-based regimens[$$]	FOS-TGC (n = 21)	7 (33.33%)	9 (42.86%)	5 (23.81%)	0 (0%)
Triple antibiotic regimens	FOS-TGC-AMI (n = 6)[*]	1 (16.67%)	1 (16.67%)	4 (66.66%)	0 (0%)
	FOS-TGC-GEN (n = 6)[**]	0 (0%)	1 (16.67%)	5 (83.33%)	0 (0%)

Abbreviations: MEM, Meropenem; IMP, Imipenem; AMK, Amikacin; GEN, Gentamicin; COL, Colistin; TGC, Tigecycline; FOS, Fosfomycin; ATM, Aztreonam; CZA, Ceftazidime-avibactam

[$] Colistin MICs ≤ 16 µg/mL; fosfomycin MICs 12-256 µg/mL;[$$] Fosfomycin MICs 12-256 µg/mL for 18 CRE isolates (n =18); fosfomycin MICs > 1,024 µg/mL for 3 CRE isolates (n = 3); [*] Colistin MICs > 2 µg/mL, fosfomycin MICs > 32 µg/mL, tigecycline MICs > 0.5 µg/mL, gentamicin MICs > 16 µg/mL and amikacin MICs ≤ 16 µg/mL

[**] Colistin MICs > 2 µg/mL, fosfomycin MICs > 32 µg/mL, tigecycline MICs > 0.5 µg/mL, gentamicin MICs ≤ 4 µg/mL and amikacin MICs >16 µg/mL

4.1.2 Genotypic results

4.1.2.1 ERIC PCR

Only CRE clinical isolates obtained from the university hospitals (n = 49) were investigated by using the ERIC PCR technique to determine their types. The results showed no discriminative DNA banding patterns in the CRE clinical isolates. Therefore, none of any isolates were classified into main groups. DNA banding patterns of the CRE clinical isolates from the university hospital were presented in Figure 26.

Figure 26 DNA banding patterns of the CRE clinical isolates from the university hospital (n = 49)

4.1.2.2 *mcr-1* PCR

One-hundred ninety-nine isolates (n = 199) were investigated by using the *mcr-1* PCR technique to determine colistin-resistance genes. The results showed that 5 of 199 (2.51%) isolates were found in *mcr-1* genes. Based on hospital levels, *mcr-1* positive isolates were only found in hospital-level A. DNA banding patterns of the *mcr-1* positive isolates were presented in Figure 27.

4.1.2.3 Multiplex PCR

Of 199 CRE isolates, NDM and OXA-48 were performed in our study, whereas KPC, VIM and IMP did not find. The prevalence of carbapenemase were as follow: OXA-48 (n = 91; 45.73%), NDM (n = 72; 36.18%), OXA-48 plus NDM (n = 31;

15.58%) and not detected any carbapenemase (n = 5; 2.51%). DNA banding patterns of the carbapenemase positive isolates were presented in Figure 28.

Figure 27 DNA banding patterns of the *mcr-1* positive isolates (n = 5)

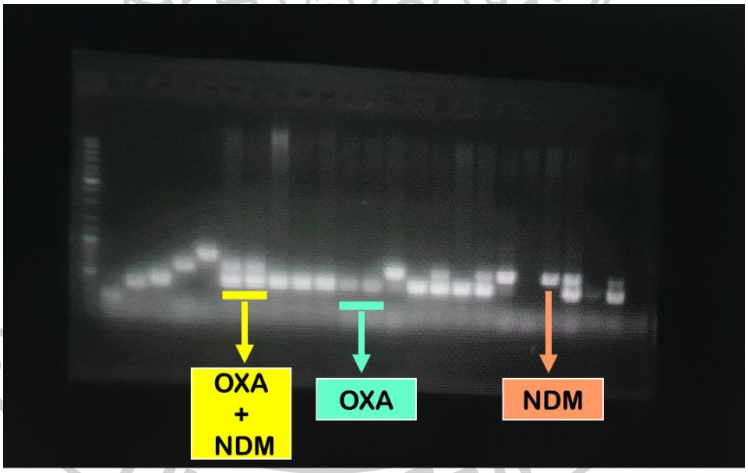


Figure 28 DNA banding patterns of the carbapenemase positive isolates

Based on the type of carbapenem resistance bacteria, CRKP and CREC were found in either NDM or OXA-48. The most common carbapenemase genes in CRKP and CREC were OXA-48 (n = 86; 52.76%) and NDM (n = 27; 81.82%), respectively. Furthermore, CREClo were found only NDM (n = 1; 33.33%). Table 20 showed the type of carbapenemase divided by carbapenem resistance bacteria.

Table 20 Type of carbapenemase divided by carbapenem resistance bacteria

Carbapenemase	CRKP	CREC	CREClo	Total
NDM	44 (26.99)	27 (81.82)	1 (33.33)	72 (36.18)
OXA-48	86 (52.76)	5 (15.15)	0 (0)	91 (45.73)
NDM plus OXA-48	30 (18.4)	1 (3.03)	0 (0)	31 (15.58)
Not detected any carbapenemase	3 (1.84)	0 (0)	2 (66.67)	5 (2.51)
Total	163 (100)	33 (100)	3 (100)	199 (100)

Abbreviations: CRKP, carbapenem resistance *Klebsiella pneumoniae;*
CREC, carbapenem resistance *Escherichia coli;* CREClo carbapenem resistance *Enterobacter cloacae*
NDM, New Delhi metallo-β-lactamase-1; OXA-48, Oxacillinases-48

For CRKP isolates, OXA-48 (n = 26; 53.06%), OXA-48 plus NDM (n = 21; 42.86%) and NDM (n = 1; 2.02%) were revealed from 49 isolates from the university hospital. In the western hospitals in health region V, of the 114 isolates of CRKP, OXA-48 (n = 60 of 114; 52.63%) was the most common carbapenemase gene detected followed by NDM (43 of 114; 37.72%), NDM plus OXA-48 (9 of 114; 7.89%) and not found any carbapenemase (2 of 114; 1.75%).

According to CREC isolates, the detected carbapenemase genes included NDM (27 of 33; 81.82%), OXA-48 (5 of 33; 15.15%) and NDM plus OXA-48 (1 of 33; 3.03%), respectively. Not found any carbapenemase gene (2 of 3; 66.7%) and NDM (1 of 3; 33.3%) was detected in CREClo isolates.

According to multiplex PCR and *mcr-1* PCR, 5 of 199 CRE clinical isolates (1.51%) contained *mcr-1* genes. Five *mcr-1* producing isolates revealed in the CRKP isolates from Hospital-level A. Moreover, they were found to co-harbor the *mcr-1* and OXA-48 genes. Table 21 showed type of carbapenemase genes and *mcr-1* divided by hospital levels.

Table 21 type of carbapenemase and *mcr-1* divided by hospital levels

Type of bacteria	Hospital levels	Total isolates	NDM	OXA-48	NDM plus OXA-48	Not found any carbapenemase	*mcr-1*
CRKP	U	49	1 (2.02%)	26 (53.06%)	21 (42.86%)	2 (4.08%)	0 (0%)
	A	65	20 (30.77%)	40 (61.54%)	5 (7.69%)	0 (0%)	5 (7.69%)
	S	37	18 (48.65%)	14 (37.84%)	4 (10.81%)	1 (2.7%)	0 (0%)
	M1	12	5 (41.67%)	6 (50%)	0 (0%)	1 (8.33%)	0 (0%)
	Total	**114**	**43 (37.72%)**	**60 (52.63%)**	**9 (7.89%)**	**2 (1.75%)**	**0 (0%)**
CREC	U	NR	NR	NR	NR	NR	NR
	A	7	6 (85.71%)	1 (14.29%)	0 (0%)	0 (0%)	0 (0%)
	S	15	12 (80%)	2 (13.33%)	1 (6.67%)	0 (0%)	0 (0%)
	M1	11	9 (81.82%)	2 (18.18%)	0 (0%)	0 (0%)	0 (0%)
	Total	**33**	**27 (81.82%)**	**5 (15.15%)**	**1 (3.03%)**	**0 (0%)**	**0 (0%)**
CREclo	U	NR	NR	NR	NR	NR	NR
	A	NR	NR	NR	NR	NR	NR
	S	2	1 (50%)	0 (0%)	0 (0%)	1 (50%)	0 (0%)
	M1	1	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)
	Total	**3**	**1 (33.33%)**	**0 (0%)**	**0 (0%)**	**2 (66.67%)**	**0 (0%)**

Type of bacteria	Hospital levels	Total isolates	NDM	OXA-48	NDM plus OXA-48	Not found any carbapenemase	*mcr-1*
All types	U	49	1 (2.02%)	26 (53.06%)	21 (42.86%)	2 (4.08%)	0 (0%)
	A	72	26 (36.11%)	41 (56.94%)	5 (6.94%)	0 (0%)	5 (6.94%)
	S	54	31 (57.41%)	16 (29.63%)	5 (9.26%)	2 (3.7%)	0 (0%)
	M1	24	14 (58.33%)	8 (33.33%)	0 (0%)	2 (8.33%)	0 (0%)
	Total	**199**	**72 (36.18%)**	**91 (45.73%)**	**31 (15.58%)**	**6 (3.02%)**	**5 (3.33%)**

Abbreviations: NR; not reported.

CRKP, carbapenem resistance *Klebsiella pneumoniae;* CREC, carbapenem resistance *Escherichia coli;* CREClo carbapenem resistance *Enterobacter cloacae;* hospital-level U , university hospital; Hospital-level A, advanced level hospital; Hospital-level S, standard level hospital; Hospital-level M1, middle level hospital; NDM, New Delhi metallo-β-lactamase-1; OXA-48, Oxacillinases-48

4.1.3 Characteristics of phenotypic results and genotypic results

The susceptible rates of tigecycline, colistin, amikacin and gentamicin were 90.28%, 84.72%, 55.56% and 55.56% for NDM positive isolates, 64.84%, 47.25%, 89.01% and 80.22% for OXA-48 isolates as well as 32.26%, 12.9%, 80.65% and 83.87% for NDM plus OXA-48 isolates, respectively. Table 22 showed MIC range, MIC_{50}, $MIC_{90,}$ and the antibiotic susceptible rates divided by each carbapenemase gene.

Interestingly, 24.12% (n = 48 of 199) of CRE clinical isolates showed no categorical agreement of interpretive criteria for aminoglycosides following to CLSI guideline. The frequencies of no agreement were NDM (n = 25 of 48; 52.08%), NDM plus OXA-48 (n = 8 of 48; 16.67%) and OXA-48 (n = 14 of 48; 29.17%), respectively. Additionally, the frequencies of agreement were OXA-48 (n = 77 of 151; 50.99%), NDM (n = 47 of 151; 31.13%), and NDM plus OXA-48 (n = 23 of 151; 15.23%), respectively. Table 23 showed categorical agreement of interpretive criteria for aminoglycosides following to CLSI guideline

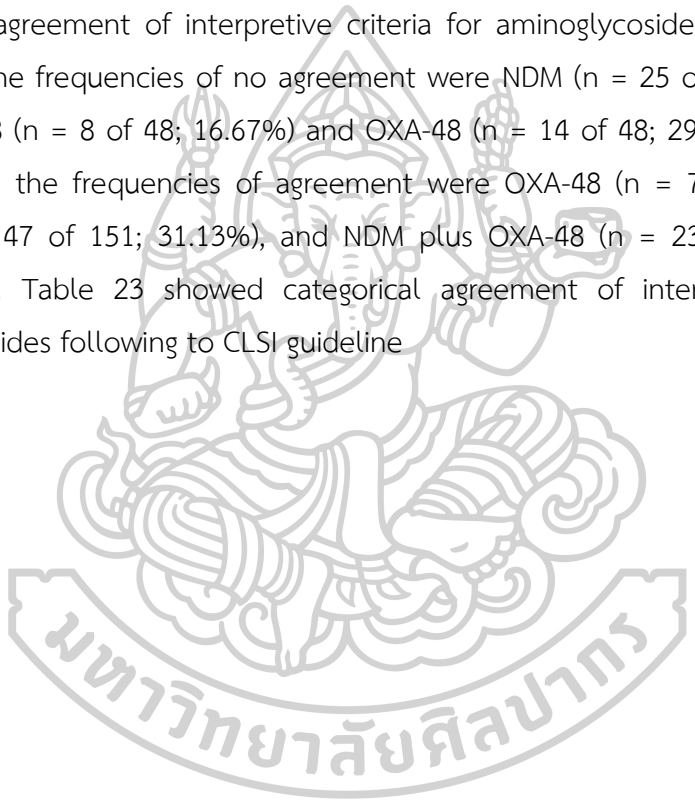


Table 22 Results of MIC range, MIC_{50}, $MIC_{90,}$ and the antibiotic susceptible rates in NDM, OXA-48, and NDM plus OXA-48 producing isolates.

Antibiotics	NDM (n = 72)				OXA-48 (n = 91)				NDM plus OXA-48 (n = 31)			
	MIC range	MIC 50	MIC 90	% S	MIC range	MIC 50	MIC 90	% S	MIC range	MIC 50	MIC 90	% S
MEM	1 - >16	>16	>16	2.78	≤0.125 - >16	>16	>16	12.09	1 - >16	>16	>16	3.23
IMP	≤0.5 - >16	16	>16	2.78	≤0.5 –>16	16	>16	15.38	≤0.5 - >16	>16	>16	3.23
AMK	≤4 - >32	<=4	16	55.56	≤4 –>32	8	32	89.01	≤4 - >32	8	32	83.87
GEN	≤0.5 - >8	2	>8	55.56	≤0.5 - >8	1	>8	80.22	≤0.5 - >8	<=0.5	8	80.65
COL	1 - >8	1	8	84.72	≤0.125 - >8	8	16	47.25	1 - >8	>8	>8	12.9
TGC	≤ 0.25 ->4	0.5	1	90.28	≤0.25 – >4	1	2	64.84	≤0.25 - 4	1	2	32.26
FOS*	-	128	128	0	16 - >1,024	>1,024	>1,024	3.85	12 - >1,024	>1,024	>1,024	14.29
ATM	≤ 0.5->32	>32	>32	6.94	4 - >32	>32	>32	4.4	-	>32	>32	0
CZA$	≤0.5/4 - >16/4	>16/4	>16/4	4.17	≤0.5/4 - >256	1/4	>16/4	72.22	1/4 - >256	>256	>256	6.45

Abbreviations: MEM, Meropenem; IMP, Imipenem; AMK, Amikacin; GEN, Gentamicin; COL, Colistin; TGC, Tigecycline; FOS, Fosfomycin; ATM, Aztreonam CZA, Ceftazidime-avibactam; NDM, New Delhi metallo-β-lactamase-1; OXA-48, Oxacillinases-48

* For fosfomycin: n = 1 for NDM; n = 26 for OXA-48; n = 21 for NDM plus OXA-48

$ For ceftazidime-avibactam: n = 89 for OXA-48; n = 28 for NDM plus OXA-48

Table 23 Categorical agreement of interpretive criteria for aminoglycosides following CLSI guideline

Categorical agreement	NDM (n = 72)	OXA-48 (n = 91)	NDM plus OXA-48 (n =31)	Not found any carbapenemase genes (n = 5)	Total
Agreement *	47 (31.13%)	77 (50.99%)	23 (15.23%)	4 (2.65%)	151 (75.88%)
No agreement **	25 (52.08%)	14 (29.17%)	8 (16.67%)	1 (2.08%)	48 (24.12%)

* Agreement, a CRE clinical isolate was susceptible to both amikacin and gentamicin

** No agreement, a CRE clinical isolate was susceptible to one of the aminoglycosides and resistant to another, e.g. susceptible to gentamicin but resistant to amikacin

4.2 PK/PD results

4.2.1. Probability of Target Attaining (PTA)

4.2.1.1 Carbapenems

4.2.1.1.1 Meropenem

At MIC_{50} and MIC_{90} (> 16 µg/mL), none of any meropenem dosing regimens were achieved in the PTA target. At MICs of 1-2 µg/mL (non-resistant breakpoints), meropenem dosing regimens (1 g) of 3-hours prolonged infusion achieved in the ≥90% PTA at 100%*f*T>MIC in general patients and patients with CrCL of > 25 mL/min. Additionally, the prolonged infusion of 3-hours given 6 hours could provide the PTA targets more than the prolonged infusion of 3-hours given 8 hours. Patient with creatinine clearance 10-25 mL/min, maintenance doses 0.75 -1 g with a 3-hours prolonged infusion every 12 hours achieved in the PTA target. None of any prolonged infusion regimens met the PTA target in a patient with CrCL < 10 mL/min. Table 24 showed the ≥90% PTA at 100%*f*T>MIC in general patients and patients with CrCL ranging from 0-9, 10-25, 26-50, 51-90 mL/min.

4.2.1.1.2 Imipenem

At MIC_{50} (16 µg/mL) and MIC_{90} (> 16 µg/mL), none of any meropenem dosing regimens were achieved in the PTA target. In general patients, only the prolonged infusion of 3-hours could provide the ≥90% PTA at 100%*f*T>MIC with a MIC of 0.5 µg/mL. Table 25 showed the ≥90% PTA at 100%*f*T>MIC in general patients.

Table 24 The Probability of Target Attaining (PTA) for meropenem dosing regimens

Patients	Regimens	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256
CrCL 0-9 mL/min	LD 2 g + MD 0.75 g inf 0.5 h q 24 h	85.19	82.52	79.14	74.93	69.27	61.43	50.38	37.34	21.32	6.74	0.04	0	0
	LD 2 g + MD 0.75 g inf 3 h q 24 h	86.46	84.02	80.84	77.15	71.44	63.37	52.86	39.2	23.52	7.72	0.09	0	0
	LD 2 g + MD 1 g inf 0.5 h q 24 h	86.29	83.69	80.66	76.29	71	64.36	54.75	42.7	27.25	11.67	0.79	0	0
	LD 2 g + MD 1 g inf 3 h q 24 h	87.49	85.22	82.34	78.33	73.17	66.32	57	44.93	29.72	12.97	1.11	0	0
CrCL 10-25 mL/min	LD 2 g + MD 0.75 g inf 0.5 h q 12 h	94.72	93.48	91.62	88.79	84.64	78.71	68.74	52.36	26.4	1.57	0	0	0
	LD 2 g + MD 0.75 g inf 3 h q 12 h	95.86	94.86	93.55	91.2	87.92	82.4	73.44	57.52	32.08	2.67	0	0	0
	LD 2 g + MD 1 g inf 0.5 h q 12 h	95.11	94.05	92.28	90.1	86.57	81.58	72.9	58.74	37.13	9.61	0	0	0
	LD 2 g + MD 1 g inf 3 h q 12 h	96.34	95.31	93.92	91.93	88.95	84.24	77.47	65.11	43.78	13.18	0	0	0
CrCL 26-50 mL/min	LD 2 g + MD 1 g inf 0.5 h q 12 h	93.28	91.3	88.7	85.03	79.2	69.31	53.17	27.43	2.16	0	0	0	0
	LD 2 g + MD 1 g inf 0.5 h q 8 h	97.8	97.1	96.16	94.54	91.69	87.55	79.34	63.82	33.33	0.66	0	0	0
	LD 2 g + MD 1 g inf 3 h q 12 h	95.42	94.01	91.92	88.94	84.17	76.08	62.29	36.17	4.6	0	0	0	0
	LD 2 g + MD 1 g inf 3 h q 8 h	98.87	98.44	97.8	96.72	95.03	91.8	86.7	74.68	47.52	2.96	0	0	0
CrCL 51-90 mL/min	LD 2 g + MD 1 g inf 0.5 h q 6 h	98.59	97.95	97.06	95.57	93.08	88.19	78.3	55.31	11.6	0	0	0	0
	LD 2 g + MD 1 g inf 0.5 h q 8 h	96.21	94.81	92.66	89.56	83.83	72.77	52.36	19.42	0.13	0	0	0	0
	LD 2 g + MD 1 g inf 3 h q 6 h	99.65	99.43	99.14	98.58	97.53	95.22	89.91	77.32	37.81	0	0	0	0
	LD 2 g + MD 1 g inf 3 h q 8 h	98.38	97.59	96.51	94.88	91.5	84.99	71.28	39.88	1.46	0	0	0	0
General	LD 0 g + MD 1 g inf 0.5 h q 6 h	97.93	97	95.68	93.41	89.63	82.95	72.58	56.39	34.24	13.78	2.6	0.16	0
	LD 0 g + MD 1 g inf 0.5 h q 8 h	94.26	92.27	89.22	84.68	78.5	69.24	55.13	38.15	18.89	5.51	0.72	0.02	0

Patients	Regimens	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256
	LD 0 g + MD 1 g inf 3 h q 6 h	99.5	99.14	98.65	97.62	95.9	92.44	85.11	70.74	47.37	19.34	3.56	0.17	0
	LD 0 g + MD 1 g inf 3 h q 8 h	97.03	95.74	93.84	90.99	86.24	78.82	66.82	48.42	25.58	7.81	0.83	0.03	0
	LD 0 g + MD 2 g inf 0.5 h q 8 h	95.72	93.98	91.96	89.22	85.04	78.83	69.48	55.5	37.55	19.07	5.89	0.72	0.02
	LD 0 g + MD 2 g inf 3 h q 8 h	98.21	97.42	96.36	94.42	91.53	86.8	78.52	66.1	48.09	25.84	7.65	0.86	0.04
	LD 2 g + MD 1 g inf 0.5 h q 6 h	97.79	96.9	95.27	92.72	89.03	82.53	72.19	56.06	34.41	13.44	2.46	0.17	0
	LD 2 g + MD 1 g inf 0.5 h q 8 h	93.98	91.84	88.65	84.41	77.69	68.48	55.34	37.59	18.85	5.68	0.72	0.05	0
	LD 2 g + MD 1 g inf 3 h q 6 h	99.49	99.2	98.68	97.64	96.03	92.71	85.86	71.66	46.58	18.99	3.41	0.2	0
	LD 2 g + MD 1 g inf 3 h q 8 h	97.16	95.89	93.95	91.04	86.31	78.66	65.94	47.29	25.47	7.91	1.02	0.06	0
	LD 2 g + MD 2 g inf 0.5 h q 8 h	95.87	94.33	92.11	89.2	84.75	78.6	68.66	55.12	37.29	18.52	5.63	0.71	0.04
	LD 2 g + MD 2 g inf 3 h q 8 h	98.1	97.35	96.16	94.23	91.36	86.73	78.9	66.52	48.24	25.64	7.35	0.89	0.03

Abbreviations: LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

Table 25 The Probability of Target Attaining (PTA) for imipenem dosing regimens

Patients	Regimens	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256
General	LD 0 g + MD 0.5 g inf 2 h q 6 h	98.3	95.54	88.73	73.07	45.81	15.11	1.29	0.01	0	0	0	0	0
	LD 0 g + MD 0.5 g inf 3 h q 6 h	99.67	98.96	95.7	85.38	61.34	24.85	2.84	0.02	0	0	0	0	0
	LD 0 g + MD 1 g inf 2 h q 6 h	99.32	98.2	95.78	88.82	72.96	45.1	14.51	1.21	0.04	0	0	0	0
	LD 0 g + MD 1 g inf 3 h q 6 h	99.93	99.71	98.62	95.46	86.09	61.55	25.6	2.93	0.01	0	0	0	0
	LD 0.5 g + MD 0.5 g inf 2 h q 6 h	98.55	95.61	87.85	72.32	45.21	14.96	1.34	0	0	0	0	0	0
	LD 0.5 g + MD 0.5 g inf 3 h q 6 h	99.61	98.67	95.49	85.47	61.94	25.36	2.7	0.01	0	0	0	0	0
	LD 0.5 g + MD 1 g inf 2 h q 6 h	99.44	98.39	95.68	88.8	72.87	44.76	14.6	1.2	0.01	0	0	0	0
	LD 0.5 g + MD 1 g inf 3 h q 6 h	99.94	99.66	98.67	95.59	86.19	61.28	25.32	2.62	0.01	0	0	0	0
	LD 1 g + MD 0.5 g inf 2 h q 6 h	98.24	95.53	88.59	72.7	45.23	14.61	1.19	0	0	0	0	0	0
	LD 1 g + MD 0.5 g inf 3 h q 6 h	99.64	98.55	95.53	85.29	61.38	25.22	2.82	0.03	0	0	0	0	0
	LD 1 g + MD 1 g inf 2 h q 6 h	99.37	98.4	95.88	88.51	72.87	44.95	14.82	1.47	0	0	0	0	0
	LD 1 g + MD 1 g inf 3 h q 6 h	99.91	99.69	98.77	95.74	85.3	61.45	24.95	2.91	0	0	0	0	0

Abbreviations: LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

4.2.1.2 Aminoglycosides

4.2.1.2.1 Amikacin

At a MIC of 1 μg/mL, all amikacin dosage regimens met the PTA target (Cmax/MIC > 8) in critically ill patients. At a MIC of 2 μg/mL, a loading dose of 25–30 mg/kg followed by a maintenance dose of 15–20 mg/kg of amikacin met the PTA target in patients with creatinine clearance levels of more than 50 mL/min. No regimens achieved the target at MIC50 (8 μg/mL) and MIC90 (32 μg/mL). The PTA for different amikacin regimens is presented in Table 26.

4.2.1.2.2 Gentamicin

For gentamicin, all dosage regimens met the PTA target in critically ill patients with a MIC of 0.5 and 1 (MIC 50) μg/mL. At a MIC of 1 μg/mL, 4 mg/kg, and 5 mg/kg every 24 and 48 hours (extended infusion) reached the PTA target in patients with creatinine clearance at 51–90 and 10–50 mL/min, respectively. None of any gentamicin regimens met the target at MIC90 (>8 μg/mL). The PTA for different gentamicin regimens is presented in Table 27.

4.2.1.3 Tigecycline

For tigecycline dosage regimens to meet the PTA at fAUC0-24/MIC ≥ 0.9, once-daily high-dose tigecycline (400 mg loading dose followed by 200 mg supplemental doses every 12 hours) achieved ≥ 90% PTA with a MIC of 1 μg/mL (MIC 50) and 2 μg/mL (MIC 90). Twice daily tigecycline dosing regimens achieved the PTA target with a MIC of 1 μg/mL (MIC 50), whereas all tigecycline dosing regimens reached the PTA target of a MIC of 0.5 μg/mL. The assessments of PTA for different tigecycline dosages are shown in Table 28.

Table 26 The Probability of Target Attaining (PTA) for the different amikacin regimens in critically ill patients according to kidney function (creatinine clearance) at steady state with targets of C_{max}/MIC > 8 (for efficacy) and AUC_{0-24} (mg × h/L) > 700

Creatinine clearance	Dosage Regimens	PTA (%)									
		Amikacin MIC (µg/mL)									AUC_{0-24} > 700
		0.0625	0.125	0.25	0.5	1	2	4	8	16	
CrCL 0-9 mL/min	LD 15 mg/kg + MD 7.5 mg/kg inf 0.5 h q 48 h	100	100	100	100	100	66.91	0.06	0	0	11.14
	LD 20 mg/kg + MD 7.5 mg/kg inf 0.5 h q 48 h	100	100	100	100	100	69.52	0.04	0	0	13.35
	LD 25 mg/kg + MD 7.5 mg/kg inf 0.5 h q 48 h	100	100	100	100	100	70.38	0.06	0	0	15.35
CrCL 10-25 mL/min	LD 20 mg/kg + MD 10 mg/kg inf 0.5 h q 48 h	100	100	100	100	100	88.94	0.36	0	0	24.89
	LD 20 mg/kg + MD 15 mg/kg inf 0.5 h q 48 h	100	100	100	100	100	99.01	5.32	0	0	53.1
	LD 25 mg/kg + MD 10 mg/kg inf 0.5 h q 48 h	100	100	100	100	100	89.88	0.34	0	0	26.8
	LD 25 mg/kg + MD 15 mg/kg inf 0.5 h q 48 h	100	100	100	100	100	99.24	5.3	0	0	55.73
CrCL 26-50 mL/min	LD 20 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	100	100	100	100	100	99.46	6.98	0	0	84.18
	LD 20 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	100	100	100	100	100	99.98	19.23	0	0	92.70
	LD 25 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	100	100	100	100	100	99.52	7.28	0	0	84.28
	LD 25 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	100	100	100	100	100	99.97	20.66	0	0	92.65
CrCL 51-90 mL/min	LD 25 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	100	100	100	100	100	99.54	6.07	0	0	81.47
	LD 25 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	100	100	100	100	100	99.94	18.49	0	0	91.94
	LD 25 mg/kg + MD 20 mg/kg inf 0.5 h q 24 h	100	100	100	100	100	100	45.98	0	0	97.62
	LD 30 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	100	100	100	100	100	99.61	6.06	0	0	81.49

Creatinine clearance	Dosage Regimens	PTA (%)									
		Amikacin MIC (µg/mL)									AUC_{0-24} > 700
		0.0625	0.125	0.25	0.5	1	2	4	8	16	
CrCL 91-130 mL/min	LD 30 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	100	100	100	100	100	100	18.29	0	0	91.65
	LD 30 mg/kg + MD 20 mg/kg inf 0.5 h q 24 h	100	100	100	100	100	100	47.43	0	0	97.76
	LD 25 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	100	100	100	100	100	99.6	7.91	0	0	81.47
	LD 25 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	100	100	100	100	100	99.97	22.11	0	0	91.94
	LD 25 mg/kg + MD 20 mg/kg inf 0.5 h q 24 h	100	100	100	100	100	100	53.43	0	0	97.62
	LD 30 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	100	100	100	100	100	99.62	8.18	0	0	81.49
	LD 30 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	100	100	100	100	100	99.95	22.57	0	0	91.65
	LD 30 mg/kg + MD 20 mg/kg inf 0.5 h q 24 h	100	100	100	100	100	99.99	52.48	0	0	97.76
General	LD 25 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	100	100	100	100	99.99	98.47	44.64	0.02	0	84.38
	LD 25 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	100	100	100	100	99.99	99.33	57.26	0.14	0	88.04
	LD 25 mg/kg + MD 20 mg/kg inf 0.5 h q 24 h	100	100	100	100	100	99.89	73.87	0.84	0	91.46
	LD 30 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	100	100	100	100	100	98.41	46.57	0.07	0	84.88
	LD 30 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	100	100	100	100	100	99.47	60.15	0.27	0	88.39
	LD 30 mg/kg + MD 20 mg/kg inf 0.5 h q 24 h	100	100	100	100	100	99.89	75.16	1.04	0	91.99

Abbreviations: LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

Table 27 The Probability of Target Attaining (PTA) for the different gentamicin regimens in critically ill patients according to kidney function (creatinine clearance) at steady state with targets of C_{max}/MIC > 8 (for efficacy) and AUC_{0-24} (mg × h/L) > 700

Creatinine clearance	Dosage Regimens	PTA (%)									
		Gentamicin MIC (µg/mL)									AUC_{0-24} > 700
		0.0625	0.125	0.25	0.5	1	2	4	8	16	
CrCL 0-9 mL/min	LD 3 mg/kg + MD 2.5 mg/kg inf 0.5 h q 48 h	100	100	100	100	98.78	42.01	0.04	0	0	42.69
	LD 5 mg/kg + MD 2.5 mg/kg inf 0.5 h q 48 h	100	100	100	100	99.09	40.6	0.11	0	0	43.27
	LD 7 mg/kg + MD 2.5 mg/kg inf 0.5 h q 48 h	100	100	100	100	99.1	41.69	0.13	0	0	43.80
CrCL 10-25 mL/min	LD 5 mg/kg + MD 4 mg/kg inf 0.5 h q 48 h	100	100	100	100	90.87	8.22	0.01	0	0	6.37
	LD 7 mg/kg + MD 3 mg/kg inf 0.5 h q 48 h	100	100	100	100	77.61	2.7	0	0	0	2.36
	LD 7 mg/kg + MD 5 mg/kg inf 0.5 h q 48 h	100	100	100	100	96.63	16.28	0.01	0	0	13.01
	LD 8 mg/kg + MD 3 mg/kg inf 0.5 h q 48 h	100	100	100	99.99	78.34	2.69	0	0	0	2.35
	LD 8 mg/kg + MD 5 mg/kg inf 0.5 h q 48 h	100	100	100	100	96.22	17.09	0.03	0	0	13.20
CrCL 26-50 mL/min	LD 5 mg/kg + MD 3 mg/kg inf 0.5 h q 24 h	100	100	100	99.98	78.24	2.48	0	0	0	1.94
	LD 5 mg/kg + MD 4 mg/kg inf 0.5 h q 24 h	100	100	100	99.99	92.09	7.5	0	0	0	6.02
	LD 7 mg/kg + MD 3 mg/kg inf 0.5 h q 24 h	100	100	100	99.98	78.93	2.66	0	0	0	2.25
	LD 7 mg/kg + MD 4 mg/kg inf 0.5 h q 24 h	100	100	100	100	91.12	8.14	0	0	0	6.51
	LD 8 mg/kg + MD 3 mg/kg inf 0.5 h q 24 h	100	100	100	99.98	78.64	2.55	0	0	0	2.34
	LD 8 mg/kg + MD 4 mg/kg inf 0.5 h q 24 h	100	100	100	99.99	91.91	8.59	0	0	0	6.55
CrCL 51-90	LD 7 mg/kg + MD 5 mg/kg inf 0.5 h q 24 h	100	100	100	100	91.61	5.95	0	0	0	3.07

Creatinine clearance	Dosage Regimens	PTA (%)									
		Gentamicin MIC (µg/mL)									AUC_{0-24} > 700
		0.0625	0.125	0.25	0.5	1	2	4	8	16	
mL/min	LD 7 mg/kg + MD 6 mg/kg inf 0.5 h q 24 h	100	100	100	100	96.13	11.7	0	0	0	5.82
	LD 8 mg/kg + MD 5 mg/kg inf 0.5 h q 24 h	100	100	100	100	91.9	6.08	0	0	0	3.61
	LD 8 mg/kg + MD 6 mg/kg inf 0.5 h q 24 h	100	100	100	100	95.75	11.91	0	0	0	6.43
	LD 8 mg/kg + MD 7 mg/kg inf 0.5 h q 24 h	100	100	100	100	97.99	19.42	0	0	0	10.61
CrCL 91-130 mL/min	LD 7 mg/kg + MD 5 mg/kg inf 0.5 h q 24 h	100	100	100	100	88.75	5.33	0	0	0	3.18
	LD 7 mg/kg + MD 6 mg/kg inf 0.5 h q 24 h	100	100	100	100	94.23	11.45	0	0	0	6.84
	LD 8 mg/kg + MD 5 mg/kg inf 0.5 h q 24 h	100	100	100	100	88.42	5.54	0	0	0	3.37
	LD 8 mg/kg + MD 6 mg/kg inf 0.5 h q 24 h	100	100	100	100	93.93	11.14	0	0	0	6.24
	LD 8 mg/kg + MD 7 mg/kg inf 0.5 h q 24 h	100	100	100	100	96.95	17.61	0.04	0	0	9.71
General	LD 7 mg/kg + MD 4 mg/kg inf 0.5 h q 24 h	100	100	100	99.94	63.41	0.5	0	0	0	0.11
	LD 7 mg/kg + MD 5 mg/kg inf 0.5 h q 24 h	100	100	100	99.99	78.99	1.64	0	0	0	0.36
	LD 7 mg/kg + MD 6 mg/kg inf 0.5 h q 24 h	100	100	100	100	78.85	1.66	0	0	0	0.99
	LD 8 mg/kg + MD 4 mg/kg inf 0.5 h q 24 h	100	100	100	99.92	64.31	0.41	0	0	0	0.20
	LD 8 mg/kg + MD 5 mg/kg inf 0.5 h q 24 h	100	100	100	99.97	79.29	1.62	0	0	0	0.50
	LD 8 mg/kg + MD 6 mg/kg inf 0.5 h q 24 h	100	100	100	100	88.33	3.66	0	0	0	1.10
	LD 8 mg/kg + MD 7 mg/kg inf 0.5 h q 24 h	100	100	100	100	93.27	7.57	0	0	0	2.15

Abbreviations: LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

Table 28 The Probability of Target Attaining (PTA) for tigecycline dosing regimens

Patients	Regimens	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256
General	LD 200 mg + MD 100 mg inf 0.5 h q 12 h	100	100	100	100	100	53.22	0	0	0	0	0	0	0
	LD 200 mg + MD 100 mg inf 0.5 h q 24 h	100	100	100	100	54.51	0.01	0	0	0	0	0	0	0
	LD 400 mg + MD 100 mg inf 0.5 h q 12 h	100	100	100	100	100	55.34	0.03	0	0	0	0	0	0
	LD 400 mg + MD 100 mg inf 0.5 h q 24 h	100	100	100	99.99	54.79	0.03	0	0	0	0	0	0	0
	LD 400 mg + MD 200 mg inf 0.5 h q 12 h	100	100	100	100	100	100	53.92	0.03	0	0	0	0	0
	LD 400 mg + MD 200 mg inf 0.5 h q 24 h	100	100	100	100	100	54.88	0.02	0	0	0	0	0	0

Abbreviations: LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram

4.2.1.4 Colistin

At a loading dose of 300 mg followed by a maintenance dose of 100-150 mg every 12 hours, achieved ≥ 90% PTA ($fAUC_{0-24}$/MIC ≥ 25) at MICs ≤ 4 µg/mL in patients with CrCL ≤ 25 mL/min as well as at MICs ≤ 2 µg/mL (MIC_{50}) in patients with CrCL of ≤ 50 mL/min, respectively. At a MIC of 0.5 µg/mL, a loading dose of 300 mg followed by a maintenance dose of 150-180 mg every 8 hours, achieved ≥ 90% PTA in patients with CrCL of > 50 mL/min. None of the colistin regimens met the target at MIC_{90} (> 8 µg/mL). The PTA for the colistin dosing regimens is presented in Table 29.

4.2.1.5 Fosfomycin

On day 1st of treatment, fosfomycin dosing regimens reached the ≥ 90% PTA ($fAUC_{0-24}$/MIC = 21.5) at MICs ≤ 256 µg/mL as follows: 24 g/day (a loading dose of 8 g, followed by 4 g drip in 1 hour every 4 hours or 6 g drip in 1 hour every 6 hours) in general patients, 16 g/day (a loading dose 8 g, followed by 4 g drip in 0.5-2 hour every 6 hours) in patients with creatinine clearance 51-90 mL/min, and 12 g/day (a loading dose 8 g, followed by 4 g drip in 0.5-2 hour every 8 hours) in patients with creatinine clearance 26-50 mL/min.

Furthermore, 12 g/day of fosfomycin dosing regimens (a loading dose of 8 g, followed by 4 g drip in 0.5-2 hours every 8 hours) in patients with CrCL 51-90 mL/min as well as all dosing regimens with CrCL ≤ 50 mL/min reached in the PTA target at MICs ≤ 128 of µg/mL on day 1st of treatment.

On day 2nd of treatment, all fosfomycin dosing regimens achieved in the ≥ 90% PTA at MICs ≤ 256 of µg/mL, except a loading dose of 4 mg, followed by 2 g drip in 0.5-2 hours every 12 hours in a patient with creatinine clearance 0-9 mL/min.

On day 5th of treatment, all fosfomycin dosing regimens achieved the ≥ 90% PTA at MICs ≤ 256 µg/mL. No regimens achieved the target at MIC_{50} and MIC_{90} (>1,024 µg/mL). The PTA for the fosfomycin dosing regimens is presented in Table 30-33.

4.2.1.6 Ceftazidime-avibactam

For ceftazidime-avibactam dosing regimens, the 6-hours and 8-hours intervals were achieved in the PTA target at MICs ≤ 4 and ≤ 8 μg/mL, respectively. Regarding PTA for various ceftazidime-avibactam regimens, for pathogens with a MIC of 8 μg/mL (the current susceptibility breakpoint for ceftazidime-avibactam), the optimal PTA target of *f*Time>MIC ≥50% was achieved in all studied regimens. For ≥90% PTA target of *f*Time>MIC 100%, the current ceftazidime-avibactam recommended dose, namely 2.5 g every 8 h, has to be infused longer time as 2-3 hours to optimally cover CRKP with a ceftazidime-avibactam MIC of 8 μg/mL. Whereas the regimen of 2.5 g every 8 hours with standard infusion time (0.5h) was effective against only isolates with ceftazidime-avibactam MICs of ≤4 μg/mL. This approach of prolonged infusion was also able to achieve PK/PD index of avibactam at avibactam *f*Ctrough exceeding 0.5 μg/mL entire time interval of ceftazidime-avibactam administration. None of the ceftazidime-avibactam dosing regimens met the PTA target at MIC 50 and MIC 90 (>16/4 μg/mL). Prolonged infusion regimens had obtained a higher PTA target than standard infusion. The PTA for the fosfomycin dosing regimens is presented in Table 34.

Table 29 The Probability of Target Attaining (PTA) for colistin dosing regimens

Patients	Regimens	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256
CrCL 0-9 mL/min	LD 300 mg + MD 100 mg inf 0.5 h q 12 h	100	100	100	99.98	99.9	99.51	96.65	86.83	66.23	41.81	20	7.13	1.79
	LD 300 mg + MD 100 mg inf 0.5 h q 24 h	100	100	100	99.94	99.49	95.48	82.01	55.91	28.24	9.85	2.62	0.4	0.02
	LD 300 mg + MD 150 mg inf 0.5 h q 12 h	100	100	99.99	99.98	99.96	99.55	96.65	85.91	66.41	40.67	19.29	6.62	1.69
	LD 300 mg + MD 150 mg inf 0.5 h q 24 h	100	100	100	99.96	99.35	95.51	81.4	54.98	27.61	9.94	2.62	0.37	0.05
	LD 300 mg + MD 180 mg inf 0.5 h q 24 h	100	100	99.98	99.93	99.38	95.71	81.12	55.52	28.16	9.81	2.34	0.39	0.03
CrCL 10-25 mL/min	LD 300 mg + MD 100 mg inf 0.5 h q 12 h	100	100	99.99	99.94	99.63	98.14	91.56	74.75	49.17	24.84	9.15	2.22	0.23
	LD 300 mg + MD 150 mg inf 0.5 h q 12 h	100	100	99.98	99.93	99.53	97.61	90.41	73.35	49.16	24.89	9.64	2.4	0.36
	LD 300 mg + MD 150 mg inf 0.5 h q 24 h	99.99	99.98	99.93	99.59	97.34	87.87	66.04	37.33	14.36	3.62	0.49	0.02	0
	LD 300 mg + MD 180 mg inf 0.5 h q 24 h	99.98	99.96	99.86	99.6	97.15	87.84	64.8	35.69	13.64	3.75	0.62	0.06	0
CrCL 26-50 mL/min	LD 300 mg + MD 100 mg inf 0.5 h q 12 h	99.98	99.92	99.74	99.22	97.4	91.36	76.8	53.82	28.9	11.36	3.18	0.46	0.03
	LD 300 mg + MD 150 mg inf 0.5 h q 12 h	99.99	99.96	99.85	99.35	97.55	91.58	77.24	54.28	28.96	11.3	3.21	0.47	0.02
	LD 300 mg + MD 150 mg inf 0.5 h q 24 h	99.96	99.81	99.11	96.74	89.07	70.46	43.46	19.18	5.6	1.05	0.18	0	0
	LD 300 mg + MD 180 mg inf 0.5 h q 24 h	99.94	99.77	99.29	97.08	89.08	71.17	43.28	19.54	5.92	1.21	0.1	0.03	0
CrCL 51-90 mL/min	LD 300 mg + MD 150 mg inf 0.5 h q 12 h	99.8	99.43	98.35	95.49	88.53	75.18	53.92	31.57	13.66	4.34	0.94	0.1	0.01
	LD 300 mg + MD 150 mg inf 0.5 h q 8 h	99.97	99.82	99.37	97.84	94.3	85.81	71.12	51.01	29.7	12.94	4.33	0.97	0.14
	LD 300 mg + MD 180 mg inf 0.5 h q 12 h	99.89	99.55	98.71	95.54	88.59	74.66	53.55	31.23	13.66	4.58	1.08	0.18	0.02
	LD 300 mg + MD 180 mg inf 0.5 h q 8 h	99.91	99.8	99.28	97.8	94.11	85.89	71.51	51.11	30.35	13.71	4.31	0.82	0.09
CrCL 91-130	LD 300 mg + MD 150 mg inf 0.5 h q 12 h	99.24	98.16	94.91	87.75	74.75	55.61	34.02	16.29	5.62	1.35	0.23	0.03	0.01

Patients	Regimens	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256
mL/min	LD 300 mg + MD 150 mg inf 0.5 h q 8 h	99.68	98.95	97.07	92.86	84.2	70.51	52.22	31.94	15.79	5.42	1.12	0.17	0
	LD 300 mg + MD 180 mg inf 0.5 h q 12 h	99.4	97.83	94.55	87.44	74.6	55.21	33.4	16.12	5.84	1.6	0.23	0.02	0
	LD 300 mg + MD 180 mg inf 0.5 h q 8 h	99.75	99.07	97.17	92.97	84.75	70.94	51.38	31.25	14.46	4.9	1.11	0.17	0.01

Abbreviations: LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

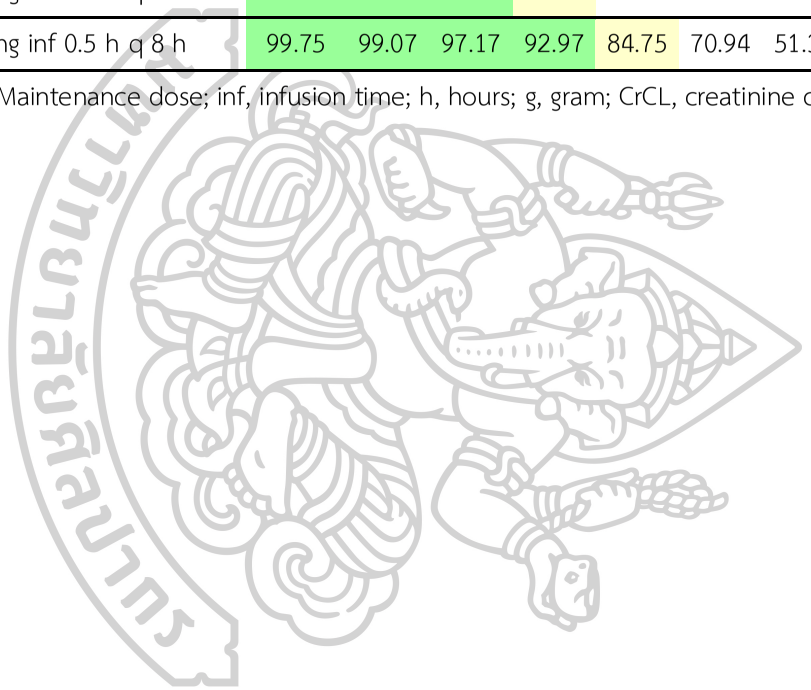


Table 30 The Probability of Target Attaining (PTA) for fosfomycin dosing regimens in general patients

Patients	Regimens	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4096
General	LD 8 g + MD 4 g inf 1 h q 4 h	100	100	100	100	100	100	100	100	100	100	99.93	98.5	90.96	64.77	14.18	0.01	0
	LD 8 g + MD 4 g inf 2 h q 4 h	100	100	100	100	100	100	100	100	100	100	99.9	98.85	91.11	64.38	13.92	0	0
	LD 8 g + MD 6 g inf 1 h q 6 h	100	100	100	100	100	100	100	100	100	100	99.91	98.74	91.11	66.42	24.26	0.44	0
	LD 8 g + MD 6 g inf 2 h q 6 h	100	100	100	100	100	100	100	100	100	100	99.87	98.79	91.27	67.76	25.38	0.45	0

Abbreviations: LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram

Table 31 The Probability of Target Attaining (PTA) for fosfomycin dosing regimens (day 1st of treatment)

Patients	Regimens	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4096
CrCL 0-9 mL/min	LD 4 g + MD 2 g inf 0.5 h q 12 h	100	100	100	100	100	100	100	100	100	100	99.99	94.76	1.29	0	0	0	0
	LD 4 g + MD 2 g inf 2 h q 12 h	100	100	100	100	100	100	100	100	100	100	99.99	93.74	0.8	0	0	0	0
	LD 8 g + MD 4 g inf 0.5 h q 12 h	100	100	100	100	100	100	100	100	100	100	100	100	94.56	1.23	0	0	0
	LD 8 g + MD 4 g inf 2 h q 12 h	100	100	100	100	100	100	100	100	100	100	100	100	94.08	1.11	0	0	0
CrCL 10-25 mL/min	LD 8 g + MD 4 g inf 0.5 h q 12 h	100	100	100	100	100	100	100	100	100	100	100	99.98	88.36	0	0	0	0
	LD 8 g + MD 4 g inf 0.5 h q 8 h	100	100	100	100	100	100	100	100	100	100	100	100	98.44	5.07	0	0	0
	LD 8 g + MD 4 g inf 2 h q 12 h	100	100	100	100	100	100	100	100	100	100	100	99.99	87.17	0	0	0	0
	LD 8 g + MD 4 g inf 2 h q 8 h	100	100	100	100	100	100	100	100	100	100	100	100	98.56	3.85	0	0	0
CrCL 26-50 mL/min	LD 8 g + MD 4 g inf 0.5 h q 12 h	100	100	100	100	100	100	100	100	100	100	100	99.99	57.09	0	0	0	0
	LD 8 g + MD 4 g inf 0.5 h q 8 h	100	100	100	100	100	100	100	100	100	100	100	100	95.76	0	0	0	0
	LD 8 g + MD 4 g inf 2 h q 12 h	100	100	100	100	100	100	100	100	100	100	100	100	52.26	0	0	0	0
	LD 8 g + MD 4 g inf 2 h q 8 h	100	100	100	100	100	100	100	100	100	100	100	100	95.22	0	0	0	0
CrCL 51-90 mL/min	LD 8 g + MD 4 g inf 0.5 h q 6 h	100	100	100	100	100	100	100	100	100	100	100	100	97.47	0	0	0	0
	LD 8 g + MD 4 g inf 0.5 h q 8 h	100	100	100	100	100	100	100	100	100	100	100	100	64.18	0	0	0	0
	LD 8 g + MD 4 g inf 2 h q 6 h	100	100	100	100	100	100	100	100	100	100	100	100	96.67	0	0	0	0
	LD 8 g + MD 4 g inf 2 h q 8 h	100	100	100	100	100	100	100	100	100	100	100	99.99	58.31	0	0	0	0

Abbreviations: LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

Table 32 The Probability of Target Attaining (PTA) for fosfomycin dosing regimens (day 2nd of treatment)

Patients	Regimens	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4096
CrCL 0-9 mL/min	LD 4 g + MD 2 g inf 0.5 h q 12 h	100	100	100	100	100	100	100	100	100	100	100	99.89	57.85	0	0	0	0
	LD 4 g + MD 2 g inf 2 h q 12 h	100	100	100	100	100	100	100	100	100	100	100	99.87	56.21	0	0	0	0
	LD 8 g + MD 4 g inf 0.5 h q 12 h	100	100	100	100	100	100	100	100	100	100	100	100	99.88	58.27	0	0	0
	LD 8 g + MD 4 g inf 2 h q 12 h	100	100	100	100	100	100	100	100	100	100	100	100	99.87	55.69	0	0	0
CrCL 10-25 mL/min	LD 8 g + MD 4 g inf 0.5 h q 12 h	100	100	100	100	100	100	100	100	100	100	100	100	99.58	7.99	0	0	0
	LD 8 g + MD 4 g inf 0.5 h q 8 h	100	100	100	100	100	100	100	100	100	100	100	100	99.99	80.47	0	0	0
	LD 8 g + MD 4 g inf 2 h q 12 h	100	100	100	100	100	100	100	100	100	100	100	100	99.55	6.55	0	0	0
	LD 8 g + MD 4 g inf 2 h q 8 h	100	100	100	100	100	100	100	100	100	100	100	100	99.99	78.96	0	0	0
CrCL 26-50 mL/min	LD 8 g + MD 4 g inf 0.5 h q 12 h	100	100	100	100	100	100	100	100	100	100	100	100	96.01	0	0	0	0
	LD 8 g + MD 4 g inf 0.5 h q 8 h	100	100	100	100	100	100	100	100	100	100	100	100	99.99	4.48	0	0	0
	LD 8 g + MD 4 g inf 2 h q 12 h	100	100	100	100	100	100	100	100	100	100	100	100	95.69	0	0	0	0
	LD 8 g + MD 4 g inf 2 h q 8 h	100	100	100	100	100	100	100	100	100	100	100	100	99.99	3.67	0	0	0
CrCL 51-90 mL/min	LD 8 g + MD 4 g inf 0.5 h q 6 h	100	100	100	100	100	100	100	100	100	100	100	100	99.98	0	0	0	0
	LD 8 g + MD 4 g inf 0.5 h q 8 h	100	100	100	100	100	100	100	100	100	100	100	100	98.53	0	0	0	0
	LD 8 g + MD 4 g inf 2 h q 6 h	100	100	100	100	100	100	100	100	100	100	100	100	99.99	0	0	0	0
	LD 8 g + MD 4 g inf 2 h q 8 h	100	100	100	100	100	100	100	100	100	100	100	100	98.06	0	0	0	0

Abbreviations: LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

Table 33 The Probability of Target Attaining (PTA) for fosfomycin dosing regimens (day 5th of treatment)

Patients	Regimens	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4096
CrCL 0-9 mL/min	LD 4 g + MD 2 g inf 0.5 h q 12 h	100	100	100	100	100	100	100	100	100	100	100	100	99.84	40.03	0	0	0
	LD 4 g + MD 2 g inf 2 h q 12 h	100	100	100	100	100	100	100	100	100	100	100	100	99.81	38.63	0	0	0
	LD 8 g + MD 4 g inf 0.5 h q 12 h	100	100	100	100	100	100	100	100	100	100	100	100	99.9	57.83	0	0	0
	LD 8 g + MD 4 g inf 2 h q 12 h	100	100	100	100	100	100	100	100	100	100	100	100	100	99.84	38.51	0	0
CrCL 10-25 mL/min	LD 8 g + MD 4 g inf 0.5 h q 12 h	100	100	100	100	100	100	100	100	100	100	100	100	100	97.23	0	0	0
	LD 8 g + MD 4 g inf 0.5 h q 8 h	100	100	100	100	100	100	100	100	100	100	100	100	100	100	23.87	0	0
	LD 8 g + MD 4 g inf 2 h q 12 h	100	100	100	100	100	100	100	100	100	100	100	100	100	96.97	0	0	0
	LD 8 g + MD 4 g inf 2 h q 8 h	100	100	100	100	100	100	100	100	100	100	100	100	100	100	22.15	0	0
CrCL 26-50 mL/min	LD 8 g + MD 4 g inf 0.5 h q 12 h	100	100	100	100	100	100	100	100	100	100	100	100	100	0	0	0	0
	LD 8 g + MD 4 g inf 0.5 h q 8 h	100	100	100	100	100	100	100	100	100	100	100	100	100	97.72	0	0	0
	LD 8 g + MD 4 g inf 2 h q 12 h	100	100	100	100	100	100	100	100	100	100	100	100	100	0	0	0	0
	LD 8 g + MD 4 g inf 2 h q 8 h	100	100	100	100	100	100	100	100	100	100	100	100	100	97.64	0	0	0
CrCL 51-90 mL/min	LD 8 g + MD 4 g inf 0.5 h q 6 h	100	100	100	100	100	100	100	100	100	100	100	100	100	0	0	0	0
	LD 8 g + MD 4 g inf 0.5 h q 8 h	100	100	100	100	100	100	100	100	100	100	100	100	100	0	0	0	0
	LD 8 g + MD 4 g inf 2 h q 6 h	100	100	100	100	100	100	100	100	100	100	100	100	100	0	0	0	0
	LD 8 g + MD 4 g inf 2 h q 8 h	100	100	100	100	100	100	100	100	100	100	100	100	100	0	0	0	0

Abbreviations: LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

Table 34 The Probability of Target Attaining (PTA) 100%*f*T > MIC for ceftazidime and *f*T> 1 µg/mL for 100% of dosing interval avibactam in general patients

CAZ regimens	AVI regimens	PTA (%) for CAZ													PTA (%) for AVI
		0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	
LD 0 g + MD 2 g inf 0.5 h q 6 h	LD 0 g + MD 0.5 g inf 0.5 h q 6 h	100	100	100	100	99	98	97	92	79	55	23	3	0	89
LD 0 g + MD 2 g inf 0.5 h q 8 h	LD 0 g + MD 0.5 g inf 0.5 h q 8 h	100	100	99	99	98	95	90	81	63	35	9	0	0	78
LD 0 g + MD 2 g inf 1 h q 6 h	LD 0 g + MD 0.5 g inf 1 h q 6 h	100	100	100	100	99	99	97	93	82	58	25	3	0	91
LD 0 g + MD 2 g inf 1 h q 8 h	LD 0 g + MD 0.5 g inf 1 h q 8 h	100	100	99	99	98	95	91	82	64	36	10	0	0	79
LD 0 g + MD 2 g inf 2 h q 6 h	LD 0 g + MD 0.5 g inf 2 h q 6 h	100	100	100	100	100	99	98	95	84	60	26	3	0	93
LD 0 g + MD 2 g inf 2 h q 8 h	LD 0 g + MD 0.5 g inf 2 h q 8 h	100	100	100	99	99	97	93	85	68	39	11	1	0	82
LD 0 g + MD 2 g inf 3 h q 6 h	LD 0 g + MD 0.5 g inf 3 h q 6 h	100	100	100	100	100	99	99	96	89	66	30	4	0	95
LD 0 g + MD 2 g inf 3 h q 8 h	LD 0 g + MD 0.5 g inf 3 h q 8 h	100	100	100	100	99	98	95	87	71	43	12	0	0	87

Abbreviations: LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CAZ, ceftazidime; AVI, avibactam

4.2.2. Cumulative Fraction of Response (CFR)

4.2.2.1 Carbapenems

4.2.2.1.1 Meropenem

None of any meropenem dosing regimens met the ≥ 90% CFR target even divided by settings, types of Enterobacterales, and hospital levels. Table 35-37 showed the percentage of CFR for the meropenem dosing regimens.

4.2.2.1.2 Imipenem

There were no imipenem dosing regimens divided by settings, types of Enterobacteriaceae and hospital levels achieved in the ≥ 90% CFR target. Table 38-40 showed the percentage of CFR for the imipenem dosing regimens.

Table 35 The Cumulative Fraction Ratio (CFR) for 100%*f*T > MIC for meropenem divided by settings

Patients	Regimens	CFR (%)	
		PMK	Health Region V
CrCL 0-9 mL/min	LD 2 g + MD 0.75 g inf 0.5 h q 24 h	9.86	16.45
	LD 2 g + MD 1 g inf 0.5 h q 24 h	13.02	21.04
	LD 2 g + MD 0.75 g inf 3 h q 24 h	10.68	17.67
	LD 2 g + MD 1 g inf 3 h q 24 h	14.12	22.53
CrCL 10-25 mL/min	LD 2 g + MD 0.75 g inf 0.5 h q 12 h	10.99	14.94
	LD 2 g + MD 1 g inf 0.5 h q 12 h	15.41	22.29
	LD 2 g + MD 0.75 g inf 3 h q 12 h	12.63	16.64
	LD 2 g + MD 1 g inf 3 h q 12 h	18.06	25.90
CrCL 26-50 mL/min	LD 2 g + MD 1 g inf 0.5 h q 8 h	13.04	15.73
	LD 2 g + MD 1 g inf 0.5 h q 12 h	4.67	11.20
	LD 2 g + MD 1 g inf 3 h q 8 h	16.67	18.95
	LD 2 g + MD 1 g inf 3 h q 12 h	6.17	12.16
CrCL 51-90 mL/min	LD 2 g + MD 1 g inf 0.5 h q 8 h	3.46	11.60
	LD 2 g + MD 1 g inf 0.5 h q 6 h	9.56	14.02
	LD 2 g + MD 1 g inf 3 h q 8 h	6.49	13.06
	LD 2 g + MD 1 g inf 3 h q 6 h	15.16	16.40
General	LD 0 g + MD 1 g inf 0.5 h q 8 h	9.71	16.57

Patients	Regimens	CFR (%)	
		PMK	Health Region V
	LD 0 g + MD 1 g inf 0.5 h q 6 h	17.17	25.76
	LD 0 g + MD 2 g inf 0.5 h q 8 h	20.32	29.55
	LD 0 g + MD 1 g inf 3 h q 8 h	12.63	19.98
	LD 0 g + MD 1 g inf 3 h q 6 h	22.61	32.01
	LD 0 g + MD 2 g inf 3 h q 8 h	25.67	36.54
	LD 2 g + MD 1 g inf 0.5 h q 8 h	9.69	16.62
	LD 2 g + MD 1 g inf 0.5 h q 6 h	16.97	25.43
	LD 2 g + MD 2 g inf 0.5 h q 8 h	19.95	29.06
	LD 2 g + MD 1 g inf 3 h q 8 h	12.58	20.04
	LD 2 g + MD 1 g inf 3 h q 6 h	22.49	31.72
	LD 2 g + MD 2 g inf 3 h q 8 h	25.56	36.38

Abbreviations: CFR, Cumulative Fraction Ratio; PMK, Phramongkutklao Hospital; LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

Table 36 The Cumulative Fraction Ratio (CFR) for 100%*f*T > MIC for meropenem in health region V divided by hospital levels

		CFR (%)		
Patients	**Regimens**	**hospital-level A**	**hospital-level S**	**hospital-level M1**
CrCL 0-9 mL/min	LD 2 g + MD 0.75 g inf 0.5 h q 24 h	20.57	12.44	13.12
	LD 2 g + MD 1 g inf 0.5 h q 24 h	25.03	17.18	17.80
	LD 2 g + MD 0.75 g inf 3 h q 24 h	21.91	13.56	14.22
	LD 2 g + MD 1 g inf 3 h q 24 h	26.61	18.57	19.19
CrCL 10-25 mL/min	LD 2 g + MD 0.75 g inf 0.5 h q 12 h	20.80	9.37	9.90
	LD 2 g + MD 1 g inf 0.5 h q 12 h	27.91	16.94	17.49
	LD 2 g + MD 0.75 g inf 3 h q 12 h	22.83	10.75	11.30
	LD 2 g + MD 1 g inf 3 h q 12 h	31.56	20.50	21.04
CrCL 26-50 mL/min	LD 2 g + MD 1 g inf 0.5 h q 8 h	22.44	9.38	9.84
	LD 2 g + MD 1 g inf 0.5 h q 12 h	15.88	6.73	7.20
	LD 2 g + MD 1 g inf 3 h q 8 h	26.21	12.09	12.64
	LD 2 g + MD 1 g inf 3 h q 12 h	17.30	7.33	7.62
CrCL 51-90 mL/min	LD 2 g + MD 1 g inf 0.5 h q 8 h	16.51	6.90	7.45
	LD 2 g + MD 1 g inf 0.5 h q 6 h	20.08	8.41	8.45
	LD 2 g + MD 1 g inf 3 h q 8 h	18.61	7.92	7.95
	LD 2 g + MD 1 g inf 3 h q 6 h	23.78	9.49	9.79
General	LD 0 g + MD 1 g inf 0.5 h q 8 h	21.27	12.01	12.73

		CFR (%)		
Patients	Regimens	hospital-level A	hospital-level S	hospital-level M1
	LD 0 g + MD 1 g inf 0.5 h q 6 h	31.02	20.75	21.27
	LD 0 g + MD 2 g inf 0.5 h q 8 h	34.11	25.18	25.72
	LD 0 g + MD 1 g inf 3 h q 8 h	25.27	14.94	15.49
	LD 0 g + MD 1 g inf 3 h q 6 h	37.70	26.63	27.03
	LD 0 g + MD 2 g inf 3 h q 8 h	41.29	32.01	32.49
	LD 2 g + MD 1 g inf 0.5 h q 8 h	21.24	12.14	12.82
	LD 2 g + MD 1 g inf 0.5 h q 6 h	30.69	20.41	20.95
	LD 2 g + MD 2 g inf 0.5 h q 8 h	33.64	24.66	25.23
	LD 2 g + MD 1 g inf 3 h q 8 h	25.31	15.00	15.58
	LD 2 g + MD 1 g inf 3 h q 6 h	37.42	26.34	26.70
	LD 2 g + MD 2 g inf 3 h q 8 h	41.14	31.84	32.31

Abbreviations: CFR, Cumulative Fraction Ratio; PMK, Phramongkutklao Hospital; LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

Table 37 The Cumulative Fraction Ratio (CFR) for 100%*f*T > MIC for meropenem in health region V divided by carbapenem-resistant Enterobacterales

Patients	Regimens	CFR (%)		
		Type of carbapenem-resistance		
		Klebsiella pneumoniae	*Escherichia coli*	*Enterobacter cloacae*
CrCL 0-9 mL/min	LD 2 g + MD 0.75 g inf 0.5 h q 24 h	16.53	14.76	32.00
	LD 2 g + MD 1 g inf 0.5 h q 24 h	21.11	19.50	35.67
	LD 2 g + MD 0.75 g inf 3 h q 24 h	17.75	15.98	33.15
	LD 2 g + MD 1 g inf 3 h q 24 h	22.59	20.99	37.05
CrCL 10-25 mL/min	LD 2 g + MD 0.75 g inf 0.5 h q 12 h	15.19	12.52	32.20
	LD 2 g + MD 1 g inf 0.5 h q 12 h	22.47	20.27	37.75
	LD 2 g + MD 0.75 g inf 3 h q 12 h	16.88	14.29	33.40
	LD 2 g + MD 1 g inf 3 h q 12 h	26.05	24.06	40.55
CrCL 26-50 mL/min	LD 2 g + MD 1 g inf 0.5 h q 8 h	16.02	13.14	32.80
	LD 2 g + MD 1 g inf 0.5 h q 12 h	11.62	8.00	30.43
	LD 2 g + MD 1 g inf 3 h q 8 h	19.14	16.88	34.78
	LD 2 g + MD 1 g inf 3 h q 12 h	12.67	8.65	31.33
CrCL 51-90 mL/min	LD 2 g + MD 1 g inf 0.5 h q 8 h	12.08	8.14	31.60
	LD 2 g + MD 1 g inf 0.5 h q 6 h	14.67	10.09	32.65
	LD 2 g + MD 1 g inf 3 h q 8 h	13.78	8.78	32.53

Patients	Regimens	CFR (%)		
		Type of carbapenem-resistance		
		Klebsiella pneumoniae	*Escherichia coli*	*Enterobacter cloacae*
General	LD 2 g + MD 1 g inf 3 h q 6 h	16.79	13.54	33.14
	LD 0 g + MD 1 g inf 0.5 h q 8 h	16.74	14.37	34.43
	LD 0 g + MD 1 g inf 0.5 h q 6 h	26.01	23.49	41.52
	LD 0 g + MD 2 g inf 0.5 h q 8 h	29.71	27.70	44.04
	LD 0 g + MD 1 g inf 3 h q 8 h	20.24	17.52	37.12
	LD 0 g + MD 1 g inf 3 h q 6 h	32.27	29.85	45.94
	LD 0 g + MD 2 g inf 3 h q 8 h	36.71	34.77	49.70
	LD 2 g + MD 1 g inf 0.5 h q 8 h	16.78	14.45	34.40
	LD 2 g + MD 1 g inf 0.5 h q 6 h	25.66	23.20	41.26
	LD 2 g + MD 2 g inf 0.5 h q 8 h	29.20	27.24	43.79
	LD 2 g + MD 1 g inf 3 h q 8 h	20.30	17.60	37.23
	LD 2 g + MD 1 g inf 3 h q 6 h	32.00	29.48	45.72
	LD 2 g + MD 2 g inf 3 h q 8 h	36.54	34.62	49.54

Abbreviations: CFR, Cumulative Fraction Ratio; PMK, Phramongkutklao Hospital; LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

Table 38 The Cumulative Fraction Ratio (CFR) for 100%fT > MIC for imipenem divided by settings

Patients	Regimens	CFR (%)	
		PMK	Health region V
General	LD 0 g + MD 0.5 g inf 2 h q 6 h	5.56	7.82
	LD 0 g + MD 0.5 g inf 3 h q 6 h	6.85	9.76
	LD 0 g + MD 1 g inf 2 h q 6 h	9.28	11.65
	LD 0 g + MD 1 g inf 3 h q 6 h	11.65	13.69
	LD 0.5 g + MD 0.5 g inf 2 h q 6 h	5.50	7.73
	LD 0.5 g + MD 0.5 g inf 3 h q 6 h	6.88	9.81
	LD 0.5 g + MD 1 g inf 2 h q 6 h	9.25	11.62
	LD 0.5 g + MD 1 g inf 3 h q 6 h	11.57	13.66
	LD 1 g + MD 0.5 g inf 2 h q 6 h	5.50	7.75
	LD 1 g + MD 0.5 g inf 3 h q 6 h	6.87	9.77
	LD 1 g + MD 1 g inf 2 h q 6 h	9.33	11.63
	LD 1 g + MD 1 g inf 3 h q 6 h	11.59	13.66

Abbreviations: CFR, Cumulative Fraction Ratio; PMK, Phramongkutklao Hospital; LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

Table 39 The Cumulative Fraction Ratio (CFR) for 100%fT > MIC for imipenem in health region V divided by hospital levels

Patients	Regimens	CFR (%)		
		Hospital-level A	hospital-level S	Hospital-level M1
General	LD 0 g + MD 0.5 g inf 2 h q 6 h	11.54	3.83	5.64
	LD 0 g + MD 0.5 g inf 3 h q 6 h	14.36	4.76	7.28
	LD 0 g + MD 1 g inf 2 h q 6 h	16.96	5.57	9.39
	LD 0 g + MD 1 g inf 3 h q 6 h	19.81	6.48	11.57
	LD 0.5 g + MD 0.5 g inf 2 h q 6 h	11.42	3.79	5.58
	LD 0.5 g + MD 0.5 g inf 3 h q 6 h	14.43	4.78	7.32
	LD 0.5 g + MD 1 g inf 2 h q 6 h	16.93	5.55	9.37
	LD 0.5 g + MD 1 g inf 3 h q 6 h	19.78	6.46	11.52
	LD 1 g + MD 0.5 g inf 2 h q 6 h	11.44	3.80	5.58
	LD 1 g + MD 0.5 g inf 3 h q 6 h	14.37	4.76	7.29
	LD 1 g + MD 1 g inf 2 h q 6 h	16.94	5.56	9.41
	LD 1 g + MD 1 g inf 3 h q 6 h	19.77	6.47	11.52

Abbreviations: CFR, Cumulative Fraction Ratio; PMK, Phramongkutklao Hospital; LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

Table 40 The Cumulative Fraction Ratio (CFR) for 100%fT > MIC for imipenem in health region V divided by carbapenem-resistant Enterobacterales

Patients	Regimens	CFR (%)		
		Type of carbapenem-resistant		
		Klebsiella pneumoniae	*Escherichia coli*	*Enterobacter cloacae*
General	LD 0 g + MD 0.5 g inf 2 h q 6 h	7.44	5.45	48.72
	LD 0 g + MD 0.5 g inf 3 h q 6 h	9.31	7.06	56.92
	LD 0 g + MD 1 g inf 2 h q 6 h	11.26	8.68	59.22
	LD 0 g + MD 1 g inf 3 h q 6 h	13.33	10.42	63.64
	LD 0.5 g + MD 0.5 g inf 2 h q 6 h	7.36	5.38	48.22
	LD 0.5 g + MD 0.5 g inf 3 h q 6 h	9.36	7.11	56.98
	LD 0.5 g + MD 1 g inf 2 h q 6 h	11.24	8.65	59.20
	LD 0.5 g + MD 1 g inf 3 h q 6 h	13.30	10.38	63.73
	LD 1 g + MD 0.5 g inf 2 h q 6 h	7.36	5.39	48.47
	LD 1 g + MD 0.5 g inf 3 h q 6 h	9.33	7.07	56.86
	LD 1 g + MD 1 g inf 2 h q 6 h	11.25	8.68	59.01
	LD 1 g + MD 1 g inf 3 h q 6 h	13.30	10.37	63.83

Abbreviations: CFR, Cumulative Fraction Ratio; PMK, Phramongkutklao Hospital; LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

4.2.2.2 Aminoglycosides

4.2.2.2.1 Amikacin

There were no amikacin dosing regimens divided by settings, types of Enterobacteriaceae, and hospital levels achieved in the ≥ 90% CFR target. Table 41-43 showed the percentage of CFR for the amikacin dosing regimens.

4.2.2.2.2 Gentamicin

There were no gentamicin dosing regimens divided by settings, types of Enterobacteriaceae, and hospital levels achieved in the ≥ 90% CFR target. Table 44-46 showed the percentage of CFR for the gentamicin dosing regimens.

Table 41 The Cumulative Fraction Ratio (CFR) for C_{max}/MIC > 8 for amikacin divided by settings

Patients	Regimens	CFR (%)		CFR for combination (%)		
		PMK	Health region V	Amikacin + Tigecycline	Amikacin + Colistin	Amikacin + Fosfomycin
CrCL 0-9 mL/min	LD 15 mg/kg + MD 7.5 mg/kg inf 0.5 h q 48 h	32.06	0.03	95.95	73.98	44.63
	LD 20 mg/kg + MD 7.5 mg/kg inf 0.5 h q 48 h	32.91	0.02	96.27	74.66	46.36
	LD 25 mg/kg + MD 7.5 mg/kg inf 0.5 h q 48 h	33.20	0.03	96.37	74.89	46.94
CrCL 10-25 mL/min	LD 20 mg/kg + MD 10 mg/kg inf 0.5 h q 48 h	39.29	0.16	98.65	79.75	59.41
	LD 20 mg/kg + MD 15 mg/kg inf 0.5 h q 48 h	43.19	2.41	99.88	82.81	67.78
	LD 25 mg/kg + MD 10 mg/kg inf 0.5 h q 48 h	39.60	0.15	98.76	80.00	60.03
	LD 25 mg/kg + MD 15 mg/kg inf 0.5 h q 48 h	43.26	2.40	99.91	82.87	67.93
CrCL 26-50 mL/min	LD 20 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	43.54	3.16	99.93	83.07	68.63
	LD 20 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	45.21	8.72	100.00	84.28	73.06
	LD 25 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	43.60	3.30	99.94	83.12	68.77
	LD 25 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	45.38	9.37	100.00	84.40	73.53
CrCL 51-90 mL/min	LD 25 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	43.45	2.75	99.94	83.02	68.38
	LD 25 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	45.11	8.38	99.99	84.20	72.79
	LD 25 mg/kg + MD 20 mg/kg inf 0.5 h q 24 h	48.49	20.84	100.00	86.61	81.99
	LD 30 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	43.48	2.75	99.95	83.03	68.43
	LD 30 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	45.10	8.29	100.00	84.20	72.76
	LD 30 mg/kg + MD 20 mg/kg inf 0.5 h q 24 h	48.67	21.50	100.00	86.73	82.48

Patients	Regimens	CFR (%)		CFR for combination (%)		
		PMK	Health region V	Amikacin + Tigecycline	Amikacin + Colistin	Amikacin + Fosfomycin
CrCL 91-130 mL/min	LD 25 mg/kg + MD 12 g inf 0.5 h q 24 h	43.70	3.59	99.95	83.19	69.04
	LD 25 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	45.56	10.02	100.00	84.52	74.02
	LD 25 mg/kg + MD 20 mg/kg inf 0.5 h q 24 h	49.41	24.22	100.00	87.25	84.48
	LD 30 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	43.74	3.71	99.95	83.22	69.14
	LD 30 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	45.61	10.23	99.99	84.56	74.16
	LD 30 mg/kg + MD 20 mg/kg inf 0.5 h q 24 h	49.29	23.79	100.00	87.17	84.15
General	LD 25 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	47.83	20.24	99.81	86.09	80.53
	LD 25 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	49.69	25.98	99.92	87.42	85.31
	LD 25 mg/kg + MD 20 mg/kg inf 0.5 h q 24 h	52.11	33.65	99.99	89.08	91.22
	LD 30 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	48.07	21.12	99.81	86.25	81.13
	LD 30 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	50.13	27.32	99.94	87.72	86.36
	LD 30 mg/kg + MD 20 mg/kg inf 0.5 h q 24 h	52.33	34.27	99.99	89.21	91.65

Abbreviations: CFR, Cumulative Fraction Ratio; PMK, Phramongkutklao Hospital; LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

Table 42 The Cumulative Fraction Ratio (CFR) for C_{max}/MIC > 8 for amikacin in health region V divided by hospital levels

Patients	Regimens	CFR (%)		
		Hospital-level A	Hospital-level S	Hospital-level M1
CrCL 0-9 mL/min	LD 15 mg/kg + MD 7.5 mg/kg inf 0.5 h q 48 h	0.03	0.03	0.02
	LD 20 mg/kg + MD 7.5 mg/kg inf 0.5 h q 48 h	0.02	0.02	0.02
	LD 25 mg/kg + MD 7.5 mg/kg inf 0.5 h q 48 h	0.03	0.03	0.02
CrCL 10-25 mL/min	LD 20 mg/kg + MD 10 mg/kg inf 0.5 h q 48 h	0.16	0.17	0.15
	LD 20 mg/kg + MD 15 mg/kg inf 0.5 h q 48 h	2.36	2.56	2.22
	LD 25 mg/kg + MD 10 mg/kg inf 0.5 h q 48 h	0.15	0.16	0.14
	LD 25 mg/kg + MD 15 mg/kg inf 0.5 h q 48 h	2.36	2.55	2.21
CrCL 26-50 mL/min	LD 20 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	3.10	3.36	2.91
	LD 20 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	8.55	9.26	8.01
	LD 25 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	3.24	3.51	3.03
	LD 25 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	9.18	9.95	8.61
CrCL 51-90 mL/min	LD 25 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	2.70	2.92	2.53
	LD 25 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	8.22	8.90	7.70
	LD 25 mg/kg + MD 20 mg/kg inf 0.5 h q 24 h	20.43	22.14	19.16
	LD 30 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	2.69	2.92	2.52
	LD 30 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	8.13	8.81	7.62
	LD 30 mg/kg + MD 20 mg/kg inf 0.5 h q 24 h	21.08	22.84	19.76

Patients	Regimens	CFR (%)		
		Hospital-level A	Hospital-level S	Hospital-level M1
CrCL 91-130 mL/min	LD 25 mg/kg + MD 12 g inf 0.5 h q 24 h	3.52	3.81	3.30
	LD 25 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	9.83	10.65	9.21
	LD 25 mg/kg + MD 20 mg/kg inf 0.5 h q 24 h	23.74	25.73	22.26
	LD 30 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	3.64	3.94	3.41
	LD 30 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	10.03	10.87	9.40
	LD 30 mg/kg + MD 20 mg/kg inf 0.5 h q 24 h	23.32	25.27	21.86
General	LD 25 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	19.84	21.50	18.60
	LD 25 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	25.48	27.60	23.88
	LD 25 mg/kg + MD 20 mg/kg inf 0.5 h q 24 h	33.00	35.72	30.91
	LD 30 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	20.71	22.44	19.41
	LD 30 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	26.79	29.01	25.10
	LD 30 mg/kg + MD 20 mg/kg inf 0.5 h q 24 h	33.62	36.38	31.49

Abbreviations: CFR, Cumulative Fraction Ratio; PMK, Phramongkutklao Hospital; LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

Table 43 The Cumulative Fraction Ratio (CFR) for C_{max}/MIC > 8 for amikacin in health region V divided by carbapenem-resistant Enterobacterales

Patients	Regimens	CFR (%)		
		Hospital-level A	Hospital-level S	Hospital-level M1
CrCL 0-9 mL/min	LD 15 mg/kg + MD 7.5 mg/kg inf 0.5 h q 48 h	0.02	0.04	0.04
	LD 20 mg/kg + MD 7.5 mg/kg inf 0.5 h q 48 h	0.02	0.03	0.03
	LD 25 mg/kg + MD 7.5 mg/kg inf 0.5 h q 48 h	0.02	0.04	0.04
CrCL 10-25 mL/min	LD 20 mg/kg + MD 10 mg/kg inf 0.5 h q 48 h	0.14	0.25	0.24
	LD 20 mg/kg + MD 15 mg/kg inf 0.5 h q 48 h	2.01	3.71	3.55
	LD 25 mg/kg + MD 10 mg/kg inf 0.5 h q 48 h	0.13	0.24	0.23
	LD 25 mg/kg + MD 15 mg/kg inf 0.5 h q 48 h	2.00	3.69	3.53
CrCL 26-50 mL/min	LD 20 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	2.63	4.87	4.65
	LD 20 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	7.25	13.40	12.82
	LD 25 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	2.75	5.07	4.85
	LD 25 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	7.79	14.40	13.77
CrCL 51-90 mL/min	LD 25 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	2.29	4.23	4.05
	LD 25 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	6.97	12.89	12.33
	LD 25 mg/kg + MD 20 mg/kg inf 0.5 h q 24 h	17.34	32.05	30.65
	LD 30 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	2.29	4.22	4.04
	LD 30 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	6.90	12.75	12.19

Patients	Regimens	CFR (%)		
		Hospital-level A	Hospital-level S	Hospital-level M1
	LD 30 mg/kg + MD 20 mg/kg inf 0.5 h q 24 h	17.89	33.06	31.62
CrCL 91-130 mL/min	LD 25 mg/kg + MD 12 g inf 0.5 h q 24 h	2.98	5.51	5.27
	LD 25 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	8.34	15.41	14.74
	LD 25 mg/kg + MD 20 mg/kg inf 0.5 h q 24 h	20.15	37.24	35.62
	LD 30 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	3.09	5.70	5.45
	LD 30 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	8.51	15.73	15.05
	LD 30 mg/kg + MD 20 mg/kg inf 0.5 h q 24 h	19.80	36.58	34.99
General	LD 25 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	16.84	31.12	29.76
	LD 25 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	21.63	39.93	38.18
	LD 25 mg/kg + MD 20 mg/kg inf 0.5 h q 24 h	28.05	51.59	49.25
	LD 30 mg/kg + MD 12 mg/kg inf 0.5 h q 24 h	17.58	32.47	31.05
	LD 30 mg/kg + MD 15 mg/kg inf 0.5 h q 24 h	22.75	41.96	40.10
	LD 30 mg/kg + MD 20 mg/kg inf 0.5 h q 24 h	28.58	52.51	50.11

Abbreviations: CFR, Cumulative Fraction Ratio; PMK, Phramongkutklao Hospital; LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

Table 44 The Cumulative Fraction Ratio (CFR) for C_{max}/MIC > 8 for gentamicin divided by settings

Patients	Regimens	CFR (%)		CFR for combination (%)		
		PMK	Health region V	Gentamicin + Tigecycline	Gentamicin + Colistin	Gentamicin + Fosfomycin
CrCL 0-9 mL/min	LD 3 mg/kg + MD 2.5 mg/kg inf 0.5 h q 48 h	76.25	58.12	100.00	85.36	100.00
	LD 5 mg/kg + MD 2.5 mg/kg inf 0.5 h q 48 h	76.02	58.09	100.00	85.17	100.00
	LD 7 mg/kg + MD 2.5 mg/kg inf 0.5 h q 48 h	76.20	58.18	100.00	85.36	100.00
CrCL 10-25 mL/min	LD 5 mg/kg + MD 4 mg/kg inf 0.5 h q 48 h	70.73	53.69	100.00	78.10	100.00
	LD 7 mg/kg + MD 3 mg/kg inf 0.5 h q 48 h	69.83	50.01	100.00	74.84	100.00
	LD 7 mg/kg + MD 5 mg/kg inf 0.5 h q 48 h	72.05	55.70	100.00	80.51	100.00
	LD 8 mg/kg + MD 3 mg/kg inf 0.5 h q 48 h	69.83	50.19	100.00	74.96	100.00
	LD 8 mg/kg + MD 5 mg/kg inf 0.5 h q 48 h	72.18	55.66	100.00	80.58	100.00
CrCL 26-50 mL/min	LD 5 mg/kg + MD 3 mg/kg inf 0.5 h q 24 h	69.79	50.15	100.00	74.90	100.00
	LD 5 mg/kg + MD 4 mg/kg inf 0.5 h q 24 h	70.61	53.94	100.00	78.19	100.00
	LD 7 mg/kg + MD 3 mg/kg inf 0.5 h q 24 h	69.82	50.33	100.00	75.05	100.00
	LD 7 mg/kg + MD 4 mg/kg inf 0.5 h q 24 h	70.72	53.75	100.00	78.13	100.00
	LD 8 mg/kg + MD 3 mg/kg inf 0.5 h q 24 h	69.80	50.25	100.00	74.99	100.00
	LD 8 mg/kg + MD 4 mg/kg inf 0.5 h q 24 h	70.79	53.97	100.00	78.35	100.00
CrCL 51-90 mL/min	LD 7 mg/kg + MD 5 mg/kg inf 0.5 h q 24 h	70.36	53.71	100.00	77.84	100.00
	LD 7 mg/kg + MD 6 mg/kg inf 0.5 h q 24 h	71.30	55.24	100.00	79.62	100.00
	LD 8 mg/kg + MD 5 mg/kg inf 0.5 h q 24 h	70.38	53.79	100.00	77.91	100.00

Patients	Regimens	CFR (%)		CFR for combination (%)		
		PMK	Health region V	Gentamicin + Tigecycline	Gentamicin + Colistin	Gentamicin + Fosfomycin
	LD 8 mg/kg + MD 6 mg/kg inf 0.5 h q 24 h	71.33	55.16	100.00	79.59	100.00
	LD 8 mg/kg + MD 7 mg/kg inf 0.5 h q 24 h	72.56	56.27	100.00	81.29	100.00
CrCL 91-130 mL/min	LD 7 mg/kg + MD 5 mg/kg inf 0.5 h q 24 h	70.26	52.96	100.00	77.23	100.00
	LD 7 mg/kg + MD 6 mg/kg inf 0.5 h q 24 h	71.26	54.76	100.00	79.25	100.00
	LD 8 mg/kg + MD 5 mg/kg inf 0.5 h q 24 h	70.29	52.89	100.00	77.21	100.00
	LD 8 mg/kg + MD 6 mg/kg inf 0.5 h q 24 h	71.21	54.66	100.00	79.14	100.00
	LD 8 mg/kg + MD 7 mg/kg inf 0.5 h q 24 h	72.27	55.88	100.00	80.79	100.00
General	LD 7 mg/kg + MD 4 mg/kg inf 0.5 h q 24 h	69.47	46.33	100.00	71.97	99.99
	LD 7 mg/kg + MD 5 mg/kg inf 0.5 h q 24 h	69.66	50.27	100.00	74.89	100.00
	LD 7 mg/kg + MD 6 mg/kg inf 0.5 h q 24 h	69.66	50.24	100.00	74.87	100.00
	LD 8 mg/kg + MD 4 mg/kg inf 0.5 h q 24 h	69.45	46.54	100.00	72.11	99.99
	LD 8 mg/kg + MD 5 mg/kg inf 0.5 h q 24 h	69.65	50.34	100.00	74.93	100.00
	LD 8 mg/kg + MD 6 mg/kg inf 0.5 h q 24 h	69.99	52.73	100.00	76.87	100.00
	LD 8 mg/kg + MD 7 mg/kg inf 0.5 h q 24 h	70.63	54.23	100.00	78.41	100.00

Abbreviations: CFR, Cumulative Fraction Ratio; PMK, Phramongkutklao Hospital; LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

Table 45 The Cumulative Fraction Ratio (CFR) for C_{max}/MIC > 8 for gentamicin in health region V divided by hospital levels

Patients	Regimens	CFR (%)		
		Hospital-level A	Hospital-level S	Hospital-level M1
CrCL 0-9 mL/min	LD 3 mg/kg + MD 2.5 mg/kg inf 0.5 h q 48 h	59.04	62.81	44.75
	LD 5 mg/kg + MD 2.5 mg/kg inf 0.5 h q 48 h	59.02	62.77	44.74
	LD 7 mg/kg + MD 2.5 mg/kg inf 0.5 h q 48 h	59.08	62.87	44.84
CrCL 10-25 mL/min	LD 5 mg/kg + MD 4 mg/kg inf 0.5 h q 48 h	55.62	57.49	39.30
	LD 7 mg/kg + MD 3 mg/kg inf 0.5 h q 48 h	52.74	53.29	34.42
	LD 7 mg/kg + MD 5 mg/kg inf 0.5 h q 48 h	57.19	59.83	41.89
	LD 8 mg/kg + MD 3 mg/kg inf 0.5 h q 48 h	52.88	53.49	34.66
	LD 8 mg/kg + MD 5 mg/kg inf 0.5 h q 48 h	57.16	59.79	41.82
CrCL 26-50 mL/min	LD 5 mg/kg + MD 3 mg/kg inf 0.5 h q 24 h	52.84	53.44	34.61
	LD 5 mg/kg + MD 4 mg/kg inf 0.5 h q 24 h	55.82	57.75	39.65
	LD 7 mg/kg + MD 3 mg/kg inf 0.5 h q 24 h	52.98	53.65	34.86
	LD 7 mg/kg + MD 4 mg/kg inf 0.5 h q 24 h	55.67	57.55	39.38
	LD 8 mg/kg + MD 3 mg/kg inf 0.5 h q 24 h	52.92	53.56	34.75
	LD 8 mg/kg + MD 4 mg/kg inf 0.5 h q 24 h	55.84	57.80	39.68
CrCL 51-90 mL/min	LD 7 mg/kg + MD 5 mg/kg inf 0.5 h q 24 h	55.64	57.48	39.36
	LD 7 mg/kg + MD 6 mg/kg inf 0.5 h q 24 h	56.84	59.27	41.34
	LD 8 mg/kg + MD 5 mg/kg inf 0.5 h q 24 h	55.70	57.57	39.47
	LD 8 mg/kg + MD 6 mg/kg inf 0.5 h q 24 h	56.78	59.18	41.24

Patients	Regimens	CFR (%)		
		Hospital-level A	Hospital-level S	Hospital-level M1
	LD 8 mg/kg + MD 7 mg/kg inf 0.5 h q 24 h	57.63	60.50	42.61
CrCL 91-130 mL/min	LD 7 mg/kg + MD 5 mg/kg inf 0.5 h q 24 h	55.05	56.63	38.35
	LD 7 mg/kg + MD 6 mg/kg inf 0.5 h q 24 h	56.45	58.72	40.69
	LD 8 mg/kg + MD 5 mg/kg inf 0.5 h q 24 h	55.00	56.56	38.26
	LD 8 mg/kg + MD 6 mg/kg inf 0.5 h q 24 h	56.38	58.61	40.56
	LD 8 mg/kg + MD 7 mg/kg inf 0.5 h q 24 h	57.33	60.04	42.11
General	LD 7 mg/kg + MD 4 mg/kg inf 0.5 h q 24 h	49.83	49.12	29.50
	LD 7 mg/kg + MD 5 mg/kg inf 0.5 h q 24 h	52.94	53.57	34.79
	LD 7 mg/kg + MD 6 mg/kg inf 0.5 h q 24 h	52.92	53.54	34.75
	LD 8 mg/kg + MD 4 mg/kg inf 0.5 h q 24 h	49.99	49.36	29.79
	LD 8 mg/kg + MD 5 mg/kg inf 0.5 h q 24 h	52.99	53.65	34.89
	LD 8 mg/kg + MD 6 mg/kg inf 0.5 h q 24 h	54.87	56.36	38.08
	LD 8 mg/kg + MD 7 mg/kg inf 0.5 h q 24 h	56.05	58.09	40.05

Abbreviations: CFR, Cumulative Fraction Ratio; PMK, Phramongkutklao Hospital; LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

Table 46 The Cumulative Fraction Ratio (CFR) for C_{max}/MIC > 8 for gentamicin in health region V divided by carbapenem-resistant Enterobacterales

Patients	Regimens	CFR (%)		
		Type of carbapenem-resistant		
		Klebsiella pneumoniae	*Escherichia coli*	*Enterobacter cloacae*
CrCL 0-9 mL/min	LD 3 mg/kg + MD 2.5 mg/kg inf 0.5 h q 48 h	69.64	20.58	33.33
	LD 5 mg/kg + MD 2.5 mg/kg inf 0.5 h q 48 h	69.62	20.53	33.33
	LD 7 mg/kg + MD 2.5 mg/kg inf 0.5 h q 48 h	69.71	20.60	33.33
CrCL 10-25 mL/min	LD 5 mg/kg + MD 4 mg/kg inf 0.5 h q 48 h	64.68	17.57	33.33
	LD 7 mg/kg + MD 3 mg/kg inf 0.5 h q 48 h	60.40	15.63	33.33
	LD 7 mg/kg + MD 5 mg/kg inf 0.5 h q 48 h	66.98	18.76	33.33
	LD 8 mg/kg + MD 3 mg/kg inf 0.5 h q 48 h	60.61	15.72	33.33
	LD 8 mg/kg + MD 5 mg/kg inf 0.5 h q 48 h	66.93	18.76	33.33
CrCL 26-50 mL/min	LD 5 mg/kg + MD 3 mg/kg inf 0.5 h q 24 h	60.56	15.69	33.32
	LD 5 mg/kg + MD 4 mg/kg inf 0.5 h q 24 h	64.97	17.68	33.33
	LD 7 mg/kg + MD 3 mg/kg inf 0.5 h q 24 h	60.77	15.79	33.32
	LD 7 mg/kg + MD 4 mg/kg inf 0.5 h q 24 h	64.74	17.60	33.33
	LD 8 mg/kg + MD 3 mg/kg inf 0.5 h q 24 h	60.68	15.74	33.32
	LD 8 mg/kg + MD 4 mg/kg inf 0.5 h q 24 h	65.00	17.72	33.33
CrCL 51-90 mL/min	LD 7 mg/kg + MD 5 mg/kg inf 0.5 h q 24 h	64.71	17.52	33.33

Patients	Regimens	CFR (%)		
		Type of carbapenem-resistant		
		Klebsiella pneumoniae	*Escherichia coli*	*Enterobacter cloacae*
	LD 7 mg/kg + MD 6 mg/kg inf 0.5 h q 24 h	66.47	18.42	33.33
	LD 8 mg/kg + MD 5 mg/kg inf 0.5 h q 24 h	64.81	17.57	33.33
	LD 8 mg/kg + MD 6 mg/kg inf 0.5 h q 24 h	66.38	18.39	33.33
	LD 8 mg/kg + MD 7 mg/kg inf 0.5 h q 24 h	67.62	19.11	33.33
CrCL 91-130 mL/min	LD 7 mg/kg + MD 5 mg/kg inf 0.5 h q 24 h	63.83	17.14	33.33
	LD 7 mg/kg + MD 6 mg/kg inf 0.5 h q 24 h	65.90	18.17	33.33
	LD 8 mg/kg + MD 5 mg/kg inf 0.5 h q 24 h	63.76	17.11	33.33
	LD 8 mg/kg + MD 6 mg/kg inf 0.5 h q 24 h	65.79	18.12	33.33
	LD 8 mg/kg + MD 7 mg/kg inf 0.5 h q 24 h	67.18	18.88	33.33
General	LD 7 mg/kg + MD 4 mg/kg inf 0.5 h q 24 h	56.09	13.77	33.31
	LD 7 mg/kg + MD 5 mg/kg inf 0.5 h q 24 h	60.71	15.73	33.33
	LD 7 mg/kg + MD 6 mg/kg inf 0.5 h q 24 h	60.68	15.72	33.33
	LD 8 mg/kg + MD 4 mg/kg inf 0.5 h q 24 h	56.34	13.87	33.30
	LD 8 mg/kg + MD 5 mg/kg inf 0.5 h q 24 h	60.79	15.77	33.32
	LD 8 mg/kg + MD 6 mg/kg inf 0.5 h q 24 h	63.58	16.99	33.33
	LD 8 mg/kg + MD 7 mg/kg inf 0.5 h q 24 h	65.32	17.82	33.33

Abbreviations: CFR, Cumulative Fraction Ratio; PMK, Phramongkutklao Hospital; LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

4.2.2.3 Tigecycline

Double high dose tigecycline (loading dose 400 mg, followed by 200 mg infusion 0.5 h every 12 hours) reached the ≥ 90% CFR target for either the university or non-university hospitals. Based on the type of carbapenem-resistant Enterobacterales and hospital levels from Health region V, double high dose tigecycline was also achieved in the CFR target. Nonetheless, the CFR of the usual dosing regimen (tigecycline 100 mg every 12 hours) ranged from 76-88%. Table 47-49 showed the percentage of CFR for the tigecyccline dosing regimens

4.2.2.4 Colistin

Although most colistin dosing regimens divided by settings could not achieve the CFR target, the colistin dosing regimens ranged from 100-150 mg every 12 hours in patients with CrCL of 0-9 mL/min (91% of CFR). Based on hospital levels, hospital-level U and A could not reach the CFR target, whereas the hospital-level S and M1 met the CFR target in patients with CrCL > 25 mL/min.

Based on CrCL, the colistin dosing regimens (loading dose 300 mg and maintenance dose 100-180 mg infusion 0.5 hours every 12 hours) met the CFR target in patients with CrCL ≤ 50 mL/min for *E. coli* and E. *cloacae*. Additionally, loading dose 300 mg and maintenance dose 150 mg infusion 0.5 hours every 8 hours) reached the CFR target in patients with CrCL 51-90 mL/min, whereas none of any colistin regimens in patients with CrCL 91-130 mL/min were achieved in the CFR. Table 50-52 showed the percentage of CFR for the colistin dosing regimens

Table 47 The Cumulative Fraction Ratio (CFR) for $fAUC_{0-24}$/MIC ≥ 0.9 for tigecycline divided by settings

Patients	Regimens	CFR (%)		CFR for combination (%)			
		PMK	Health region V	Tigecycline + Amikacin	Tigecycline + Gentamicin	Tigecycline + Colistin	Tigecycline + Fosfomycin
General	LD 200 mg + MD 100 mg inf 0.5 h q 12 h	76.70	88.07	96.05	98.09	95.93	88.25
	LD 200 mg + MD 100 mg inf 0.5 h q 24 h	40.45	70.11	85.52	82.92	79.44	74.88
	LD 400 mg + MD 100 mg inf 0.5 h q 12 h	77.48	88.34	96.14	98.18	96.12	88.35
	LD 400 mg + MD 100 mg inf 0.5 h q 24 h	40.55	70.18	85.57	83.00	79.51	74.95
	LD 400 mg + MD 200 mg inf 0.5 h q 12 h	94.98	95.44	99.06	100.00	100.00	95.61
	LD 400 mg + MD 200 mg inf 0.5 h q 24 h	77.31	88.28	96.12	98.16	96.08	88.33

Abbreviations: CFR, Cumulative Fraction Ratio; PMK, Phramongkutklao Hospital; LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

Table 48 The Cumulative Fraction Ratio (CFR) for $fAUC_{0-24}$/MIC ≥ 0.9 for tigecycline in health region V divided by hospital levels

Patients	Regimens	CFR (%)		
		Hospital-level A	Hospital-level S	Hospital-level M1
General	LD 200 mg + MD 100 mg inf 0.5 h q 12 h	81.82	95.67	89.71
	LD 200 mg + MD 100 mg inf 0.5 h q 24 h	62.74	75.58	79.91
	LD 400 mg + MD 100 mg inf 0.5 h q 12 h	82.21	95.86	89.80
	LD 400 mg + MD 100 mg inf 0.5 h q 24 h	62.79	75.67	79.95
	LD 400 mg + MD 200 mg inf 0.5 h q 12 h	92.52	100.00	93.91
	LD 400 mg + MD 200 mg inf 0.5 h q 24 h	82.12	95.82	89.78

Abbreviations: CFR, Cumulative Fraction Ratio; PMK, Phramongkutklao Hospital; LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

Table 49 The Cumulative Fraction Ratio (CFR) for *f*AUC_{0-24}/MIC ≥ 0.9 for tigecycline in health region V divided by carbapenem-resistant Enterobacterales

Patients	Regimens	CFR (%)		
		Type of carbapenem-resistant		
		Klebsiella pneumoniae	*Escherichia coli*	*Enterobacter cloacae*
General	LD 200 mg + MD 100 mg inf 0.5 h q 12 h	84.31	100.00	100.00
	LD 200 mg + MD 100 mg inf 0.5 h q 24 h	62.27	97.24	69.67
	LD 400 mg + MD 100 mg inf 0.5 h q 12 h	84.67	100.00	100.00
	LD 400 mg + MD 100 mg inf 0.5 h q 24 h	62.35	97.26	69.86
	LD 400 mg + MD 200 mg inf 0.5 h q 12 h	94.00	100.00	100.00
	LD 400 mg + MD 200 mg inf 0.5 h q 24 h	84.59	100.00	100.00

Abbreviations: CFR, Cumulative Fraction Ratio; PMK, Phramongkutklao Hospital; LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

Table 50 The Cumulative Fraction Ratio (CFR) for *f*AUC/MIC ≥ 25 for colistin divided by settings

Patients	Regimens	CFR (%)		CFR for combination (%)			
		PMK	Health region V	Colistin + Amikacin	Colistin + Gentamicin	Colistin + Tigecycline	Colistin + Fosfomycin
CrCL 0-9 mL/min	LD 300 mg + MD 100 mg inf 0.5 h q 24 h	34.42	80.23	100.00	99.98	99.66	99.86
	LD 300 mg + MD 100 mg inf 0.5 h q 12 h	53.30	91.63	100.00	99.89	98.08	99.04
	LD 300 mg + MD 150 mg inf 0.5 h q 12 h	52.84	91.64	100.00	99.99	99.67	99.91
	LD 300 mg + MD 150 mg inf 0.5 h q 24 h	34.23	79.94	100.00	99.86	97.99	98.92
	LD 300 mg + MD 180 mg inf 0.5 h q 24 h	34.28	80.12	99.99	99.86	97.98	98.97
CrCL 10-25 mL/min	LD 300 mg + MD 100 mg inf 0.5 h q 12 h	43.98	86.68	100.00	99.92	99.07	99.46
	LD 300 mg + MD 150 mg inf 0.5 h q 12 h	43.84	86.43	99.99	99.90	98.91	99.32
	LD 300 mg + MD 150 mg inf 0.5 h q 24 h	27.82	73.47	99.96	99.41	95.67	96.29
	LD 300 mg + MD 180 mg inf 0.5 h q 24 h	27.51	73.05	99.95	99.35	95.49	96.12
CrCL 26-50 mL/min	LD 300 mg + MD 100 mg inf 0.5 h q 12 h	34.19	78.45	99.91	99.39	96.73	96.73
	LD 300 mg + MD 150 mg inf 0.5 h q 12 h	34.28	78.62	99.94	99.45	96.84	96.89
	LD 300 mg + MD 150 mg inf 0.5 h q 24 h	21.95	63.12	99.70	97.52	90.13	87.00
	LD 300 mg + MD 180 mg inf 0.5 h q 24 h	22.08	63.28	99.72	97.51	90.18	87.09
CrCL 51-90 mL/min	LD 300 mg + MD 150 mg inf 0.5 h q 12 h	25.68	66.17	99.41	97.19	90.87	87.05
	LD 300 mg + MD 150 mg inf 0.5 h q 8 h	33.84	75.90	99.79	98.67	94.91	93.36
	LD 300 mg + MD 180 mg inf 0.5 h q 12 h	25.70	66.12	99.53	97.29	90.89	87.04
	LD 300 mg + MD 180 mg inf 0.5 h q 8 h	34.10	75.96	99.73	98.59	94.85	93.20

Patients	Regimens	CFR (%)		CFR for combination (%)			
		PMK	Health region V	Colistin + Amikacin	Colistin + Gentamicin	Colistin + Tigecycline	Colistin + Fosfomycin
CrCL 91-130 mL/min	LD 300 mg + MD 150 mg inf 0.5 h q 12 h	19.49	52.81	98.13	93.45	83.03	72.62
	LD 300 mg + MD 150 mg inf 0.5 h q 8 h	25.64	63.61	99.01	96.01	88.87	82.68
	LD 300 mg + MD 180 mg inf 0.5 h q 12 h	19.47	52.69	98.18	93.39	82.94	72.45
	LD 300 mg + MD 180 mg inf 0.5 h q 8 h	25.30	63.62	99.09	96.20	89.02	83.22

Abbreviations: CFR, Cumulative Fraction Ratio; PMK, Phramongkutklao Hospital; LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

Table 51 The Cumulative Fraction Ratio (CFR) for *f*AUC/MIC ≥ 25 for colistin in health region V divided by hospital levels

Patients	Regimens	CFR (%)		
		Hospital-level A	Hospital-level S	Hospital-level M1
CrCL 0-9 mL/min	LD 300 mg + MD 100 mg inf 0.5 h q 24 h	74.64	82.37	92.18
	LD 300 mg + MD 100 mg inf 0.5 h q 12 h	88.57	93.10	97.51
	LD 300 mg + MD 150 mg inf 0.5 h q 12 h	88.63	93.05	97.53
	LD 300 mg + MD 150 mg inf 0.5 h q 24 h	74.33	82.07	91.94
	LD 300 mg + MD 180 mg inf 0.5 h q 24 h	74.56	82.26	91.99
CrCL 10-25 mL/min	LD 300 mg + MD 100 mg inf 0.5 h q 12 h	82.38	88.56	95.36
	LD 300 mg + MD 150 mg inf 0.5 h q 12 h	82.22	88.22	95.04
	LD 300 mg + MD 150 mg inf 0.5 h q 24 h	67.71	75.28	86.68
	LD 300 mg + MD 180 mg inf 0.5 h q 24 h	67.30	74.83	86.29
CrCL 26-50 mL/min	LD 300 mg + MD 100 mg inf 0.5 h q 12 h	73.31	80.32	89.65
	LD 300 mg + MD 150 mg inf 0.5 h q 12 h	73.45	80.52	89.85
	LD 300 mg + MD 150 mg inf 0.5 h q 24 h	58.31	64.04	75.42
	LD 300 mg + MD 180 mg inf 0.5 h q 24 h	58.49	64.24	75.50
CrCL 51-90 mL/min	LD 300 mg + MD 150 mg inf 0.5 h q 12 h	61.41	67.43	77.59
	LD 300 mg + MD 150 mg inf 0.5 h q 8 h	71.32	77.43	86.20
	LD 300 mg + MD 180 mg inf 0.5 h q 12 h	61.40	67.34	77.53

Patients	Regimens	CFR (%)		
		Hospital-level A	Hospital-level S	Hospital-level M1
	LD 300 mg + MD 180 mg inf 0.5 h q 8 h	71.43	77.45	86.15
CrCL 91-130 mL/min	LD 300 mg + MD 150 mg inf 0.5 h q 12 h	49.08	53.34	62.74
	LD 300 mg + MD 150 mg inf 0.5 h q 8 h	59.34	64.67	74.03
	LD 300 mg + MD 180 mg inf 0.5 h q 12 h	49.03	53.20	62.53
	LD 300 mg + MD 180 mg inf 0.5 h q 8 h	59.22	64.74	74.27

Abbreviations: CFR, Cumulative Fraction Ratio; PMK, Phramongkutklao Hospital; LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

Table 52 The Cumulative Fraction Ratio (CFR) for *f*AUC/MIC ≥ 25 for colistin in health region V divided by carbapenem-resistant Enterobacterales

Patients	Regimens	CFR (%)		
		Type of carbapenem-resistant		
		Klebsiella pneumoniae	*Escherichia coli*	*Enterobacter cloacae*
CrCL 0-9 mL/min	LD 300 mg + MD 100 mg inf 0.5 h q 24 h	75.36	95.30	99.49
	LD 300 mg + MD 100 mg inf 0.5 h q 12 h	89.32	98.86	99.90
	LD 300 mg + MD 150 mg inf 0.5 h q 12 h	89.34	98.86	99.96
	LD 300 mg + MD 150 mg inf 0.5 h q 24 h	75.03	95.11	99.35
	LD 300 mg + MD 180 mg inf 0.5 h q 24 h	75.25	95.17	99.38
CrCL 10-25 mL/min	LD 300 mg + MD 100 mg inf 0.5 h q 12 h	83.22	97.45	99.63
	LD 300 mg + MD 150 mg inf 0.5 h q 12 h	82.98	97.16	99.53
	LD 300 mg + MD 150 mg inf 0.5 h q 24 h	67.86	90.66	97.34
	LD 300 mg + MD 180 mg inf 0.5 h q 24 h	67.41	90.34	97.15
CrCL 26-50 mL/min	LD 300 mg + MD 100 mg inf 0.5 h q 12 h	73.80	92.78	97.40
	LD 300 mg + MD 150 mg inf 0.5 h q 12 h	73.96	92.97	97.55
	LD 300 mg + MD 150 mg inf 0.5 h q 24 h	57.59	79.82	89.07
	LD 300 mg + MD 180 mg inf 0.5 h q 24 h	57.78	79.92	89.08
CrCL 51-90 mL/min	LD 300 mg + MD 150 mg inf 0.5 h q 12 h	61.18	81.36	88.53
	LD 300 mg + MD 150 mg inf 0.5 h q 8 h	71.55	89.24	94.30

Patients	Regimens	CFR (%)		
		Type of carbapenem-resistant		
		Klebsiella pneumoniae	*Escherichia coli*	*Enterobacter cloacae*
CrCL 91-130 mL/min	LD 300 mg + MD 180 mg inf 0.5 h q 12 h	61.13	81.30	88.59
	LD 300 mg + MD 180 mg inf 0.5 h q 8 h	71.66	89.14	94.11
	LD 300 mg + MD 150 mg inf 0.5 h q 12 h	48.28	66.42	74.75
	LD 300 mg + MD 150 mg inf 0.5 h q 8 h	59.06	77.44	84.20
	LD 300 mg + MD 180 mg inf 0.5 h q 12 h	48.20	66.21	74.60
	LD 300 mg + MD 180 mg inf 0.5 h q 8 h	58.95	77.81	84.75

Abbreviations: CFR, Cumulative Fraction Ratio; PMK, Phramongkutklao Hospital; LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

4.2.2.5 Fosfomycin

None of the fosfomycin dosing regimens for all patients divided by creatinine clearance on day 1, day 2, and day 5 met the CFR target. Table 53 showed the percentage of CFR for the fosfomycin dosing regimens

4.2.2.6 Ceftazidime-avibactam

Based on a CFR of ≥90%, no ceftazidime-avibactam regimens were effective against all studied CRKP isolates with various types of carbapenemase. Focusing on only CRKP isolates carrying only bla_{OXA-48}, the optimal CFR target of *f*T>MIC ≥50% was achieved in all studied ceftazidime-avibactam regimens. Whereas, for *f*T>MIC 100%, regimens of 2.5 g infused longer time as 2-3 hours every 8 hours gave CFR ≥90% in critically ill patients with OXA-48 type CRKP infection or isolates giving susceptible to ceftazidime-avibactam based on CLSI guideline. Table 54-56 showed the percentage of CFR for the ceftazidime-avibactam dosing regimens

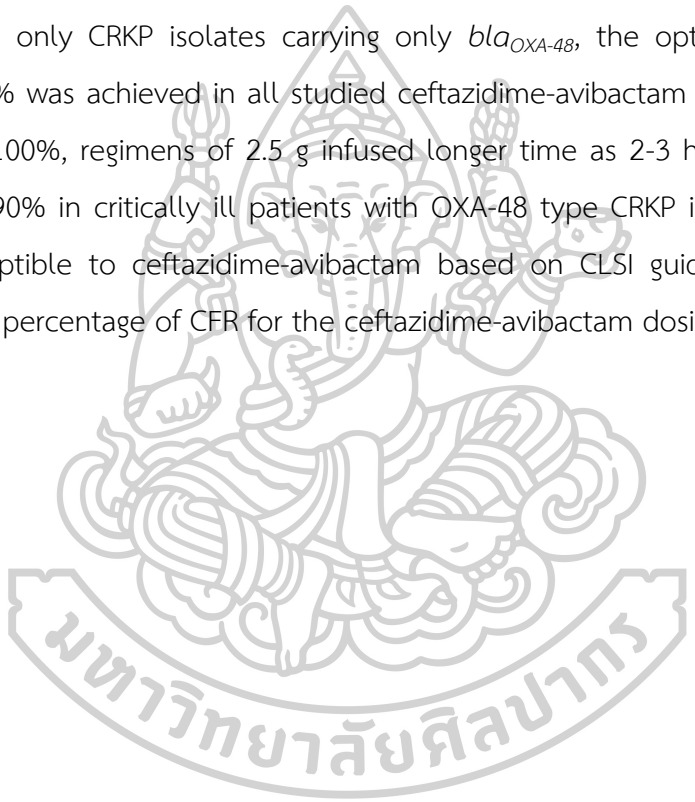


Table 53 The Cumulative Fraction Ratio (CFR) for $fAUC_{0\text{-}24h}$/MIC = 21.5 for fosfomycin

Patients	Regimens	CFR (%)			CFR for combination (%)											
		PMK			Fosfomycin + Amikacin			Fosfomycin + Gentamicin			Fosfomycin + Colistin			Fosfomycin + Tigecycline		
		Day 1	Day 2	Day 5	Day 1	Day 2	Day 5	Day 1	Day 2	Day 5	Day 1	Day 2	Day 5	Day 1	Day 2	Day 5
CrCL 0-9 mL/min	LD 8 g + MD 4 g inf 0.5 h q 12 h	36.51	36.73	36.73	99.71	99.99	100.00	100.00	100.00	100.00	100.00	100.00	100.00	93.74	95.21	97.14
	LD 8 g + MD 4 g inf 2 h q 12 h	36.49	36.72	36.73	99.65	99.99	100.00	100.00	100.00	100.00	100.00	100.00	100.00	93.45	95.20	97.08
	LD 4 g + MD 2 g inf 0.5 h q 12 h	31.95	34.99	36.72	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	95.30	98.01	97.99
	LD 4 g + MD 2 g inf 2 h q 12 h	31.79	34.92	36.72	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	95.29	97.89	99.99
CrCL 10-25 mL/min	LD 8 g + MD 4 g inf 0.5 h q 8 h	36.67	36.73	36.73	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	95.23	95.62	99.87
	LD 8 g + MD 4 g inf 2 h q 8 h	36.67	36.73	36.73	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	95.48	99.07	100.00
	LD 8 g + MD 4 g inf 0.5 h q 12 h	36.25	36.71	36.73	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	95.24	95.55	99.86
	LD 8 g + MD 4 g inf 2 h q 12 h	36.21	36.71	36.73	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	95.42	99.00	100.00
CrCL 26-50 mL/min	LD 8 g + MD 4 g inf 0.5 h q 8 h	36.56	36.73	36.73	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	95.24	95.24	95.24
	LD 8 g + MD 4 g inf 2 h q 8 h	36.53	36.73	36.73	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	95.24	95.45	99.89

Patients	Regimens	CFR (%)			CFR for combination (%)											
		PMK			Fosfomycin + Amikacin			Fosfomycin + Gentamicin			Fosfomycin + Colistin			Fosfomycin + Tigecycline		
		Day 1	Day 2	Day 5	Day 1	Day 2	Day 5	Day 1	Day 2	Day 5	Day 1	Day 2	Day 5	Day 1	Day 2	Day 5
	LD 8 g + MD 4 g inf 0.5 h q 12 h	34.98	36.57	36.73	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	95.24	95.24	95.24
	LD 8 g + MD 4 g inf 2 h q 12 h	34.78	36.55	36.73	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	95.24	95.41	99.89
CrCL 51-90 mL/min	LD 8 g + MD 4 g inf 0.5 h q 6 h	36.63	36.73	36.73	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	95.24	95.24	95.24
	LD 8 g + MD 4 g inf 2 h q 6 h	36.59	36.73	36.73	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	95.24	95.24	95.24
	LD 8 g + MD 4 g inf 0.5 h q 8 h	35.27	36.67	36.73	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	95.24	95.24	95.24
	LD 8 g + MD 4 g inf 2 h q 8 h	35.03	36.65	36.73	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	95.24	95.24	95.24
General	LD 8 g + MD 4 g inf 1 h q 4 h	36.15	-	-	99.92	-	-	100.00	-	-	100.00	-	-	97.87	-	-
	LD 8 g + MD 4 g inf 2 h q 4 h	36.19	-	-	99.94	-	-	100.00	-	-	100.00	-	-	97.95	-	-
	LD 8 g + MD 6 g inf 1 h q 6 h	36.46	-	-	99.93	-	-	100.00	-	-	100.00	-	-	98.02	-	-
	LD 8 g + MD 6 g inf 2 h q 6 h	36.47	-	-	99.93	-	-	100.00	-	-	100.00	-	-	98.08	-	-

Abbreviations: CFR, Cumulative Fraction Ratio; PMK, Phramongkutklao Hospital; LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

Table 54 The Cumulative Fraction Ratio (CFR) for Ceftazidime (CAZ): 100%*f*T > MIC and Avibactam (AVI): *f*T> 1 μg/mL for 100% of dosing interval divided by settings

Patients	Ceftazidime regimens	Avibactam regimens	CFR (%)	
			PMK	Health Region V
General	LD 0 g + MD 2 g inf 0.5 h q 6 h	LD 0 g + MD 0.5 g inf 0.5 h q 6 h	49.52	65.94
	LD 0 g + MD 2 g inf 1 h q 6 h	LD 0 g + MD 0.5 g inf 1 h q 6 h	49.64	68.24
	LD 0 g + MD 2 g inf 2 h q 6 h	LD 0 g + MD 0.5 g inf 2 h q 6 h	49.89	69.93
	LD 0 g + MD 2 g inf 3 h q 6 h	LD 0 g + MD 0.5 g inf 3 h q 6 h	49.89	74.45
	LD 0 g + MD 2 g inf 0.5 h q 8 h	LD 0 g + MD 0.5 g inf 0.5 h q 8 h	48.82	50.45
	LD 0 g + MD 2 g inf 1 h q 8 h	LD 0 g + MD 0.5 g inf 1 h q 8 h	48.82	51.22
	LD 0 g + MD 2 g inf 2 h q 8 h	LD 0 g + MD 0.5 g inf 2 h q 8 h	49.34	53.74
	LD 0 g + MD 2 g inf 3 h q 8 h	LD 0 g + MD 0.5 g inf 3 h q 8 h	49.52	56.86

Abbreviations: CFR, Cumulative Fraction Ratio; PMK, Phramongkutklao Hospital; LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

Table 55 The Cumulative Fraction Ratio (CFR) for Ceftazidime (CAZ): 100%*f*T > MIC and Avibactam (AVI): *f*T> 1 μg/mL for 100% of dosing interval in health region V divided by hospital levels

Patients	Ceftazidime regimens	Avibactam regimens	CFR (%)		
			Hospital-level A	Hospital-level S	Hospital-level M1
General	LD 0 g + MD 2 g inf 0.5 h q 6 h	LD 0 g + MD 0.5 g inf 0.5 h q 6 h	86.22	81.46	83.13
	LD 0 g + MD 2 g inf 1 h q 6 h	LD 0 g + MD 0.5 g inf 1 h q 6 h	88.19	84.11	85.50
	LD 0 g + MD 2 g inf 2 h q 6 h	LD 0 g + MD 0.5 g inf 2 h q 6 h	89.69	85.96	87.25
	LD 0 g + MD 2 g inf 3 h q 6 h	LD 0 g + MD 0.5 g inf 3 h q 6 h	92.90	90.33	91.25
	LD 0 g + MD 2 g inf 0.5 h q 8 h	LD 0 g + MD 0.5 g inf 0.5 h q 8 h	75.44	67.18	70.00
	LD 0 g + MD 2 g inf 1 h q 8 h	LD 0 g + MD 0.5 g inf 1 h q 8 h	76.10	68.07	70.84
	LD 0 g + MD 2 g inf 2 h q 8 h	LD 0 g + MD 0.5 g inf 2 h q 8 h	79.00	71.72	74.21
	LD 0 g + MD 2 g inf 3 h q 8 h	LD 0 g + MD 0.5 g inf 3 h q 8 h	81.08	74.41	76.71

Abbreviations: CFR, Cumulative Fraction Ratio; PMK, Phramongkutklao Hospital; LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

Table 56 The Cumulative Fraction Ratio (CFR) Ceftazidime (CAZ): 100%*f*T > MIC and Avibactam (AVI): *f*T> 1 μg/mL for 100% of dosing interval in health region V divided by carbapenem-resistant Enterobacterales

Patients	Ceftazidime regimens	Avibactam regimens	CFR (%)		
			Type of carbapenem-resistant		
			Klebsiella pneumoniae	*Escherichia coli*	*Enterobacter cloacae*
General	LD 0 g + MD 2 g inf 0.5 h q 6 h	LD 0 g + MD 0.5 g inf 0.5 h q 6 h	84.70	59.06	84.33
	LD 0 g + MD 2 g inf 1 h q 6 h	LD 0 g + MD 0.5 g inf 1 h q 6 h	86.89	61.79	85.33
	LD 0 g + MD 2 g inf 2 h q 6 h	LD 0 g + MD 0.5 g inf 2 h q 6 h	88.51	63.64	86.67
	LD 0 g + MD 2 g inf 3 h q 6 h	LD 0 g + MD 0.5 g inf 3 h q 6 h	92.09	69.09	88.67
	LD 0 g + MD 2 g inf 0.5 h q 8 h	LD 0 g + MD 0.5 g inf 0.5 h q 8 h	72.76	40.79	77.00
	LD 0 g + MD 2 g inf 1 h q 8 h	LD 0 g + MD 0.5 g inf 1 h q 8 h	73.50	41.70	77.34
	LD 0 g + MD 2 g inf 2 h q 8 h	LD 0 g + MD 0.5 g inf 2 h q 8 h	76.66	44.45	79.00
	LD 0 g + MD 2 g inf 3 h q 8 h	LD 0 g + MD 0.5 g inf 3 h q 8 h	78.94	48.15	80.34

Abbreviations: CFR, Cumulative Fraction Ratio; PMK, Phramongkutklao Hospital; LD, Loading dose; MD, Maintenance dose; inf, infusion time; h, hours; g, gram; CrCL, creatinine clearance

4.3 Clinical results

4.3.1 Baseline characteristics

Of the 121 patients with positive hemocultures for CRE, 102 patients met the inclusion criteria, and 19 patients were excluded from the study. A total of participants in the final analysis included 88 patients (n = 88) that divided the 48 patients into retrospective group (n = 48) and 40 patients into prospective group (n = 40). The flowchart of patients in the study is presented in Figure 29.

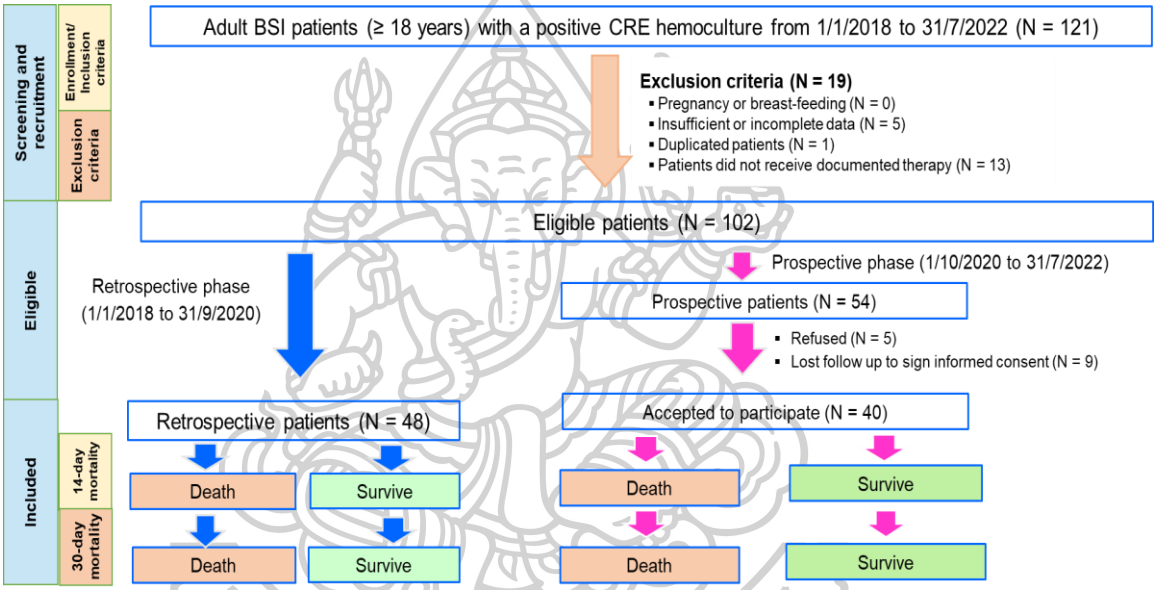


Figure 29 Flowchart of patients in the study

Table 57 showed baseline characteristics of all pateints (n = 88). In the retrospective groups, 26 patients (54.17%) were male, the median age was 66 years, the mean CCI score was 4.67 ± 2.58, the mean Pitt bacteremia score was 5.72 ± 2.86, 29 patients (60.42%) had sepsis, 17 patients (35.42%) had septic shock, 44 patients (91.67%) used mechanical ventilators, 38 patients (79.17%) received vasopressors. Vascular catheter-related infections were the most common types of infection (n = 20; 41.67%) in retrospective, followed by IAIs (n = 9; 18.75%). In the prospective group, 26 patients (50.00%) were male, the median age was 68 years, the mean CCI score was 4.18 ± 2.71, the mean Pitt bacteremia score was 5.36 ± 3.45, 20 patients

(50.00%) had sepsis, 14 patients (35.00%) had septic shock, 32 patients (80.00%) used mechanical ventilators, 26 patients (72.22%) received vasopressors. Vascular catheter-related infections were the most common types of infection (n = 21; 52.50%) in prospective groups, followed by IAIs (n = 7; 17.50%).

In the antimicrobial susceptibility testing, CRKP and CREC in the retrospective group were susceptible to tigecycline (89.58%), amikacin (89.58%), and gentamicin (66.67%) as well as intermediate to colistin (47.92%). CRKP in the prospective group was susceptible to gentamicin (87.50%), amikacin (83.33%), tigecycline (60.00%) as well as intermediate to colistin (60.00%).

All patients included to analyzed at 14-day mortality divided to survived and non-survived patients. Of 63 survived patients (71.59%), 38 patients (60.32%) were male, the mean Pitt bacteremia score was 5.11 ± 3.45, 32 patients (50.79%) had sepsis, 21 patients (33.33%) had septic shock, 51 patients (80.95%) used mechanical ventilators, 44 patients (69.84%) received vasopressors. In 25 non-survived patients (28.41%), 14 patients (56.00%) were male. mean Pitt bacteremia score was 6.61 ± 1.85, 17 patients (68.00%) had sepsis, 10 patients (40.00%) had septic shock, 25 patients (100%) used mechanical ventilators, 24 patients (96.00%) received vasopressors. Pitt bacteremia score, mechanical use, and vasopressor use were statistically significant between survived and non-survived groups.

All patients included to analyzed at 30-day mortality also divided to survived and non-survived patients. In 47 survived patients (43.41%), 26 patients (55.32%) were male, the mean Pitt bacteremia score was 4.75 ± 3.62, 27 patients (46.81%) had sepsis, 13 patients (27.66%) had septic shock, 35 patients (74.47%) used mechanical ventilators, 30 patients (63.83%) received vasopressors. In 41 non-survived patients (46.59%) at 30-day, 26 patients (63.41%) were male, the mean Pitt bacteremia score was 6.41 ± 2.24, 27 patients (65.85%) had sepsis, 18 patients (43.90%) had septic shock, 41 patients (53.95%) used mechanical ventilators, 38 patients (92.68%) received vasopressors. Table 59 showed survived and non-survived patients at 14-day and 30-day mortality.

4.3.2 Treatment regimens

Among the antibiotic regimens in the study, 6.82% (n = 6 of 88) and 93.18% (n = 82 of 88) were treated with monotherapy and combination therapy, respectively. The most frequent therapies were aminoglycoside-based regimen (n = 40 of 88; 45.45%) and colistin-based regimens (n = 40 of 72; 45.45%). None of the carbapenem-based regimens were shown in the study.

Table 58 showed treatment characteristics of patients. In retrospective group, the most antibiotic-based regimens were colistin-based regimens (n = 24 of 48; 50.00%) and the most frequent antibiotic regimens were colistin plus meropenem (n = 9). In prospective group, gentamicin-based regimens were the most antibiotic-based regimens (n = 14 of 40; 63.63%) and gentamicin plus tigecycline were the most frequent antibiotic regimens (n = 8). Fosfomycin-based regimens and triple-based regimens were only used in the retrospective group. The duration of treatment was not significant.

Table 60 showed treatment characteristics of survive and non-survive patients of 14-day mortality and 30-day mortality. For 14-day mortality, there was 63 (n = 63; 71.59%) and 25 patients (n = 25; 28.41%) in survive and non-survive groups, respectively. In survive group, 38 patients (60.32%) were male, the median age was 66 years, the mean CCI score was 4.30 ± 2.70, the mean Pitt bacteremia score was 5.11 ± 3.45, 32 patients (50.79%) had sepsis, 21 patients (33.33%) had septic shock, 51 patients (80.95%) used mechanical ventilators, 44 patients (69.84%) received vasopressors. Vascular catheter-related infections were the most common types of infection (n = 29; 46.03%) in retrospective, followed by IAIs (n = 10; 15.87%). In the non-survive group, 14 patients (56.00%) were male, the median age was 68 years, the mean CCI score was 4.8 ± 2.50, the mean Pitt bacteremia score was 6.60 ± 1.85, 17 patients (68%) had sepsis, 10 patients (40%) had septic shock, 25 patients (100.00%) used mechanical ventilators, 24 patients (96%) received vasopressors. Vascular catheter-related infections were the most common types of infection (n = 12; 48%) in prospective groups, followed by IAIs (n = 6; 24%). In the antimicrobial susceptibility testing, CRKP and CREC in the survive group were susceptible to amikacin (82.54%), gentamicin (76.19%) and tigecycline (73.02%), as well as intermediate to colistin

(52.38%). CRKP and CREC in the non-survive group was susceptible amikacin (92.00%), tigecycline (84.00%) and gentamicin (76.00%) and as well as intermediate to colistin (56.00%).

For 30-day mortality, there was 47 (n = 47; 53.41%) and 41 patients (n = 41; 46.59%) in survive and non-survive groups, respectively. In survive group, 26 patients (55.32%) were male, the median age was 63 years, the mean CCI score was 4.09 ± 2.87, the mean Pitt bacteremia score was 4.75 ± 3.62, 22 patients (46.81%) had sepsis, 13 patients (27.66%) had septic shock, 35 patients (74.47%) used mechanical ventilators, 30 patients (63.83%) received vasopressors. Vascular catheter-related infections were the most common types of infection (n = 25; 53.19%) followed by IAIs (n = 5; 10.64%). In the non-survive group, 41 patients (46.51%) were male, the median age was 68 years, the mean CCI score was 4.85 ± 2.31, the mean Pitt bacteremia score was 6.41 ± 2.24, 27 patients (65.85%) had sepsis, 18 patients (43.90%) had septic shock, 41 patients (53.95%) used mechanical ventilators, 38 patients (92.68%) received vasopressors. Vascular catheter-related infections were the most common types of infection (n = 16; 39.02%), followed by IAIs (n = 11; 26.83%). In the antimicrobial susceptibility testing, CRKP and CREC in the survive group were susceptible to amikacin (82.98%), gentamicin (74.47%) and tigecycline (74.47%), as well as intermediate to colistin (55.32%). CRKP and CREC in the non-survive group was susceptible amikacin (87.80%), tigecycline (78.05%) and gentamicin (78.05%) and as well as intermediate to colistin (51.22%).

Table 61 showed the frequency and the percentage of all antibiotic combination regimens, including dual therapy and triple therapy. The most antibiotic combination regimens were dual therapy either retrospective or prospective groups. The most antibiotic combination regimens in retrospective and prospective groups were colistin-based regimens and aminoglycoside-based regimens, respectively. There were no differences in each combination regimens.

Table 57 Baseline characteristics of patients

	Variables	Retrospective (n = 48)	Prospective (n = 40)	P-value
a. Demographic information	Age (y), median (IQR)	66 (30)	68 (24)	0.5829 $$
	Male, n (%)	26 (54.17)	26 (50.00)	0.303 #
b. Underlying conditions	CCI, mean (SD)	4.67 (2.58)	4.18 (2.71)	0.3872 $
	CCI ≥ 3, n (%)	32 (66.67)	24 (60.00)	0.517 #
	History of leukemia or metastasis cancer, n (%)	11 (22.92)	8 (20.00)	0741 #
	Chronic liver disease, n (%)	5 (10.42)	3 (7.50)	0.636 ##
	Chronic Kidney Disease, n (%)	9 (18.75)	7 (17.50)	0.880 #
	Diabetes mellitus, n (%)	12 (25.00)	11 (27.50)	0.790 #
	COPD, n (%)	3 (6.25)	5 (12.50)	0.460 ##
	Neutropenia, n (%)	4 (8.33)	2 (5.00)	1.000 ##
	Recent surgery 7 days before infection, n (%)	2 (4.17)	2 (5.00)	1.000 ##
c. Severity	Pitt bacteremia score, mean (SD)	5.72 (2.86)	5.36 (3.45)	0.6014 $
	Pitt bacteremia score ≥ 4, n (%)	41 (89.13)	29 (72.50)	0.219 #
	SIRs, median (IQR)	3 (1)	2 (1)	0.1034 $$
	Sepsis, n (%)	29 (60.42)	20 (50.00)	0.327 #
	Septic shock, n (%)	17 (35.42)	14 (35.00)	0.968 #
	Mechanical ventilator use, n (%)	44 (91.67)	32 (80.00)	0.112 ##
	Vasopressor use, n (%)	38 (79.17)	26 (72.22)	0.460 #

	Variables	Retrospective (n = 48)	Prospective (n = 40)	P-value
n (%)	UTIs	1 (2.08)	4 (10.00)	0.113 ##
	IAIs	9 (18.75)	7 (17.50)	
	Vascular catheter-related infections	20 (41.67)	21 (52.50)	
	Pneumonia	11 (22.92)	5 (12.50)	
	SSTI/wound	5 (10.42)	0 (0)	
	Primary Bacteriemia	2 (4.17)	3 (7.50)	
d. Antibiotic use ≤ 90 days before admission, n (%)	Cephalosporins	13 (27.08)	5 (12.50)	0.115 ##
	Carbapenems	14 (29.17)	4 (10.00)	0.034 *, ##
	BLBIs	7 (14.58)	4 (10.00)	0.748 ##
	Fluoroquinolones	5 (10.42)	0 (0)	0.061 ##
	Vancomycin	9 (18.75)	1 (2.50)	0.019 *, ##
e. Type of CRE, n (%)				0.015 *, ##
	CRKP	41 (85.42)	40 (100)	
	CREC	7 (14.58)	0 (0)	
f. Susceptibility (susceptible/samples), n (%)	MER	2 (4.17)	5 (12.50)	0.238 ##
	IMI	2 (4.17)	6 (15.00)	0.134 ##
	ERT	0 (0)	0 (0)	
	COL (Intermediate)	23 (47.92)	24 (60.00)	0.258 #

	Variables	Retrospective (n = 48)	Prospective (n = 40)	P-value
	AMI	43 (89.58)	32 (83.33)	0.207 [#]
	GEN	32 (66.67)	35 (87.50)	0.022 [*, #]
	TIG	43 (89.58)	24 (60)	0.001 [*, #]
	CZA	NR	18 (45.00)	
d. Serum creatinine (mg/dL)	Day 0, median (IQR)	1.50 (2.14) [n = 47]	1.55 (1.67) [n = 40]	0.4484 [$$]
	Day 3, median (IQR)	1.33 (1.25) [n = 41]	1.54 (2.04) [n = 37]	0.4962 [$$]
	Day 7, median (IQR)	1.33 (1.40) [n = 31]	1.30 (1.24) [n = 34]	0.8696 [$$]
	Day at the end of treatment, median (IQR)	1.40 (1.31) [n = 46]	1.27 (1.70) [n = 38]	0.4639 [$$]
e. ALT (IU/mL)	Day 0, median (IQR)	32.6 (50.2) [n = 38]	41.2 (42.6) [n = 37]	0.3820 [$$]
	Day 3, median (IQR)	43.2 (54.3) [n = 23]	34.9 (36.7) [n = 25]	0.2043 [$$]
	Day 7, median (IQR)	40.0 (81.3) [n = 20]	23.7 (33.2) [n = 23]	0.1650 [$$]
	Day at the end of treatment, median (IQR)	35.8 (66.9) [n = 25]	19.6 (20.9) [n = 29]	0.0460 [*, $$]

* Significance if *P-value* ≤ 0.05; [#] P-value using chi-square test [##] P-value using fisher's exact test; [$] P-value using independent t-test [$$] Mann-Whitney U test

Abbreviations: CCI, Charlson comorbidity index; DM, Diabetes mellitus; COPD, Chronic obstructive pulmonary disease; CKD, Chronic Kidney Disease; SIRS, Systemic inflammatory response syndrome; UTIs, Urinary tract infections; IAIs, Intra-abdominal infections; SSTIs, Skin and soft tissue infection; BLBIs; Betalactam/betalactamase inhibitors; CRKP, Carbapenem-resistant *Klebsiella pneumoniae*; CREC, Carbapenem-resistant *Escherichia coli*; MER, meropenem; IMI, imipenem; ERT, ertapenem; COL, colistin; AMI, amikacin; GEN, gentamicin; TIG, tigecycline

Table 58 Treatment characteristics of patients

	Variable	Retrospective (n = 48)	Prospective (n = 40)	P-value
a. Appropriate therapy	Appropriate empirical therapy, n (%)	13 (27.08)	24 (60.00)	0.002*, #
	Appropriate documented therapy, n (%)	41 (85.42)	36 (90.00)	0.748 ##
b. Antibiotic regimens, n (%)				0.310 ##
	b.1 Monotherapy	4 (8.33)	2 (5.00)	
	b.2 Combination therapy	44 (91.67)	38 (95.00)	
	b.2.1 Colistin-based	24 (50)	16 (40.00)	
	COL + Aminoglycosides [a]	5 (20.83)	3 (18.75)	
	COL + Carbapenems [b]	10 (41.67)	7 (29.17)	
	COL + AMI + Carbapenems [c]	3 (12.50)	0 (0.00)	
	COL + TIG	2 (8.33)	4 (25.00)	
	COL + FOS	4 (16.67)	2 (12.50)	
	b.2.2 Aminoglycoside-based	18 (37.50)	22 (55.00)	
	b.2.2.1 Amikacin-based	13 (72.22)	8 (36.36)	
	AMI+ Carbapenems [d]	4 (30.77)	6 (75.00)	
	AMI + TIG	5 (38.46)	2 (25.00)	
	AMI + FOS	3 (23.08)	0 (0.00)	
	AMI + TIG + MER	1 (7.69)	0 (0.00)	
	b.2.2.2 Gentamicin-based	5 (10.42)	14 (63.63)	

	Variable	Retrospective (n = 48)	Prospective (n = 40)	P-value
	GEN + Carbapenems [e]	1 (20.00)	4 (28.57)	
	GEN + FOS	4 (80.00)	4 (28.57)	
	GEN + TIG	0 (0.00)	6 (42.86)	
	b.2.3 Carbapenem-based	0 (0)	0 (0.00)	
	b.2.4 Fosfomycin-based	2 (4.17)	0 (0.00)	
	FOS + MER	2 (100.00)	0 (0.00)	
c. Treatment duration (days)	Treatment duration (days), median (IQR)	8 (9)	7 (5)	0.6742 [$$]

[a] COL + Aminoglycosides: retrospective groups: COL + AMI 5 (20.83); prospective groups: COL + GEN 3 (18.75)

[b] COL + Carbapenems: retrospective groups: COL + MER 9 (37.50), COL + MER 5 (31.25); prospective groups: COL + IMI 1 (4.17), COL + IMI 2 (12.50)

[C] COL + AMI + Carbapenems: retrospective groups: COL + AMI + MER 2 (8.33), COL + AMI + IMI 1 (4.17)

d AMI+ Carbapenems: retrospective groups: AMI + MER 2 (11.11), AMI + IMI 2 (11.11); prospective groups: AMI + MER 1 (4.55), AMI + IMI 5 (22.73)

[e] GEN + Carbapenems: retrospective groups: GEN + IMI 1 (5.56); prospective groups: GEN + MER 2 (9.09), GEN + IMI 2 (9.09)

* Significance if *P-value* ≤ 0.05; [#] P-value using chi-square test [##] P-value using fisher's exact test; [$] P-value using independent t-test [$$] Mann-Whitney U test

Abbreviations: MER, meropenem; IMI, imipenem; ERT, ertapenem; COL, colistin; AMI, amikacin; GEN, gentamicin; TIG, tigecycline

Table 59 Baseline characteristics of survive and non-survive patients of 14-day mortality and 30-day mortality

Variables		14-day mortality			30-day mortality		
		Survive (n = 63)	Non-survive (n = 25)	P-value	Survive (n = 47)	Non-Survive (n = 41)	P-value
a. Demographic information	Age (y), median (IQR)	66 (33)	68 (17)	0.2850 $$	63 (40)	68 (17)	0.1247 $$
	Male, n (%)	38 (60.32)	14 (56)	0.710 #	26 (55.32)	26 (63.41)	0.441 #
b. Underlying conditions	CCI, mean (SD)	4.30 (2.70)	4.8 (2.5)	0.4274 $	4.09 (2.87)	4.85 (2.31)	0.1743 $
	CCI ≥ 3, n (%)	40 (63.49)	16 (64.00)	0.964 #	26 (55.32)	30 (73.17)	0.082 #
	History of leukemia or metastasis cancer, n (%)	13 (20.63)	6 (24.00)	0.729 #	11 (23.40)	8 (19.51)	0.658 #
	Chronic liver disease, n (%)	5 (7.94)	3 (12.00)	0.683 ##	3 (6.38)	5 (12.20)	0.465
	Chronic Kidney Disease, n (%)	13 (20.63)	3 (12.00)	0.541 #	9 (19.15)	7 (17.07)	0.801 #
	Diabetes mellitus, n (%)	17 (26.98)	6 (24.00)	0.774 #	13 (27.66)	10 (24.39)	0.728 #
	COPD, n (%)	6 (9.52)	2 (8.00)	1.000 ##	4 (8.51)	4 (9.76)	1.000 ##
	Neutropenia, n (%)	4 (6.35)	0 (0.00)	0.574 ##	3 (6.38)	1 (2.44)	0.620 ##
	Recent surgery 7 days before infection, n (%)	3 (4.76)	1 (4.00)	1.000 ##	3 (6.38)	1 (2.44)	0.620 ##

	Variables	14-day mortality			30-day mortality		
		Survive (n = 63)	Non-survive (n = 25)	P-value	Survive (n = 47)	Non-Survive (n = 41)	P-value
c. Severity	Pitt bacteremia score, mean (SD)	5.11 (3.45)	6.6 (1.85)	0.0456 *, $$	4.75 (3.62)	6.41 (2.24)	0.0133 *, $$
	Pitt bacteremia score ≥ 4, n (%)	45/60 (75.00)	25/25 (100.00)	0.004 *, ##	30/44 (68.18)	40/41 (97.56)	0.000 *, ##
	SIRs, mean (SD)	2.5 (0.99)	2.8 (0.95)	0.1995 $	2.44 (1.05)	2.76 (0.89)	0.1288 $
	Sepsis, n (%)	32 (50.79)	17 (68)	0.143 #	22 (46.81)	27 (65.85)	0.073 #
	Septic shock, n (%)	21 (33.33)	10 (40.00)	0.555 #	13 (27.66)	18 (43.90)	0.112 #
	Mechanical ventilator use, n (%)	51 (80.95)	25 (100)	0.017 *, ##	35 (74.47)	41 (53.95)	0.000 *, ##
	Vasopressor use, n (%)	44 (69.84)	24 (96)	0.01 *,##	30 (63.83)	38 (92.68)	0.002 *, ##
	Type of infection, n (%)			0.652 ##			0.316 ##
	UTIs	4 (6.35)	1 (4)	1.000 ##	3 (6.38)	2 (4.88)	1.000 ##
	IAIs	10 (15.87)	6 (24)	0.375 #	5 (10.64)	11 (26.83)	0.058 #
	Vascular catheter-related infections	29 (46.03)	12 (48)	0.867 #	25 (53.19)	16 (39.02)	0.184 #

Variables		14-day mortality			30-day mortality		
		Survive (n = 63)	Non-survive (n = 25)	P-value	Survive (n = 47)	Non-Survive (n = 41)	P-value
	Pneumonia	11 (17.46)	5 (20)	0.781 #	8 (17.02)	8 (19.51)	0.762 #
	SSTI/Wound	4 (6.35)	1 (4)	1.000 ##	2 (4.26)	3 (7.32)	0.661 ##
	Primary Bacteriemia	5 (7.94)	0 (0)	0.396 ##	4 (8.51)	1 (2.44)	0.366 ##
d. Antibiotic use ≤ 90 days before admission, n (%)	Cephalosporins	10 (15.87)	8 (32.00)	0.091 #	7 (14.89)	11 (26.83)	0.193 #
	Carbapenems	10 (15.87)	8 (32.00)	0.091 #	7 (14.89)	11 (26.83)	0.193 #
	BLBIs	7 (63.64)	4 (36.36)	0.500 ##	5 (10.64)	6 (14.63)	0.799 ##
	Fluoroquinolones	3 (4.76)	2 (8.00)	0.620 ##	3 (6.38)	2 (4.88)	1.000 ##
	Vancomycin	6 (9.52)	2 (8.00)	0.461 ##	6 (12.77)	4 (9.76)	0.745 ##
e. Type of CRE, n (%)				0.400 ##			0.700 ##
	CRKP	59 (93.65)	22 (88)		44 (93.62)	37 (90.24)	
	CREC	4 (6.78))	3 (12)		3 (6.67)	4 (10.26)	
f. Susceptibility, n (%)	MER	5 (7.94)	2 (8)	1.000 ##	4 (8.51)	3 (7.32)	1.000 ##
	IMI	6 (9.52)	2 (8)	1.000 ##	4 (8.51)	4 (9.76)	1.000 ##
	ERT	0 (0)	0 (0)		0 (0)	0 (0)	

Variables	14-day mortality			30-day mortality		
	Survive (n = 63)	Non-survive (n = 25)	P-value	Survive (n = 47)	Non-Survive (n = 41)	P-value
COL (Intermediate)	33 (52.38)	14 (56)	0.759 ##	26 (55.32)	21 (51.22)	0.701 ##
AMI	52 (82.54)	23 (92)	0.334 ##	39 (82.98)	36 (87.80)	0.524 ##
GEN	48 (76.19)	19 (76)	0.985 #	35 (74.47)	32 (78.05)	0.694 ##
TIG	46 (73.02)	21 (84.00)	0.407 ##	35 (74.47)	32 (78.05)	0.694 #
CZA, n/N (%)	17/33 (51.52)	1/7 (14.29)	0.105 ##	12/48 (48.00)	6/15 (40.00)	0.622 #

* Significance if *P-value* ≤ 0.05

P-value using chi-square test ## P-value using fisher's exact test

$ P-value using independent t-test $$ Mann-Whitney U test

Abbreviations: CCI, Charlson comorbidity index; DM, Diabetes mellitus; COPD, Chronic obstructive pulmonary disease; CKD, Chronic Kidney Disease; SIRS, Systemic inflammatory response syndrome; UTIs, Urinary tract infections; IAIs, Intra-abdominal infections; SSTIs, Skin and soft tissue infection; BLBIs; Betalactam/betalactamase inhibitors; CRKP, Carbapenem-resistant *Klebsiella pneumoniae*; CREC, Carbapenem-resistant *Escherichia coli*; MER, meropenem; IMI, imipenem; ERT, ertapenem; COL, colistin; AMI, amikacin; GEN, gentamicin; TIG, tigecycline

Table 60 Treatment characteristics of survive and non-survive patients of 14-day mortality and 30-day mortality

Variables		14-day mortality			30-day mortality		
		Survive (n = 63)	Non-survive (n = 25)	P-value	Survive (n = 47)	Non-Survive (n = 41)	P-value
a. Appropriate therapy	Appropriate empirical therapy, n (%)	26 (41.27)	11 (44.00)	0.815 #	20 (42.55)	17 (41.46)	0.918 #
	Appropriate documented therapy, n (%)	56 (88.89)	21 (84)	0.500 #	42 (89.36)	35 (85.37)	0.572 #
b. Antibiotic regimens, n (%)	Monotherapy *vs* Dual therapy *vs* Triple therapy			1.000 ##			0.474 ##
	Monotherapy	4 (6.35)	2 (8.00)		4 (8.51)	2 (4.88)	
	Dual therapy	56 (88.89)	22 (88.00)		40 (85.11)	38 (92.68)	
	Triple therapy	3 (4.76)	1 (4.00)		3 (6.38)	1 (2.44)	
c. Treatment duration (days)	Treatment duration (days), median (IQR)	9 (8)	6 (4)	0.0008 *, $$	9 (8)	7 (5)	0.1341 $$

Table 61 Comparison of antibiotic-based regimens of survive and non-survive patients of 14-day mortality and 30-day mortality

Antibiotic-based regimens	Survive (n = 59)	Non-survive (n = 23)	P-value	Survive (n = 43)	Non-Survive (n = 39)	P-value
Colistin + carbapenems *vs* other combination regimens			0.426 #			0.444 #
Colistin + Carbapenems	46 (77.97)	7 (30.43)		9 (20.93)	11 (28.21)	
Other combination regimens	13 (22.03)	16 (69.57)		34 (79.07)	28 (71.79)	
Tigecycline + aminoglycosides *vs* other combination regimens			0.329 ##			0.148 ##
Tigecycline + aminoglycosides	12 (35.59)	8 (34.78)		10 (23.36)	4 (10.26)	
Other combination regimens	47 (79.66)	21 (91.30)		33 (76.74)	35 (89.74)	
Amikacin combination regimens *vs* other combination regimens			0.945 #			0.714 #
Amikacin combination regimens	21 (35.59)	8 (34.78)		16 (37.21)	13 (33.33)	
Other combination regimens	38 (64.41)	15 (65.22)		27 (62.79)	26 (66.67)	
Gentamicin combination regimens *vs* other combination regimens			0.278 #			0.219 #
Gentamicin combination regimens	18 (30.51)	4 (17.39)		14 (32.56)	8 (20.51)	
Other combination regimens	41 (69.49)	19 (82.61)		29 (67.44)	31 (79.45)	
Carbapenems combination regimens *vs*			0.866 #			

Antibiotic-based regimens	Survive (n = 59)	Non-survive (n = 23)	P-value	Survive (n = 43)	Non-Survive (n = 39)	P-value
other combination regimens						
Carbapenems combination regimens	27 (45.76)	11 (47.83)		21 (48.84)	17 (43.59)	
Other combination regimens	32 (54.24)	12 (52.17)		22 (51.16)	22 (56.41)	

* Significance if *P-value* ≤ 0.05

[#] P-value using chi-square test [##] P-value using fisher's exact test [$] P-value using Mann-Whitney U test

Abbreviations: MER, meropenem; IMI, imipenem; ERT, ertapenem; COL, colistin; AMI, amikacin; GEN, gentamicin; TIG, tigecycline; FOS, fosfomycin

4.3.2 Treatment outcomes

Table 62 showed overall treatment outcomes of the study. They are divided into mortality, clinical outcomes, microbiological outcome, and process outcome.

4.3.2.1 Mortality

4.3.2.1.1 Mortality rates

At 14 days, patients in the retrospective groups died 18, whereas Patients in the prospective groups died 7. There was statistically difference in the 14-day mortality rate (p-value = 0.038). At 30 days, patients in the retrospective groups died 26, whereas patients in the prospective groups died 15. There was no difference in the 30-day mortality rate (p-value = 0.119). Figure 30 showed flowchart of patients in the study and the frequency of mortality at 14 and 30 days.

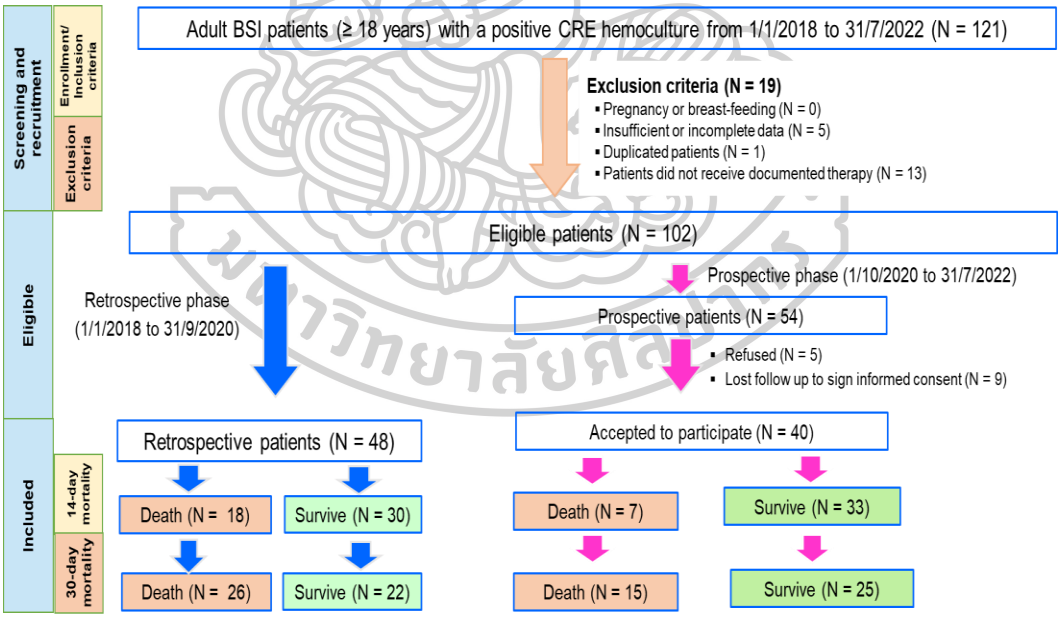


Figure 30 Flowchart of patients in the study and the frequency of mortality at 14 and 30 days

4.3.2.1.2 Kaplan-Meier curves and log-rank test

Figure 31 and Figure 32 showed Kaplan-Meier curves of survival to 14-day and 30-day divided by retrospective and prospective groups, respectively.

At 14-day mortality, the retrospective group were significantly different in the prospective group when using log-rank test analysis (p-value = 0.0368), whereas there was no difference in 30-day mortality (p-value = 0.0778).

4.3.2.1.3 Factors associated with mortality

The risk factors were associated with 14-day mortality in univariate analysis, including receiving PK/PD dose optimization with pharmacists (intervention) (OR = 0.35; 95%CI = 0.13-0.96; p-value = 0.042) and using vasopressors (OR = 10.36; 95%CI = 1.31-82.25; p-value = 0.027). When using multivariate analysis, both receiving intervention (OR = 0.35; 95%CI = 0.12-0.98; p-value = 0.046) and using vasopressors were significantly associated with 14-day mortality (OR = 10.54; 95%CI = 1.31-85.00; p-value = 0.027).

The risk factors were associated with 30-day mortality in univariate analysis, including receiving intervention (OR = 0.51; 95%CI = 0.21-1.19; p-value = 0.120), age (OR = 1.02; 95%CI = 1.00-1.05; p-value = 0.040), using vasopressors (OR = 7.18; 95%CI = 1.92-26.80; p-value = 0.003), CCI score ≥ 3 (OR = 2.21; 95%CI = 0.90-5.41; p-value = 0.085), Sepsis (OR = 2.19; 95%CI = 0.93-5.19; p-value = 0.075) and Intra-abdominal infections (OR = 3.53; 95%CI = 1.01-12.38; p-value = 0.048). When using multivariate analysis, only using vasopressors were significantly associated with 30-day mortality (OR = 6.05; 95%CI = 1.48-24.70; p-value = 0.012).

4.3.2.2 Clinical outcomes

Clinical failure was 54.17% (n = 26 of 48) in retrospective group and 32.50% (n = 13 of 40) in prospective group. Clinical outcomes between retrospective and prospective groups were statistically significance (p-value = 0.042). At 14-day clinical failure (Figure 33), the retrospective group were significantly different in the prospective group when using log-rank test analysis (p-value = 0.0463).

4.3.2.3 Microbiological outcome

Microbiological eradication was 90.63% (n = 29 of 32) in retrospective patients and 83.78% (n = 31 of 37) in prospective patients. No significant differences between retrospective and prospective groups were found (p-value = 0.489).

4.3.2.4 Process outcomes

The physician acceptance following protocols was acceptance with minimum requirement of protocol (77.50%; n = 31 of 40) and fully acceptance (22.50; n = 9 of 40). The most acceptance with minimum requirement of protocol were antibiotic combination options, whereas antibiotic dosing regimens occasionally followed the protocol, especially tigecycline dosing regimens at a MIC of 0.5 µg/mL.

Table 62 Overall treatment outcomes of the study

	Variables	Retrospective (n = 48)	Prospective (n = 40)	P-value
a. Clinical outcomes	14-day mortality	18 (37.50)	7 (17.50)	0.038 *, #
	30-day mortality	26 (54.17)	15 (37.50)	0.119 #
	Clinical failure, n (%)	26 (54.17)	13 (32.50)	0.042 *, #
	Length of stay (days), median (IQR)	50 (83.5)	51.5 (47.5)	0.8275 $$
b. Process outcomes	Fully acceptance of protocol, n (%)	NA	9 (22.50)	
	Acceptance with minimum requirement of protocol, n (%)	NA	31 (77.50)	
	No acceptance, n (%)	NA	0 (0)	
C. Microbiological outcomes, n (%)				0.489 ##
	Microbiological eradication	29/32 (90.63)	31/37 (83.78)	
	Microbiological persistent	3/32 (9.38)	6/37 (16.22)	

* Significance if *P-value* ≤ 0.05 # P-value using chi-square test ## P-value using fisher's exact test

Abbreviations: NA, not analyze

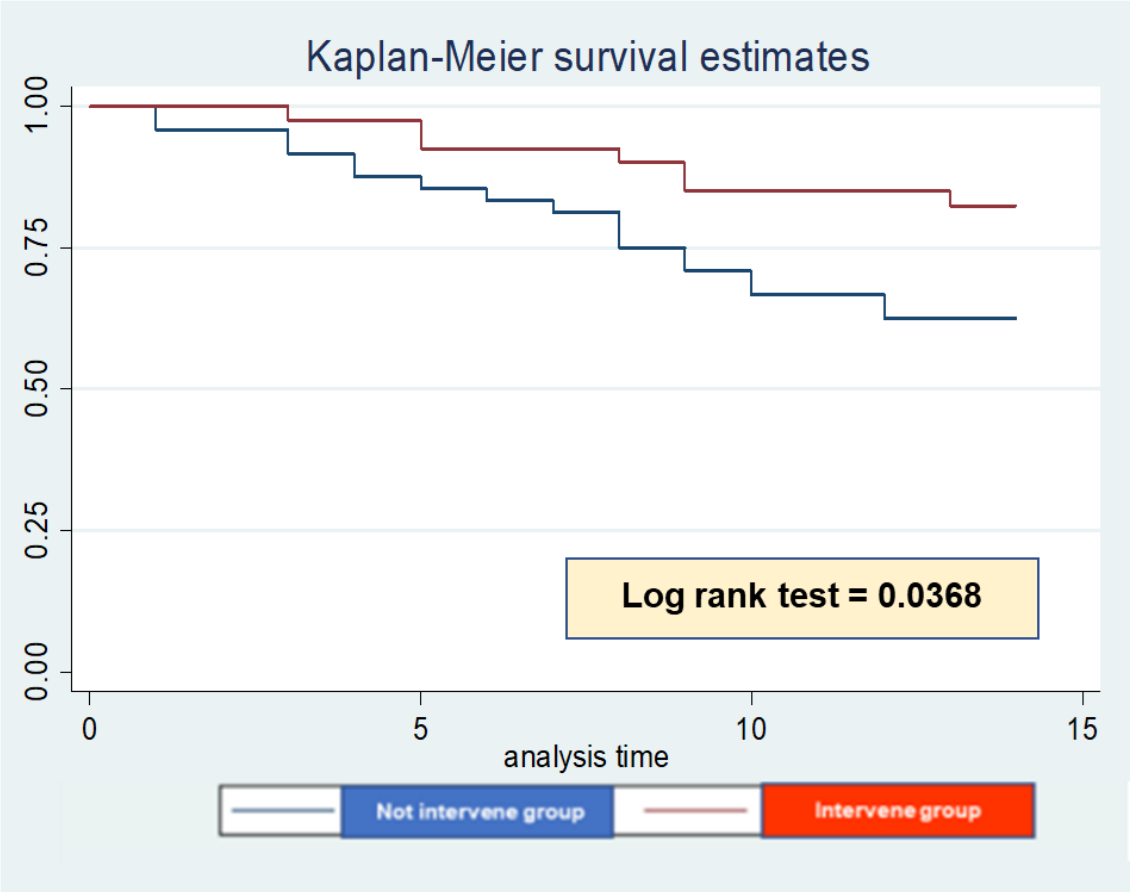


Figure 31 Kaplan-Meier and log-rank test analysis of survival at 14-day

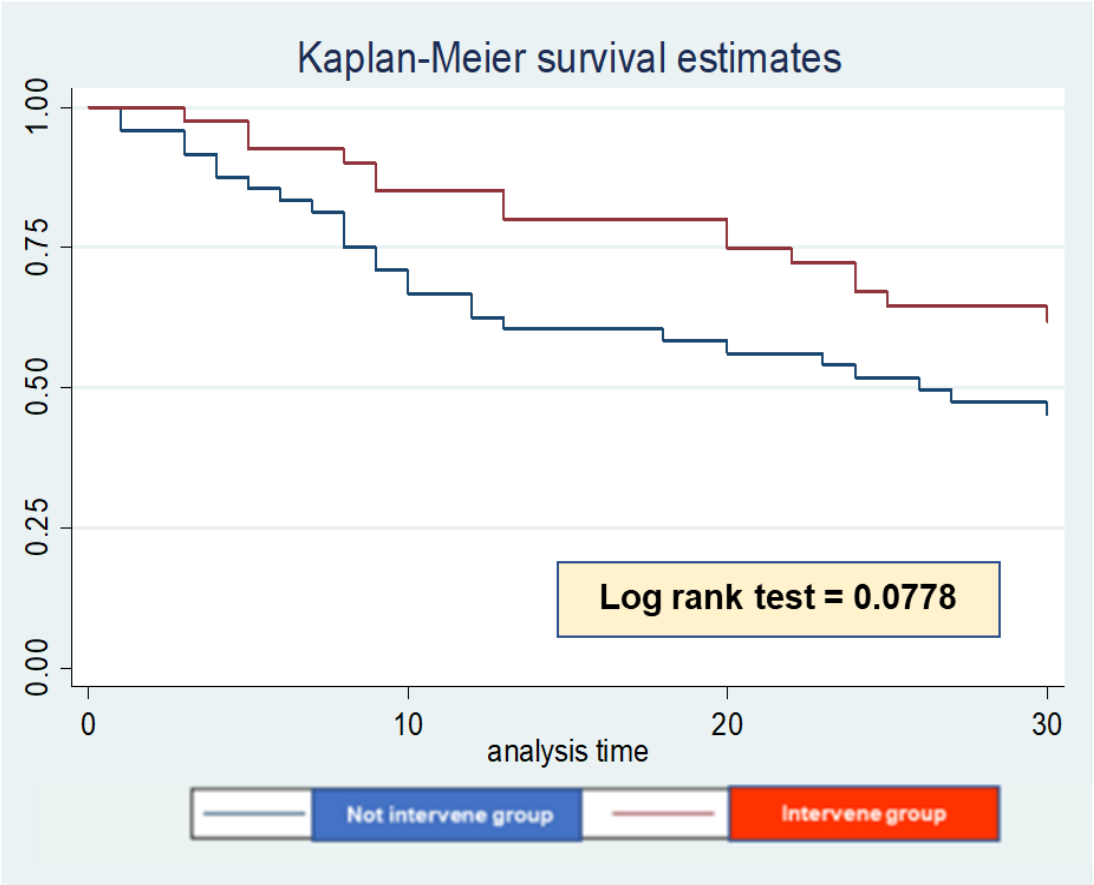


Figure 32 Kaplan-Meier and log-rank test analysis of survival at 30-day

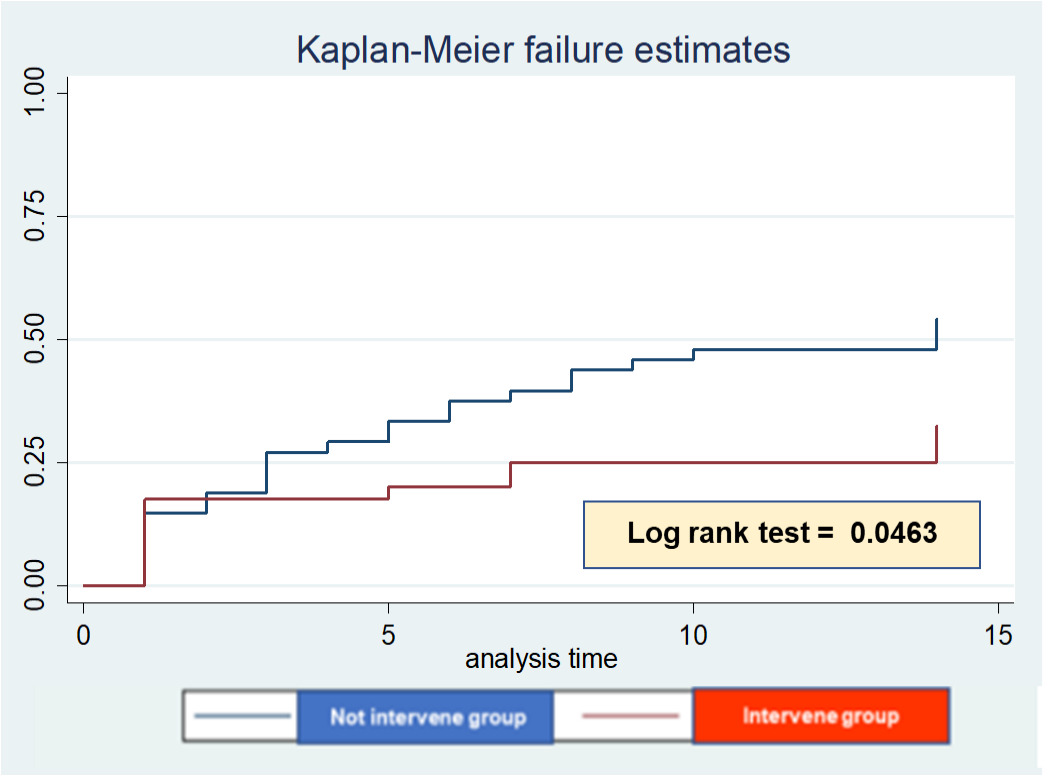


Figure 33 Kaplan-Meier and log-rank test analysis of 14-day clinical failure

Table 63 Univariate and multivariate logistic regression analyses of variables associated with mortality

	Variable	Univariate analysis		Multivariate analysis [a]	
		Crude OR (95%CI)	*P-value*	Adjusted OR (95%CI)	*P-value*
14-day mortality	**Intervention**	**0.35 (0.13 – 0.96)**	**0.042**	**0.35 (0.12 – 0.98)**	**0.046**
	Vasopressor use	**10.36 (1.31 – 82.25)**	**0.027**	**10.54 (1.31 – 85.00)**	**0.027**
30-day mortality	Intervention	0.51 (0.21 – 1.19)	0.120		
	Age	1.02 (1.00 – 1.05)	0.040		
	Vasopressor use	**7.18 (1.92 – 26.80)**	**0.003**	**6.05 (1.48-24.70)**	**0.012**
	CCI score ≥ 3	2.21 (0.90 - 5.41)	0.085		
	Sepsis	2.19 (0.93 – 5.19)	0.075		
	Intra-abdominal infections	3.53 (1.01 – 12.38)	0.048		
Clinical failure	**Intervention**	**0.41 (0.17 – 0.97)**	**0.044**	**0.38 (0.15 – 1.00)**	**0.049**
	History of COPD	4.27 (0.81 - 22.50)	0.087		
	PITT ≥ 4	4.24 (1.10 – 16.32)	0.036		
	Sepsis	2.26 (0.95 – 5.40)	0.066		

[a] The univariate and multivariate analysis was analyzed by logistic regression. The variables with *p-value* < 0.1 in the univariate analysis were included in the multivariate analysis. [b] Intervention: using PK/PD dose-optimization protocol

CHAPTER V
DISCUSSION AND CONCLUSION

Nowadays, limited available antibiotic options for treating infections caused by CRE are a critical problem for antibiotic resistance gram-negative bacteria worldwide. Mostly, second-line antibiotics have been used in clinical settings. In Thailand, the carbapenem-resistant rates of gram-negative bacteria have continually increased over time. Nonetheless, there is a lack of linkage between an in vitro study involving antibiotic susceptibility with molecular epidemiology and a clinical study in multicenter. This is the first study to report the optimal antibiotic options and appropriate antibiotic dosing regimens through clinical study towards CRE across multiple hospitals in Thailand.

In this study, carbapenem resistance among a total of CRE isolates was mainly found among *K. pneumoniae* (n = 163 of 199; 81.91%), followed by *E. coli* (n = 33 of 199; 16.58%) and *E. Cloacae* (n = 3 of 199; 1.51%). Our findings are consistent with those of Thongkoom et al., who found that the most common CRE isolates from a tertiary care hospital in Thailand, were *K. pneumoniae* (n = 290 of 411; 71%), followed by *E. Coli* (n = 47 of 411; 11.4%) and *E. Cloacae* (n = 31 of 411; 7.5%) [118]. In contrast, a systematic review and meta-analysis found that the majority of CRE were *K. pneumoniae* (63.6%), followed by *E. Cloacae* and *E. Coli.*, respectively [119].

The prevalence rates of susceptible to studied antibiotics among CRE isolates were determined as follows: amikacin (n = 175 of 199; 87.84%), gentamicin (n = 141 of 199; 70.85%), colistin (n = 112 of 199; 56.28%), tigecycline (n = 95 of 199; 47.74%), CZA (n = 59 of 194; 30.41%), imipenem (n = 20 of 199; 10.05%), fosfomycin (n = 4 of 49; 8.16%), meropenem (n = 15 of 199; 7.54%) and aztreonam (n = 7 of 199; 3.52%). Our susceptibility results of studied antibiotics were reported as low compared with Thongkoom P et al in 2017. The previous study showed the susceptibility testing in CRE isolates from a tertiary hospital in Thailand; the susceptibility rates of CRKP, CREC and CrCLo isolates to meropenem, imipenem, amikacin, gentamicin and tigecycline were 40-80%, 1-7%, 0%, 0-7%, 12-53%, respectively [118].

In Thailand, there was the diversity of carbapenemase genes found over time. The presence of carbapenemase types in our study was not inconsistent with previous studies. Most isolates harbored one of carbapenemase genes, including OXA-48 (n = 91 of 199; 45.73%), NDM (n =72 of 199; 36.18%) NDM plus OXA-48 (n = 31 of 199; 15.58%). In 2012, Rimrang et al collected CRE clinical isolates from Srinagarind Hospital, Khon Kaen University from 2010 to 2011. CRKP isolates found two of the $bla_{IMP\text{-}14a}$ and two of $bla_{NDM,}$ whereas CREC found two of bla_{NDM}. NDM-producing isolates had higher carbapenem MICs than IMP-14a-producing isolates. NDM-producing isolates were highly susceptible to amikacin (100%) and gentamicin (50%), whereas IMP-14a producers were susceptible to amikacin and gentamicin (50%) [5]. In 2015, Netikul and Kiratisin conducted a surveillance study of CRE clinical isolates at Siriraj hospital, Bangkok during 2009-2011. They found that 12.1% of CRKP isolates (n = 4 of 36) only carried bla_{IMP}, whereas 5.9% of CREC (n = 1 and 0.8% of CREclo carried $bla_{KPC,}$ respectively. None of the CRE isolates found bla_{NDM} or $bla_{OXA\text{-}48}$ [120].

OXA-48 and co-existence of OXA-48 also reported since 2016. In 2016, Weewan A collected 53 CRE isolates at Prapokklao Hospital, Chanthaburi. She found the carbapenemase enzyme, including NDM (43,4%), IMP (35.8 %),and NDM plus OXA (5.7%) [6]. In 2018, Laolerd W et al reported 223 non-duplicated clinical strains of CRE isolates from Ramathibodi Hospital between March 2012 and September 2016. Of the 168 CRKP isolates, 27.80% (n = 62) were bla_{NDM}, followed by bla_{NDM} plus $bla_{OXA\text{-}48\text{-}like}$ (24.66%; n = 55) and $bla_{OXA\text{-}48\text{-}like}$ (21.97%; n = 49). Of 27 CREC isolates, 9.87% (n = 22) were bla_{NDM}, followed by $bla_{OXA\text{-}48\text{-}like}$ (1.79%; n = 4) and bla_{NDM} plus $bla_{OXA\text{-}48\text{-}like}$ (0.45%; n = 1). Of 17 CREclo, 4.04% (n = 9) were bla_{NDM}, followed by bla_{IMP} (2.24%; n = 5) and $bla_{OXA\text{-}48\text{-}like}$ (1.35%; n = 3) [121]. In 2019, Prawang A et al reported 30 coRKP isolates collected from patients admitted at Pharmongkutklao hospital; of 93.33% (n = 28) carried $bla_{OXA\text{-}48}$. None of the other carbapenamase found [122]. In 2021, Paveenkittiporn W et al obtained 3,946 CRE clinical isolates from multicenter hospitals in health region V using data from a national antimicrobial resistance surveillance system developed by the Thailand National Institute of Health (NIH) from 2016 to 2018. The most common carbapenemase types in CRKP (n =

2,659) were $bla_{OXA-48-like}$ (59%), followed by bla_{NDM} (55%) and bla_{IMP} (2%). The most common carbapenemase types in CREC (n = 799) were bla_{NDM} (94%), followed by $bla_{OXA-48-like}$ (18%) and bla_{IMP} (<1%), whereas the most common carbapenemase types in CREClo (n = 113) were bla_{NDM} (59%), followed by bla_{IMP} (25%), and $bla_{OXA-48-like}$ (22%). The results also concordance with this study which 16% (n = 634) of the CRE isolates showed coexistence of bla_{NDM} plus $bla_{OXA-48-like}$ [123].

As mentioned above, although the most common identified carbapenemase are NDM and OXA-48, NDM and OXA-48-producing strains have continually reported over the last 5 years. It seems to be that the carbapenemase genes will continue to evolve. The features related to the evolution of carbapenmase genes are as follows: 1) geographical conditions, such as infectious control (hygiene), high selective pressure reflected to antibiotic use (overuse or misuse) 2) genetic structures of the carbapenemase genes 3) human exchange e.g. foreign travel, tourism or medical tourism [124]. Indeed, carbapenemase genes can be carried the mobile genetic elements (such as plasmid, transposons and integrons). The conjugation of NDM and OXA-48 may occur even the same or different bacterial species, especially *Acinetobacter* spp, which found the co-producing of NDM and OXA-48. In China, Yin C et al found the co-producing of KPC-2 and NDM or 5 in CRE isolates because the carbapenemase genes could be transferable plasmids [125]. Thus, Horizontal gene transfer (HGT) may occur and can transfer the mobile genetic elements between bacteria with same bacterial species or different bacterial species [126, 127].

Different carbapenemase types in each hospital may affect carbapenem-resistant levels. The higher virulence of CRE clinical isolates may also be associated with carbapenemase genes which were investigated by Tamma P.D. et al in 2017 [128]. Our CRE clinical isolates seem to be more virulent due to containing at least one of the carbapenemase-producing CRE isolates. At meropenem MICs ≤ 4 μg/mL, our majority of the carbapenemase positive isolates were OXA-48 producing isolates (16 of 65; 24.6%), followed by NDM (2 of 71; 2.82%) and NDM plus OXA-48 (2 of 10; 20%). In 2016, Fattouh R et al showed types of carbapenemase having meropenem MICs ≤ 4 μg/mL as follows: 66.6% of OXA-48 positive (n = 20 of 30), 50.1% of KPC positive (n = 32 of 63), and 8.8% of NDM-positive (n = 20 of 90). Furthermore, NDM-

positive isolates had meropenem MICs ≥ 2 μg/mL, whereas OXA-48 and KPC-positive isolates were relatively distributed across the meropenem MIC, ranging from ≤ 0.12 to ≥ 16 μg/mL [23]. It seems to be containing NDM-producing isolates are likely to have stronger resistance levels than OXA-48 and KPC-producing isolates, respectively. The molecular resistance mechanisms should also be considered for designing antibiotic dosing regimens.

Carbapenems have a role in the treatment of infections caused by CRE based on MIC levels. The prolonged infusion with shorten interval regimens (loading dose 2 g followed by 1 g infusion 3 hours every 6 hours) reached the ≥ 90% PTA target of 100%*f*T >MIC at carbapenem MICs ≤ 2 μg/mL. The results are in concordance with a systematic review and meta-analysis conducted by Yu Z et al in 2018. The results favored the prolonged infusion because it significantly had a higher clinical improvement rate (OR = 2.10, 95% CI = 1.31-3.38) and a lower mortality (RR = 0.66, 95% CI = 0.50-0.88) compared with intermittent bolus [129]. In 2019, Thunyapituk N et al also performed the dosing regimens of 3 hours of prolonged infusion every 6 hours could reach the PTA target of 100%*f*T >MIC at meropenem MICs ≤ 2 μg/mL [65]. Nonetheless, Del Bono V et al in 2017 assessed the achievement of 40% T>MIC measured by meropenem-levels in 19 critically ill patients with BSI caused by KPC-KP were 95% (n = 18 of 19), 68% (n = 13 of 19) and 32% (n = 6 of 19) at MICs of 8 μg/mL, 16 μg/mL and 32 μg/mL, respectively. The difference in PK/PD target of meropenem in each study may affect the achievement of the target at each MIC.

Based on the current evidence, administration of high dose prolonged infusion carbapenem-based regimen (e.g. double carbapenem regimens) for the treatment of infections caused by KPC-producing isolates could be recommended if carbapenem MICs ≤ 8 μg/mL [41, 130]. Our findings showed that more than 80% of CRE isolates being non-susceptible to meropenem and imipenem (14.57% (n = 29 of 199) for meropenem MICs ≤ 8 μg/mL and 29.15% (n = 58 of 199) for imipenem MICs ≤ 8 μg/mL). None of the KPC-producing isolates were found in the study. Thus, carbapenem-based regimens may not be considered the first antibiotic regimen for treating CRE in Thailand.

For colistin, our findings showed 56.28% of all isolates (n = 112 of 199) were intermediate to colistin (colistin MICs ≤ 2 μg/mL). Based on hospital levels, university hospital (*n* = 9 of 49; 18.37%) had lower-intermediate to colistin than non-university hospital (*n* = 103 of 150; 68.67%). In 2019, Jitaree K et al showed intermediate rates to colistin for 116 CRE isolates (n = 96 for CRKP and n = 20 for CREC) in a tertiary hospital were 78.44% (n = 91 of 116 isolates) [77]. Our CRE isolates may be higher resistance to colistin than the previous study because of carrying OXA-48 in most CRE clinical isolates (n = 132 of 199; 66.33%). The resistant genes associated with colistin-resistance isolates were OXA-48 and *mcr-1* Interestingly, we observed the coexistence of *mcr-1* with OXA-48 in carbapenem-resistant *K. pneumoniae.* Among these coexistence resistance gene, 80% (4 of 5) had colistin MICs > 8 μg/mL. Previous studies have demonstrated the co-occurrence of *mcr-1* and OXA-48 is common in colistin-resistance isolates; these isolates had colistin MICs ranged of 32-64 μg/mL [131]. The reason may be most pathogens containing the carbapenemase and *mcr-1* positive genes on their plasmids or integrons can carry, transfer, and move the genetic elements to another pathogen, leading to a high level of resistance in colistin and carbapenem [132]. Moreover, increasing the use of colistin for the treatment of infections caused by Gram-negative bacteria as well as horizontal gene transfer between drug-resistant Gram-negative bacteria might be principal factors that led to the emergence of CoRKP worldwide [133]. The molecular mechanism of these clinical isolates should be warranted for further investigation.

Colistin is recommended as a backbone of treatment for patients infected by CRE when the CRE clinical isolates were susceptible to at least 2 antibiotic agents, including colistin [83]. Selecting suitable colistin dosage regimens for the treatment has led to maximizing the clinical efficacy. Based on colistin MICs, a higher MIC could lead to treatment failure and the development of antibiotic resistance. Our results found that 35.17% (n = 70 of 199) of CRE isolates had high MICs of colistin (MICs ≥ 16 μg/mL). Colistin revealed an extremely high rate of resistance (81.63%) in our studied CRE isolates compared with other studies in which the resistance rate did not exceed 15% [134, 135]. Resulted of PK/PD study recommended colistin MICs to achieve PTA in all patients with various creatinine clearances ranging from 0.5 to 16 μg/mL [77].

For CFR, some dosing regimens in patients with CrCL below 25 mL/min met ≥ 90% CFR targets in hospital-level S and M1; the colistin dosing regimens in all patients with creatinine clearance below 50 and 90 mL/min reached ≥ 90% CFR targets in CREC and CREclo isolates, respectively. None of any regimens achieved ≥ 90% CFR targets in hospital-level U and A or CRKP isolates. The results were consistence with Jitaree K et al study in 2019, which determined the CFR using CRE isolates from a tertiary hospital. Their results reported CFR of overall colistin regimens is approximately 70–86% [77]. Although high rates of colistin resistance were reported, colistin remains the activity against the CRE isolates depending on antibiotic resistance rate or types of pathogens. Selecting colistin-containing regimens for the therapy of CRE may be used with caution.

When colistin resistance occurs, tigecycline may be an interesting option for the treatment of infections. Our finding showed 47.74% (n = 95 of 199) of CRE isolates were susceptible to tigecycline at tigecycline MICs ≤ 0.5 μg/mL. Nonetheless, previous studies reported wide tigecycline susceptibility rates ranging from 12 to 100% [87, 118, 136, 137] in Thailand, as well as 58.0–86.1% in other countries [40, 138]. There may be differences in the cutoff tigecycline breakpoints (in a range of between 0.5 and 2 μg/mL). The fact that type of carbapenemase genes may affect wide disparities of tigecycline MICs among CRE isolates. Compared to MIC_{50} and MIC_{90}, the CRE isolates harboring OXA-48 and OXA-48 plus NDM had higher tigecycline MICs than the CRE isolates harboring NDM. Similar to colistin, there was resistance to tigecycline in OXA-48 positive isolates (susceptibility rate ranging from 64.84% for OXA-48 and 32.26% for OXA-48 plus NDM), whereas NDM-positive isolates were susceptible (90.28%). Thus, tigecycline may be an interesting option for NDM-positive isolates if knowing carbapenemase genes.

To date, a high-dose tigecycline-based combination may be considered in critically ill patients for the treatment options for CRE infections. The potential benefit of the tigecycline regimens is non-nephrotoxicity compared to colistin or aminoglycosides [41]. According to the tigecycline PK/PD study, all high dose tigecycline regimens reached the PTA target for isolates with a MIC of 0.5 μg/mL. At MIC = 1 μg/mL, a loading dose of 200 mg of tigecycline followed by a maintenance

dose of 100 mg every 12 h (400 mg/day) was able to reach the PTA target; these results were consistent with those of previous studies [75, 139]. At MIC = 2 μg/mL, our results showed that double high-dose tigecycline regimens (a loading dose of 400 mg tigecycline followed by a maintenance dose of 200 mg every 12 h: 400 mg/day) also achieved the PTA targets. Once-daily high doses of tigecycline (loading dose of 400 mg followed by maintenance doses of 200 mg every 24 h) also met the PTA target with a MIC of 1 μg/mL, owning to it have a concentration-dependent killing property and long half-life (~27–42 hours) [140]. For CFR, double high-dose tigecycline regimens achieved the ≥ 90% CFR in all hospital levels. Besides, the double-dosing regimens remained activity against CRKP, CREC, and CREclo. Using a CFR > 90% for $fAUC_{0\text{-}24}$/MIC ≥ 0.9, only high-dose tigecycline regimens achieved the target: a loading dose of 400 mg with a maintenance dose of 200 mg every 12 hours, whereas the usual regimen (a loading dose of 200 mg with a maintenance dose of 100 mg every 12 hours) met almost 90% CFR. The results were consistent with the previous study, a high dose (tigecycline 200 mg initially, followed by 100 mg every 12 hours) achieved in a favorable CFR target leads to reduce 30-day and ICU mortality when compared with the standard dose (tigecycline 100 mg initially, followed by 50 mg every 12 hours) (OR (95%CI) = 2.25 (0.55-9.24) and 12.48 (2.06-75.43), respectively), respectively [47, 75]. Although differences in the cutoff tigecycline breakpoints (ranging from 0.5 to 2 μg/mL), hospital levels, and types of CRE pathogens, using high-dose tigecycline dosing regimens could be a potential activity for the treatment of CRE infections, especially the higher colistin MICs or renal failure occurs [41]. The efficacy and safety of various high-dose tigecycline against CRE should be investigated in large-scale clinical studies.

Fosfomycin is a therapeutic alternative for the treatment of infections caused by CRE. The appropriate intravenous fosfomycin dosing regimens should be used to increase clinical outcomes, especially in critically ill patients. In this study, all dosing regimens yielded ≥ 90% PTA target at a MIC of 128 μg/mL. Furthermore, every fosfomycin dosing regimen consisted of a loading dose of 8 g followed by a maintenance dose based on CrCL, including 4-6 g every 6 hours (16-24 g/day) in patients with CrCL 51-90 mL/min, 4 g every 8 hours (12 g/day) in patients with CrCL

10-50 mL/min and 4 g every 12 hours (8 g/day) in patients with CrCL < 10 mL/min, can reach ≥ 90% PTA target for AUC_{0-24}/MIC of > 21.5 at a MIC of 256 μg/mL. Considering the highest dosing regimens (24/day), our findings showed the Fosfomycin dosing regimens achieved the desired target at different fosfomycin MICs compared with previous studies. Of 24 g/day achieved ≥ 90% PTA for 70%*f*T>MIC, Albiero J et al in 2016 showed the target at a MIC of ≤ 16 μg/mL [80], whereas Rodriguez-Gascon A et al in 2019 met the target at a MIC of ≤ 64 μg/mL [81]. In Thailand, a study conducted by Leelawattanachai P et al in 2020 also showed 24 g/day (8 g every 8 hours infusion 1 hour) in patients with CrCL ≥ 50 mL/min yield to optimal PK/PD target (≥ 90% PTA for AUC_{0-24}/MIC of > 21.5) at MICs of ≤ 128 μg/mL [82]. Differences in PK/PD index and target may affect reaching the PTA target in each MIC.

According to CFR, any fosfomycin dosing regimens in our study could not reach ≥ 90% CFR whether be it settings, hospital levels, or types of carbapenemase. These results were consistent with previous studies, performing none of any fosfomycin dosing regimens in any studies met ≥ 90% CFR [80, 82]. Interesting, our findings harboring OXA-48 (n = 26 of 49; 53%) and NDM + OXA-48 (n = 21 of 49; 43%) showed the fosfomycin MIC range, MIC_{50} and MIC_{90} against CRE were 12 to >1,024 μg/mL, > 1,024 μg/mL. Leelawattanachai P et al study using PK/PD target for NDM producing isolates performed fosfomycin MIC range, MIC_{50} and MIC_{90} were 0.38 to > 1,024 μg/mL, 48 μg/mL, and >1,024 μg/mL in 129 non-duplicated CRE clinical isolates in a university hospital. For Albiero J et al study harboring KPC-2 (n = 18) showed the fosfomycin MIC range, MIC_{50} and MIC_{90} were 16 to 1,024 μg/mL, 64 μg/mL and 8-fold higher [80]. It is noteworthy that KPC-producing isolates had lower fosfomycin MICs than NDM or OXA-48 producing isolates. Thus, intravenous fosfomycin should be considered when determining the fosfomycin susceptibility that differently depends on carbapenemases-producing CRE.

Amikacin and gentamicin were the top two antibiotics being susceptible to aminoglycosides. The susceptible rates of amikacin were 87.94% (n = 175 of 199), whereas 70.85% (n = 141 of 199) of a total of CRE isolates were susceptible to gentamicin. Data from NARST 2020 showed the susceptibility rates to amikacin and

gentamicin ranged from 93.0 - 96.8% for amikacin and 74.1-87.6% for gentamicin [141]. Although the susceptibility rates of amikacin and gentamicin in the study had lower than the susceptibility rates from NARST, there was more susceptible than other antibiotics.

Noteworthy is CRE clinical isolates were resistant to one of the two aminoglycosides tested (e.g. resistant to gentamicin, but susceptible to amikacin), especially among NDM-producing isolates (n = 25 of 48; 52.08%). In 2011, Bercot B et al evaluated the prevalence of 16S rRNA methylase genes in 16 NDM producing isolates; they found that 75% of those enterobacterial isolates performed 16S rRNA methylase genes [142]. In 2017, Nordmann P et al used a total of 48 enterobacterial isolates, including 28 isolates resistant to aminoglycosides and 10 isolates susceptible to aminoglycosides to develop and evaluate a rapid colorimetric test for identifying aminoglycoside (amikacin and gentamicin) resistance. The results showed that 10 aminoglycosides-susceptible enterobacterial isolates were susceptible to both amikacin and gentamicin. For aminoglycosides-resistant enterobacterial isolates, all clinical isolates that produced methylases (n = 18) were resistant to both amikacin and gentamicin at high MIC levels (amikacin and gentamicin MICs > 256 μg/mL), whereas most aminoglycoside-resistant isolates produced aminoglycoside-modifying enzymes (AMEs) performed non-susceptible to one of the two aminoglycosides tested [143]. In 2019, Upadhyaya P and colleagues found the loss of *rmtF* methylase genes may be associated with loss of amikacin resistance [144]. The mobile genetic elements related to carbapenemase gene transmission on chromosomes or plasmids may be involved in the presence of highly spreading antibiotic resistance [145]. As described above, our CRE clinical isolates being not cross-resistant to aminoglycosides may be mainly associated with aminoglycoside-modifying enzymes (AMEs).

The antibiotic susceptibility pattern may be associated with types of carbapenemase genes. Our study showed the susceptibility to aminoglycosides, colistin and tigecycline were different among the carbapenemase genes. The NDM-producing isolates were more susceptible to colistin and tigecycline than OXA-48 and NDM plus OXA-48 producing isolates. Additionally, the antibiotic-resistant pattern in

the OXA-48 producing isolates was similar to NDM plus OXA-48-producing isolates, which be highly resistant to all antibiotics, except amikacin. Nonetheless, the number of carbapenemase genes may not be proportional to the percentage of susceptibility. A similar finding was similar to previous studies. Most NDM-positive isolates remained susceptible to colistin and tigecycline [144, 146] but resistant to aminoglycosides owning to carrying 16S rRNA methylase genes (*rmtF*) [147]. Thus, recognizing the types of carbapenemase genes may predict the characteristic of the aminoglycoside susceptibility pattern and aminoglycoside-resistance mechanism.

High-dose regimens of aminoglycosides were simulated for critically ill patients in order to increase the probability of achieving target peak concentrations. Our findings showed the regimens of amikacin and gentamicin met the PTA targets (at C_{max}/MIC ≥ 8) with MICs of no more than 2 and 1 μg/mL, respectively. In 2017, Kato et al recommended the initial total daily dose of amikacin required to achieve a C_{max}/MIC ≥ 8 μg/mL was 15 mg/kg daily, and also recommended 15 mg/kg/day as the maintenance dosage for amikacin MICs ≤ 4 μg/mL [69]. Additionally, Rea RS et al in 2008 showed aminoglycoside dosage regimens (ranging from 5 to 30 mg/kg) achieved the PTA target at MICs of 0.5 μg/mL with the corresponding target of C_{max}/MIC ≥ 10 [148]. Although our study used the minimum PK/PD target of aminoglycosides when compared with the previous study, the dosage regimens did not achieve the targets within the non-susceptibility breakpoints. Considering our aminoglycoside susceptibility, MIC50 of amikacin and gentamicin were 8 and 1 μg/mL. Using high-dose regimens of aminoglycosides as the adjuncts in the antibiotic combination-based regimens may be beneficial when synergism occurs because the aminoglycoside MICs were reduced to 1-2-fold contributing to achieving the PTA target.

Aztreonam is new hope antibiotic for combating antibiotic resistance to gram-negative bacteria. Aztreonam was not hydrolyzed by NDM, whereas ceftazidime-avibactam was not hydrolyzed by OXA-48 [149]. According to carbapenemase types, NDM and OXA-48 were the majority in Thailand, respectively [5, 121]. We hypothesized both antibiotics may have good activity against CRE. Our findings were not consistence with the hypothesis, 96% of total CRE isolates were resistant to

aztreonam because of the background of resistance, particularly ESBLs which have activity to hydrolyze most penicillin, cephalosporins and monobactam (aztreonam).

For a new BLBI, the ceftazidime-avibactam susceptibility rates of ceftazidime-avibactam were 30.41% (n = 59 of 194), ranging from 11.11% to 55.10% (55.10% for hospital-level U , 36.11% for Hospital-level A, 11.11% for hospital-level S and 20.83% for Hospital-level M1). In a university hospital, most CRE clinical isolates harbored OXA-48 (n = 24 of 44; 89.55%). Only 2 of 24 isolates had ceftazidime-avibactam MICs > 256 µg/mL, whereas 22 of 24 isolates had ceftazidime-avibactam MICs, ranging from 0.125 to 2 µg/mL. According to non-university hospitals, the main carbapenemase genes were NDM (n = 71 of 150; 47.7%), whereas the CRE isolates which contained OXA-48 (n = 65 of 150; 43%) which the 37 of 65 (56.92%) CRE isolates had ceftazidime-avibactam MICs ≥ 8/4 µg/mL. It is noteworthy that ceftazidime-avibactam MICs in the non-university hospitals were higher resistant than in the university hospital. Compared with other studies the susceptible rate of ceftazidime-avibactam in CRE was diverse across the region, ranging from 53.3 to 96.6% [150-153]. In the region with a highly susceptible rate of ceftazidime/avibactam, KPCs were the major epidemic genotype [150, 152, 153]. In Arabian Peninsula, the susceptible rate of ceftazidime-avibactam was 53.3%; OXA-48 was the most common CRE genotype that resembles our result [151]. Hence, the genotype of epidemic CRE strain in each hospital region was the key contributor to the ceftazidime-avibactam susceptible rate.

Although, it is highly active against class D of carbapenemase, 41.57% (n = 37 of 89) of all OXA-48 positive isolates had high levels of ceftazidime-avibactam (≥ 16/4 µg/mL). These isolates developed ceftazidime-avibactam resistance led to an increase in the MIC values. The resistance mechanisms may be associated with the amino acid mutation. Typically, naïve OXA-48 contained Proline (Pro) at position 68 and tyrosine (Tyr) at position 211. Substitution of Proline (Pro) by alanine (Ala) (Ala68 Pro) at position 68 and Tyr by serine (Ser) (Ser211Tyr) at position 211 may occur in the ceftazidime-avibactam resistance isolates [154].

PK/PD profile of ceftazidime-avibactam provided a rationale for optimization regimens. For optimal ceftazidime-avibactam dosage regimens for *f*Time>MIC 100%,

the current recommended dose was effective against isolates with ceftazidime-avibactam MICs of ≤ 4 μg/mL despite ceftazidime-avibactam MIC susceptible breakpoint specified at ≤ 8 μg/mL. Additionally, this dosage was not only enough for all range of susceptible strains, particularly the nearest MIC susceptible breakpoint but it also did not reach ≥ 90% PTA of AVI at 100%*f*T ≥ 0.5 μg/mL. The critical threshold of AVI at 0.5 μg/mL was considered appropriate to completely inhibit the various types of beta-lactamases in Enterobacterales [155]. Besides, the recommended dosing regimens cannot meet the CFR targets because of harboring the NDM-positive isolates (n = 91 of 194; 46.91%). The ceftazidime-avibactam dosing regimens recommended from IDSA in 2020 were 2.5 g every 8 hours infusion 2 hours if OXA-48 positive CRE isolates were detected and are susceptible to ceftazidime-avibactam [156]. Additionally, ceftazidime-avibactam was recommended as the first-line antimicrobial agent against CRE in the KPC epidemic region in some guidelines [157]. Thus, as our finding simulated ceftazidime-avibactam dosing regimens supported by a previous study [158], the current recommended ceftazidime-avibactam dose of 2.5 g every 8 hours has to be infused longer time as 2-3 h in order to optimally cover CRE with an ceftazidime-avibactam MIC of ≤ 8 μg/mL and also achieved optimal PK/PD index of AVI.

Based on hospital levels, the university hospital was mostly higher resistance rates to antibiotics than the other hospital levels. Furthermore, the antibiotic-resistant rates had higher in the regional hospital than in the others. The resistant rates of colistin, tigecycline, ceftazidime-avibactam, and aztreonam were higher in hospital-level A than in other levels, whereas aminoglycoside seldom changed in the resistant rates. The diverse resistance rates may be caused by the antibiotic use for the treatment of patients with the difference in complicated infections at each hospital level. Further study should be investigated the relationship between defined daily dose (DDD) and antibiotic resistance rates.

As mentioned above, the combination of antibiotics with MIC-based dose optimization is a recommended strategy in clinical practice due to reduce mortality [9, 47, 159]. When selecting antibiotics for combination, one of the active antibiotics should be susceptible to producing synergistic or additive effects when antibiotics are

combined. When synergistic or additive effects occur, MICs of one of the active antibiotics in a combination antibiotic regimen are decreased by at least 1-fold compared to single antibiotic regimens. Consequently, the MICs moved from resistant to susceptible, leading to the achievement of PTA and CFR targets with usual dosing regimens [160].

Notably, the CRKP isolates producing OXA-48-like showed more antibiotic synergy than the CRKP isolates producing NDM. Coproduction of bla_{NDM} and aminoglycoside resistance genes (e.g., 16S Ribosomal RNA Methyltransferases F: RmtF) may be associated with mobile genetic elements in CRKP isolates and lead to a high level of resistance to aminoglycosides [161]. Based on epidemic spreading characteristics, aminoglycoside-based regimens may be used as the first option for the treatment of infections caused by OXA-48-like-producing isolates.

Aminoglycoside-based regimens may be selected as the appropriate antibiotic regimens owning to being susceptible to amikacin and gentamicin in our clinical setting. Overall, the combination of amikacin and fosfomycin also demonstrated the highest synergism. Synergy rates of the CRE clinical isolates for aminoglycoside-based regimens included amikacin-fosfomycin (n = 8 of 18; 44.44%), gentamicin-fosfomycin (n = 3 of 18; 16.67%), amikacin-tigecycline (n = 4 of 49; 8.16%) and gentamicin-tigecycline (n = 4 of 49; 8.16%). In 2019, Prawang A et al evaluated the synergistic effects in the same setting for the 30 CoRKP clinical isolates that showed 100% (n = 30 of 30) of susceptibility rates for gentamicin and amikacin and 26.27% (n = 8 of 30) for fosfomycin. Their findings showed the greater synergistic effects of CoRKP included gentamicin-fosfomycin (n = 3 of 10; 30.00%) and gentamicin-tigecycline (n = 4 of 30; 13.33%) [137]. Although both studies had different antibiotic susceptibility rates, the results are concordant. The synergy mechanism was aminoglycosides interfere with protein synthesis at 30S ribosomal subunit and affect to decrease expression of the protein (i.e. carbapenemase - KPC or NDM); besides, they can increase permeabilizing of the outer membrane and affect to penetrate of a second antibiotic to the inner membrane [43]. For PK/PD study, none of any aminoglycoside regimens met both ≥ 90% PTA at their resistant breakpoints and reached the ≥ 90% CFR targets. However, aminoglycosides are mostly used as an adjunct to mainstay

antibiotics [83]. When combined with antibiotics, the aminoglycoside MIC values are shifted from high to low levels (approximately a 2-fold reduction in the MICs compared with MICs of single agents) [160]. Therefore, amikacin or gentamicin should be considered as an option in the combination antibiotic regimens in our clinical settings for the CRE treatment both documented and empirical therapy; nephrotoxicity and ototoxicity need to be closely monitored.

Colistin-based regimens may be a treatment option for the CRE clinical isolates. Our results demonstrated that the CRE isolates exhibited synergistic effect ranged of 8.70-22.22%, including colistin-fosfomycin (n = 2 of 9; 22.22%), colistin-gentamicin (n = 4 of 23; 17.39%), colistin-tigecycline (n = 4 of 23; 17.39%) and colistin-amikacin (n = 2 of 23; 8.70%). Furthermore, none of any antibiotic regimens showed antagonism. In 2013, Evren E et al assessed *in vitro* activity of colistin in combination with fosfomycin against 12 OXA-48 producing CRKP isolates. The MICs of colistin and fosfomycin ranged from 1-32 µg/mL and 64-512 µg/mL, respectively. The results revealed fully antagonistic against all isolated tested [40]. Furthermore, Gaibani P et al in 2014 revealed the synergistic effects of colistin with tigecycline (n = 2 of 17; 11.76%) in KPC-producing *K. pneumoniae* isolates [29]. In 2018, Brennan-Krohn T et al investigated *in vitro* antibiotic synergy of colistin-containing combinations in 20 colistin-resistant Enterobacteriaceae isolates, which mostly being KPC-producing isolates (n = 6). Synergy rates by using the checkboard technique for the combination of colistin with tigecycline, and amikacin were 25.0% and 15%, respectively. Overall, the synergy rates revealed in the range of 0-25% even carbapenemase resistance genes may not be similar to previous studies in both synergy rates and synergistic testing patterns. Zusman O et al in 2017 reviewed the proposed synergistic mechanism of colistin-based regimens [162]. Colistin showed a detergent-like effect. Colistin containing positively charged binds to the negatively charged phosphate groups of lipid A on the lipopolysaccharide (LPS) at the outer membrane of gram-negative bacteria, leading to displacing the calcium and magnesium cations into LPS as well as disrupting the LPS structure. Consequently, a change in permeability of the outer membrane allows colistin to penetrate the inner membrane [163]. Furthermore, other antibiotics also get through of outer membrane [162]. In my viewpoint, colistin may be beneficial as the last resort for salvage use in

combination; nephrotoxicity should be concerned, especially combined with aminoglycosides.

The combination of fosfomycin with tigecycline showed the synergy rates against 7 CRE isolates (n = 7 of 21; 33.33%) which most CRE isolates had fosfomycin MICs ranging from 16 - 256 µg/mL. In 2013, Evren E et al also evaluated *in vitro* activity of fosfomycin in combination with tigecycline against 12 OXA-48-producing CRKP isolates. The MICs of fosfomycin and tigecycline ranged from 64-512 µg/mL and 0.25-16 µg/mL, respectively. The results revealed 25% of synergistic effects (n = 4 of 12) [40]. In 2019, Prawang A et al demonstrated the synergistic effects against 30 OXA-48 -producing CoRKP clinical isolates. The fosfomycin and tigecycline MICs ranged of ≤16 - >2,024 µg/mL and 0.5-1 µg/mL, respectively. The synergy rates of fosfomycin-tigecycline were 30% (n = 3 of 10) [137]. Overall, the synergy rate was similar to previous studies due to harboring the same carbapenemase genes. In my opinion, fosfomycin-based regimens may be beneficial use for OXA-48 producing isolates if one of these antibiotics had less than or equal to 1-2- fold MICs above resistance breakpoints.

Notably, high synergy rates occur when any antibiotics are combined through different targets. For all antibiotics tested, antibiotics tested could be classified into three groups based on drug targets of bacteria. Group 1 includes the antibiotics targeting the cell wall, including fosfomycin. Group 2 includes the antibiotics targeting the outer membrane, including colistin. Group 3 includes the antibiotics targeting the 30S ribosomal subunit, including amikacin, gentamicin, and tigecycline. Indeed, we hypothesize that colistin-based regimens had a greater synergy rate than other combination-based regimens owning to *in vitro* and clinical data from the systematic review and meta-analysis studies support synergy use for colistin-based regimens [159, 162]. However, the results were not similar to the hypothesis. High colistin resistance occurs in the CRE clinical isolates, affecting lower synergy rates than expected results.

According to combination regimens, aminoglycoside combined with fosfomycin may be higher synergy rates than other combination regimens. The proposed mechanism for synergy is that fosfomycin disrupted the cell wall by interfering with the formation of the peptidoglycan precursor UDP N-acetylmuramic acid (UDPMurNAc), which will allow penetration of aminoglycosides to increase

permeability at the cell membrane and will inhibit protein synthesis at 30S ribosomal subunit. On the other hand, aminoglycoside combined with tigecycline had the lowest synergy rates. The proposed mechanism for synergy by Zavascki AP et al in 2017 is that aminoglycosides may increase the permeability of cell membrane and will penetrate tigecycline to the ribosome [43]. In a comparison of two aminoglycosides, amikacin can increase the permeability of the cell membrane more than gentamicin [164]. Then both aminoglycosides and tigecycline bind to 30S ribosomal subunits. Aminoglycosides inhibit protein synthesis which targets the mechanisms of translation elongation of amino acid, whereas tigecycline inhibits protein synthesis which targets the mechanisms of blocking the interaction of aminoacyl-tRNA with the A site of the ribosome. The error of protein synthesis will lead to a reduction in β-lactamase expression [43].

Different drug targets may be a factor to increase the success of synergy. Additionally, amikacin combined with fosfomycin had a potential activity against OXA-48, NDM, and NDM plus OXA-48 producing isolates. The high synergy rates for NDM plus OXA-48 producing isolates included 75% (n = 3 of 4) for colistin-tigecycline, 57.15 % (n = 4 of 7) for amikacin-fosfomycin and 42.86% (n = 3 of 7) for gentamicin-fosfomycin. Of OXA-48 producing isolates, the high synergy rates were performed 33.34% (n = 3 of 9) for amikacin-fosfomycin, 33.34% (n = 3 of 9) fosfomycin-tigecycline and 23.53% (n = 4 of 17), whereas amikacin-fosfomycin also had synergistic effects against NDM-producing isolates. Nonetheless, the synergy rate is likely to be low when aminoglycosides are combined with tigecycline or triple combination regimens. That may emphasize that an antibiotic combined with the difference in drug targets may increase the synergistic effect, affecting to maximize their therapeutic effects.

As mentioned above, several studies showed effective antibiotic combination regimens from *in vitro* studies and PK/PD studies, however, the most effective combination antibiotic regimens for treating CRE patients in the clinical study remain to be further studied. The differences in antimicrobial susceptibility testing and resistance mechanism in each region affect the selection of antibiotic agents. In Thailand, the most effective antibiotics for these patients included colistin, amikacin, gentamicin, and tigecycline. Generally, evidence-based clinical decision

making based on either in vitro antimicrobial susceptibility testing or physician experience affects selecting one or more of these antibiotics to treat these patients.

In 2020, IDSA recommended the new treatment options if detected carbapenemase enzymes. Ceftazidime-avibactam is preferred for OXA-48-like-producing isolates, whereas ceftazidime-avibactam plus aztreonam, or cefiderocol are preferred for MBL-producing isolates. In cases of carbapenemase enzymens cannot be identified, colistin-based regimens may be recommended as a last resort for the CRE isolates [157]. In Thailand, a patients infected with a CRE strain is unknown types of carbapenamase enzymes in the routine practice. Furthermore, the new antibiotic options may not be selected as the first option in the antibiotic lists. Thus, antibiotic combination regimens might be considered in the routine practice.

Based on the clinical outcome results, colistin-based regimens might not be considered as the first option following to IDSA recommendation. Although, using each antibiotic-based regimen in the study was not statistically different, the mortality rates revealed that aminoglycoside-based regimens had lower mortality rates at 14-days and 30-days than other based regimens. The lowest mortality rates of aminoglycoside-based regimens at 14-day were 17.39% in gentamicin combination regimens and 10.26% of aminoglycosides plus tigecycline. The results were concordance with previous studies. In 2015, Gonzalez-Padill M et al conducted a retrospective cohort study. They found that colistin plus tigecycline or colistin plus meropenem had higher mortality rates than gentamicin plus tigecycline (colistin-based regimens vs aminoglycoside-based regimens = 66.67%; n = 6 vs 23.80%; n = 25) [166]. In 2021, Effah, C.Y., et al conducted a systematic review and meta-analysis included 21 studies (n = 2,841) between 2015 and 2020 to evaluate the clinical impact of various antibiotic regimens among patients infected with CRKP. The antibiotic susceptibility rates included 47.6% for colistin, 63.1% for gentamicin and 25.7% for tigecycline. The results showed mortality rates on polymyxin-containing regimens and polymyxin-sparing regimens (n = 576 from 3 studies) were not different (OR = 0.99; 95%CI, 0.26-3.01; I^2 = 87%; p < 0.001), whereas aminoglycoside-containing regimens and aminoglycoside-sparing regimens (n = 913 from 5 studies) were significantly different (OR = 0.86; 95%CI, 0.35-1.13; I^2 = 45%; p = 0.71) [167]. Thus,

aminoglycoside-based regimens should be considered as an option for the CRE treatment in our setting.

Although amikacin or gentamicin combined with other antibiotics had lower synergistic effects than other combined regimens, aminoglycoside-based regimens revealed the good treatment outcomes in a patient received aminoglycoside-based regimens, especially aminoglycoside combined with tigecycline. The reasons consisted of 1) the isolates remained susceptible to amikacin and gentamicin 2) the association of non-β-lactams and carbapenemase 3) the antibiotic dosing regimens. Firstly, more than 80% of the CRE clinical isolates remained susceptible to amikacin and gentamicin, contributing to decrease from high MICS to lower MICs. Secondly, the CRE clinical isolates produced carbapenemase enzymes for destroying β-lactams antibiotics, whereas non-β-lactams being not associated with carbapenemase enzymes (such as aminoglycosides and tigecycline) play a role to combat all carbapenemases of CRE strains. Lastly, high-dose antibiotic regimen adjustments based on the PK/PD principle for high-MIC pathogens are often used in clinical study. The benefits of these regimens can be achieved with more PK/PD targets of 90% at each MIC, contributing to increasing penetration to organ targets and improving treatment success. Thus, high-dose aminoglycoside-based combination therapy may be an optimal regimen for the treatment of CRE infections.

Overall, the 14-day and 30-day mortality rates, clinical outcomes, and microbiological outcomes in prospective groups had lower than in retrospective groups due to differences in antibiotic options in combination therapy. Overall, the selected antibiotic regimen for the CRE treatment was such combination therapy than monotherapy, with 93.06% (n = 67 of 72) receiving combination antibiotic regimens. Colistin-based regimens and aminoglycoside-based regimens were the most frequent combination antibiotic regimens in retrospective and prospective groups, respectively. Our protocol based on *in vitro* study showed low susceptibility to colistin (18.37%) and highly susceptible to aminoglycoside (91.84%), recommended aminoglycoside-based regimens if resistant to colistin. Our recommendation was similar to the previous study. Bano RJ. et al in 2018 also

recommended aminoglycoside-based regimens for the treatment of patients with septic shock, bloodstream infections severe sepsis, or shock (high-risk) if resistant to all carbapenem and colistin but susceptible to colistin [83].

CRE infection was a high risk associated with mortality in the clinical study. CRE patients in Prawang A et al study died 40%-60.71%, aged 66 years, median Pitt score of 3.5-5, 78.57% of patients with shock and 17.86% of patients receiving appropriate empirical antibiotics, whereas CRE patients in our study aged 66-68 years, median Pitt score 5.36-5.72, 35% with shock and 60% of patients receiving appropriate empirical antibiotics [49]. In 2019, Li C et al found the 30-day mortality rate in BSI patients infected with CRE in China was 53.1% (n = 52 of 98) and only 26.5% of all patients (n = 26 of 98) received appropriate empirical therapy within 48 hours after the onset of bacteremia [168]. In 2017, Gradel KO et al showed patients received appropriate empirical therapy had lower mortality rate than patients received inappropriate empirical therapy either short-term mortality (day 2-30) (15.1% *vs* 17.4%; aORs 0.85, 95%CI 0.70-1.02) or long-term mortality (day 31-365) (22.3% *vs* 30.7%; aORs 1.35, 95%CI 1.13-1.60) [169]. Although receiving appropriate empirical therapy is not a difference in short-term mortality, receiving appropriate empirical antibiotics may be involved in the mortality rates in clinical practice. Nonetheless, it would also be difficult to compare because of differences in study design and resistance mechanism. Our study used a quasi-experimental study that could manipulate independent variables before independent variables are measured, whereas the previous study used an observational study that cannot manipulate. Additionally, the most CRE in the previous study was KPC (74%), followed by OXA-48 (17%) and VIM (9%), whereas our study included OXA-48 (53.06%) and OXA-48 plus NDM (42.86%). The differences in results should be interpreted with caution.

The reported mortality among the patients with CRE infections is high, ranging from 17.50% to 54.17% (37.50% to 54.17% in retrospective groups and 17.50% to 37.50% in prospective groups). Focusing on 14-day mortality, the mortality rate was 17.50% to 37.50% (37.50% in retrospective groups and 17.50% in prospective groups). Nonetheless, the overall mortality and 14-day mortality in this present study

seems to have a lower rate than the previous study in the same setting, ranging from 40% to 60.71% (40% in CRKP and 60.71% in CoRKP for 14-day mortality) [49].

I hypothesized that intervention may be associated with the improved treatment outcomes. The study also demonstrated that patients received PK/PD dosing regimen protocol (intervention) in prospective groups had significant difference in lower 14-day mortality rate (P-value = 0.038), overall clinical failure (P-value = 0.042) and 14-day clinical failure (P-value = 0.0463) than patients not received intervention However, there was no difference in 30-day mortality rate and microbiologic outcomes.

Receiving intervention is also a statistically significant predictor of the 14-day mortality in multivariate analysis (OR = 0.35; 95%CI 0.12-0.98; p-value = 0.046) and 14-day clinical failure (OR = 0.38; 95%CI 0.15-1.00; p-value = 0.049). Furthermore, physicians accepted to the minimum requirement of protocol in all prospective patients may result to decrease 14-day mortality. The most regimen that are likely to not comply with the PK/PD dosing protocol was tigecycline dosing regimens at MIC ≤ 0.5 μg/mL. The recommended tigecycline dosing regimens are loading dose 200 mg, followed by maintenance dose 100 mg every 24 hours; however, general tigecycline dosing regimen in routine practice is high-dose regimens (loading dose 200 mg, followed by maintenance dose 100 mg every 12 hours).

The intervention in the present study is designed by integrating the concept of “individualized optimal antibiotic dosing regimens based on PK/PD properties”. The optimal dosing regimens should be selected on: 1) optimal antibiotic options from *in vitro* results, including mono antibiotic activities and combined antibiotic activities, should be sufficient for covering the CRE strains; 2) optimal antibiotic dosing from PK/PD analysis affects more achievement PK/PD target and index and achieve adequate drug levels at the infection site. The concept can be applied to individual-level and hospital-level. In individual-level, all patients may not use the same pattern of antibiotic dosing regimen, they should be received their optimal antibiotic dosing regimen based on MIC-adjust following the PK/PD dosing protocol. In hospital-level, accumulating the optimal antibiotic dosing regimens in individuals can reasonably select the more precise pattern for empiric or documented therapy.

Vasopressor use is associated with increased risk of 14-day mortality, 30-day mortality and 14-day clinical failure in all patients. Using vasopressor were different between survivors and non-survivors (63.83% to 69.84% in survivors and 92.68 to 96% in non-survivors). Similar to previous studies, vasopressor use are important predictors of survival (OR = 4.31; 95%CI 0.83–22.53; p-value = 0.08) in multivariate logistic regression [170]. The most frequent of vasopressor agents in the study were norepinephrine. In 2016, Surviving Sepsis Campaign (SSC) Bundle recommends the sepsis management within the first hour, including administering broad-spectrum antibiotics, administering crystalloid (30 ml/kg) for hypotension or lactate ≥ 4 mmol/L, and administering vasopressors to maintain MAP ≥ 65 mmHg if the patient occurs hypotension during or after fluid resuscitation [94]. In 2020, Li Y et al conducted a systematic review and meta-analysis to evaluate the clinical outcomes of early and late initiation of norepinephrine in patients with septic shock. The results showed that early start of norepinephrine (definition based on each reference study) was associated with reduced short-term mortality (OR = 0.45; 95% CI, 0.34 to 0.61; P < 0.00001; x^2= 3.74; I^2 = 0%). Early start of norepinephrine can increase cardiac output and improve microcirculation and tissue oxygenation, whereas high dose and prolonged exposure to vasopressors may potentially add to the increased mortality rate [171]. However, the study did not focus on timing of start vasopressor. Future research could investigate.

Our study has several limitations. Firstly, *in vitro* study was only investigated at Pramongkutkloa hospital and hospitals in the western region of Thailand; it cannot generalize to others. Secondly, our colleague can control any system becoming a static laboratory of *in vitro* study, whereas there are complex dynamics in humans due to the immune response. Thirdly, we focused on the antibiotic resistance mechanisms from carbapenemase production; non-carbapenemase production, e.g. efflux pump or porin loss, do not investigate. Lastly, results from the clinical study were obtained from a single center with relatively small sample size. Therefore, the power was not enough to detect a different effect (power = 74.75%), and the outcome may not apply in different hospitals in Thailand.

Further studies on CRE infections will be performed as follows: 1) investigating a synergistic activity of other combination antibiotic regimens (e.g. ceftazidime-

avibactam plus aztreonam) against NDM plus OXA-48 positive isolates 2) investigating whole-genome sequencing in the CRE clinical isolates with negative detecting carbapenemase enzymes to obtain further information on the genetic variants associated with antibiotic resistance genes of other resistance mechanisms 3) simulating PTA and CFR if covariates of tigecycline are identified 4) developing population pharmacokinetic model to characterize the pharmacokinetic parameters in Thai patients 5) using the methodology in a large multicenter to confirm the results from the clinical study 6) antibiotic levels to confirm the achievement of PK/PD of 90%, especially aminoglycosides and 7) selecting appropriate antibiotic regimens to be more accurate and precise for individual patients based on PK/PD principle with MICs and genetic polymorphism.

In conclusion, CRE, including CRKP, CREC, and CREclo isolates were resistant to aztreonam, ceftazidime-avibactam, tigecycline, and colistin, whereas they remained susceptible to amikacin and gentamicin. University hospitals had higher resistance rates than non-university hospitals. NDM and OXA-48 enzymes were the most common carbapenemase among the CRE clinical isolates. All CRE clinical isolates carrying the *mcr-1* gene also co-existed with bla_{OXA-48}. High-dose aminoglycosides-based regimens should be considered the optimal antibiotic regimens for the treatment of infections caused by CRE in critically ill patients. The benefit of the aminoglycosides combined with other antibiotics might improve the PTA and CFR achievement and reduce the mortality rates when compared with other combination regimens. Nonetheless, a large sample of CRE clinical isolates should be assessed to confirm the combination activity of these antibiotics against CRKP, CREC, and CREclo isolates.

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REFERENCES

1. Bartsch, S.M., et al., *Potential economic burden of carbapenem-resistant Enterobacteriaceae (CRE) in the United States.* Clinical Microbiology and Infection, 2017. **23**(1): p. 48.e9-48.e16.
2. Centers for Disease Control and Prevention, *Antibiotic resistacce threats in the United States, 2013*. 2013.
3. Tacconelli, E., et al., *Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis.* Lancet Infect Dis, 2018. **18**(3): p. 318-327.
4. National Antimicrobial Resistance Surveillance Center (NARST), *Antimicrobial Resistance 2000-2018 (12M)*. 2019.
5. Rimrang, B., et al., *Emergence of NDM- and IMP-14a-producing Enterobacteriaceae in Thailand.* J Antimicrob Chemother, 2012. **67**(11): p. 2626-30.
6. Arsheewa W, *Prevalence of Carbapenemase Enzyme in Clinical Isolates of Carbapenem-Resistant Enterobacteriaceae from Prapokklao Hospital in 2012 - 2013.* J Prapokklao Hosp Clin Med Educat Center, 2016. **33**(4): p. 315-24.
7. Duangkaw W, et al., *Identification of extended-spectrum beta-lactamase (ESBL) and carbapenemase genes in carbapenemresistant Enterobacteriaceae (CRE) clinical isolates from hospitals in Nan province.* Chiang Mai Medical J, 2018. **57**(4).
8. Dellit, T.H., et al., *Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America guidelines for developing an institutional program to enhance antimicrobial stewardship.* Clin Infect Dis, 2007. **44**(2): p. 159-77.
9. Barlam, T.F., et al., *Implementing an Antibiotic Stewardship Program: Guidelines by the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America.* Clin Infect Dis, 2016. **62**(10): p. e51-77.
10. Asin-Prieto, E., A. Rodriguez-Gascon, and A. Isla, *Applications of the*

pharmacokinetic/pharmacodynamic (PK/PD) analysis of antimicrobial agents. J Infect Chemother, 2015. **21**(5): p. 319-29.

11. Rodriguez-Bano, J., et al., *Treatment of Infections Caused by Extended-Spectrum-Beta-Lactamase-, AmpC-, and Carbapenemase-Producing Enterobacteriaceae.* Clinical Microbiology Reviews, 2018. **31**(2).
12. Zavascki, A.P., J.B. Bulitta, and C.B. Landersdorfer, *Combination therapy for carbapenem-resistant Gram-negative bacteria.* Expert Rev Anti Infect Ther, 2013. **11**(12): p. 1333-53.
13. Falagas, M.E., et al., *Antibiotic treatment of infections due to carbapenem-resistant Enterobacteriaceae: systematic evaluation of the available evidence.* Antimicrob Agents Chemother, 2014. **58**(2): p. 654-63.
14. Menegucci, T.C., et al., *Pharmacodynamic Effects of Sulbactam/Meropenem/Polymyxin-B Combination Against Extremely Drug Resistant Acinetobacter baumannii Using Checkerboard Information.* Microb Drug Resist, 2019. **25**(9): p. 1266-1274.
15. Centers for Disease Control and Prevention, *Facility Guidance for Control of Carbapenem-resistant Enterobacteriaceae (CRE) – November 2015 Update CRE Toolkit*. 2015.
16. Clinical and Laboratory Standards Institute (CLSI), *Performance Standards for Antimicrobial Susceptibility Testing*. 30th ed. Thirtieth Informational Supplement M100-S30. 2020, Wayne, PA, USA: Clinical and Laboratory Standards Institute.
17. Livorsi, D.J., et al., *A systematic review of the epidemiology of carbapenem-resistant Enterobacteriaceae in the United States.* Antimicrob Resist Infect Control, 2018. **7**: p. 55.
18. Kostyanev, T., et al., *Detection and prevalence of carbapenem-resistant Gram-negative bacteria among European laboratories in the COMBACTE network: a COMBACTE LAB-Net survey.* Int J Antimicrob Agents, 2019. **53**(3): p. 268-274.
19. Xu, Y., et al., *Epidemiology of carbapenem resistant Enterobacteriaceae (CRE) during 2000-2012 in Asia.* J Thorac Dis, 2015. **7**(3): p. 376-85.
20. National Antimicrobial Resistance Surveillance Center (NARST), *Antimicrobial resistance 2000-2019*. 2019.

21. Soontaros, S. and N. Leelakanok, *Association between carbapenem-resistant Enterobacteriaceae and death: A systematic review and meta-analysis.* American Journal of Infection Control, 2019. **47**(10): p. 1200-1212.
22. Tamma, P.D., et al., *Comparing the Outcomes of Patients With Carbapenemase-Producing and Non-Carbapenemase-Producing Carbapenem-Resistant Enterobacteriaceae Bacteremia.* Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 2017. **64**(3): p. 257-264.
23. Fattouh, R., et al., *What Is the Appropriate Meropenem MIC for Screening of Carbapenemase-Producing Enterobacteriaceae in Low-Prevalence Settings?* Antimicrob Agents Chemother, 2015. **60**(3): p. 1556-9.
24. Morrill, H.J., et al., *Treatment options for carbapenem-resistant Enterobacteriaceae infections.* Open Forum Infectious Diseases, 2015. **2**(2).
25. Lapponampai W and Pamonsinlapatham P, *Application Program for Anti-HIV Drug Self-Management on Android Smart Phone.* Thai Journal of Science and Technology, 2019. **27**(4): p. 738-750.
26. Karaiskos, I., A. Antoniadou, and H. Giamarellou, *Combination therapy for extensively-drug resistant gram-negative bacteria.* Expert Review of Anti-Infective Therapy, 2017. **15**(12): p. 1123-1140.
27. Schmid, A., et al., *Monotherapy versus combination therapy for multidrug-resistant Gram-negative infections: Systematic Review and Meta-Analysis.* Sci Rep, 2019. **9**(1): p. 15290.
28. Yim, H., et al., *Time-kill synergy tests of tigecycline combined with imipenem, amikacin, and ciprofloxacin against clinical isolates of multidrug-resistant Klebsiella pneumoniae and Escherichia coli.* Ann Clin Lab Sci, 2011. **41**(1): p. 39-43.
29. Gaibani, P., et al., *In vitro activity and post-antibiotic effects of colistin in combination with other antimicrobials against colistin-resistant KPC-producing Klebsiella pneumoniae bloodstream isolates.* J Antimicrob Chemother 2014. **69**(7): p. 1856-1865.
30. Fredborg, M., T.E. Sondergaard, and M. Wang, *Synergistic activities of*

meropenem double and triple combinations against carbapenemase-producing Enterobacteriaceae. Diagn Microbiol Infect Dis, 2017. **88**(4): p. 355-360.

31. Oliva, A., et al., *In vitro evaluation of different antimicrobial combinations against carbapenemase-producing Klebsiella pneumoniae: the activity of the double-carbapenem regimen is related to meropenem MIC value.* J Antimicrob Chemother, 2017. **72**(7): p. 1981-1984.
32. Del Bono, V., et al., *Meropenem for treating KPC-producing Klebsiella pneumoniae bloodstream infections: Should we get to the PK/PD root of the paradox?* Virulence, 2017. **8**(1): p. 66-73.
33. Brennan-Krohn, T., et al., *Screening for synergistic activity of antimicrobial combinations against carbapenem-resistant Enterobacteriaceae using inkjet printer-based technology.* J Antimicrob Chemother, 2017. **72**(10): p. 2775-2781.
34. Wiskirchen, D.E., et al., *In vitro pharmacodynamics of simulated pulmonary exposures of tigecycline alone and in combination against Klebsiella pneumoniae isolates producing a KPC carbapenemase.* Antimicrob Agents Chemother, 2011. **55**(4): p. 1420-1427.
35. Bulik, C.C. and D.P. Nicolau, *Double-carbapenem therapy for carbapenemase-producing Klebsiella pneumoniae.* Antimicrob Agents Chemother, 2011. **55**(6): p. 3002-3004.
36. Poirel, L., N. Kieffer, and P. Nordmann, *In vitro evaluation of dual carbapenem combinations against carbapenemase-producing Enterobacteriaceae.* J Antimicrob Chemother, 2016. **71**(1): p. 156-61.
37. Nath, S., et al., *In vitro and in vivo activity of single and dual antimicrobial agents against KPC-producing Klebsiella pneumoniae.* J Antimicrob Chemother, 2018. **73**(2): p. 431-436.
38. Betts, J.W., et al., *In vitro and in vivo activities of tigecycline-colistin combination therapies against carbapenem-resistant Enterobacteriaceae.* Antimicrob Agents Chemother, 2014. **58**(6): p. 3541-3546.
39. Kulengowski, B., et al., *Effect of the meropenem MIC on the killing activity of meropenem and polymyxin B in combination against KPC-producing Klebsiella pneumoniae.* J Antibiot (Tokyo), 2017. **70**(9): p. 974-978.

40. Evren, E., et al., *In vitro activity of fosfomycin in combination with imipenem, meropenem, colistin and tigecycline against OXA 48-positive Klebsiella pneumoniae strains.* Diagn Microbiol Infect Dis, 2013. **76**(3): p. 335-8.
41. Sheu, C.C., et al., *Infections Caused by Carbapenem-Resistant Enterobacteriaceae: An Update on Therapeutic Options.* Front Microbiol, 2019. **10**: p. 80.
42. Bulik, C.C. and D.P. Nicolau, *Double-carbapenem therapy for carbapenemase-producing Klebsiella pneumoniae.* Antimicrobial agents and chemotherapy, 2011. **55**(6): p. 3002-3004.
43. Zavascki, A.P., B.O. Klee, and J.B. Bulitta, *Aminoglycosides against carbapenem-resistant Enterobacteriaceae in the critically ill: the pitfalls of aminoglycoside susceptibility.* Expert Rev Anti Infect Ther, 2017. **15**(6): p. 519-526.
44. Gutierrez-Gutierrez, B., et al., *Effect of appropriate combination therapy on mortality of patients with bloodstream infections due to carbapenemase-producing Enterobacteriaceae (INCREMENT): a retrospective cohort study.* Lancet Infectious Diseases, 2017. **17**(7): p. 726-734.
45. Hawkey, P.M., et al., *Treatment of infections caused by multidrug-resistant gram-negative bacteria: Report of the British society for antimicrobial chemotherapy/healthcare infection society/british infection association joint working party.* Journal of Antimicrobial Chemotherapy, 2018. **73**: p. iii2-iii78.
46. Zusman, O., et al., *Polymyxin monotherapy or in combination against carbapenem-resistant bacteria: Systematic review and meta-analysis.* Journal of Antimicrobial Chemotherapy, 2017. **72**(1): p. 29-39.
47. Ni, W., et al., *Tigecycline Treatment for Carbapenem-Resistant Enterobacteriaceae Infections: A Systematic Review and Meta-Analysis.* Medicine (Baltimore), 2016. **95**(11): p. e3126.
48. Thamlikitkul V and Popum S, *Monitoring of Effectiveness and Safety of Colistin for Therapy in Resistant Gram-Negative Bacterial Infections in Hospitalized Patients at Siriraj Hospital.* J Med Assoc Thai, 2016. **99**: p. 301-7.
49. Prawang, A., et al., *Treatment and Clinical Outcomes Among Infected Patients with Colistin-resistant Klebsiella pneumoniae Bacteremia.* Open Forum Infect

Dis, 2019. **6**(Suppl 2): p. S782-3.

50. Cotta, M.O., J.A. Roberts, and J. Lipman, *Antibiotic dose optimization in critically ill patients.* Med Intensiva, 2015. **39**(9): p. 563-72.
51. Varghese, J.M., J.A. Roberts, and J. Lipman, *Antimicrobial pharmacokinetic and pharmacodynamic issues in the critically ill with severe sepsis and septic shock.* Crit Care Clin, 2011. **27**(1): p. 19-34.
52. Shah, S., G. Barton, and A. Fischer, *Pharmacokinetic considerations and dosing strategies of antibiotics in the critically ill patient.* J Intensive Care Soc, 2015. **16**(2): p. 147-153.
53. Reller, L.B., et al., *Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices.* Clinical Infectious Diseases, 2009. **49**(11): p. 1749-1755.
54. Balouiri, M., M. Sadiki, and S.K. Ibnsouda, *Methods for in vitro evaluating antimicrobial activity: A review.* Journal of Pharmaceutical Analysis, 2016. **6**(2): p. 71-79.
55. Khan, Z.A., M.F. Siddiqui, and S. Park, *Current and Emerging Methods of Antibiotic Susceptibility Testing.* Diagnostics (Basel, Switzerland), 2019. **9**(2): p. 49.
56. Clinical and Laboratory Standards Institute (CLSI), *Performance Standards for Antimicrobial Susceptibility Testing*. 29 th ed. Twenty-Eigthth Informational Supplement M100-S29. 2019, Wayne, PA, USA: Clinical and Laboratory Standards Institute.
57. European Committee on Antimicrobial Susceptibility Testing (EUCAST), *Breakpoint tables for interpretation of MICs and zone diameters*. 2019.
58. Baietto, L., et al., *A 30-years review on pharmacokinetics of antibiotics: is the right time for pharmacogenetics?* Curr Drug Metab, 2014. **15**(6): p. 581-98.
59. US FDA, *MERREM IV (meropenem for injection)*. 2016.
60. US FDA, *PRIMAXIN® (imipenem and cilastatin) for Injection,*. 2016.
61. Neuner, E.A. and J.C. Gallagher, *Pharmacodynamic and pharmacokinetic considerations in the treatment of critically Ill patients infected with carbapenem-resistant Enterobacteriaceae.* Virulence, 2017. **8**(4): p. 440-452.

62. Guilhaumou, R., et al., *Optimization of the treatment with beta-lactam antibiotics in critically ill patients—guidelines from the French Society of Pharmacology and Therapeutics (Société Française de Pharmacologie et Thérapeutique—SFPT) and the French Society of Anaesthesia and Intensive Care Medicine (Société Française d'Anesthésie et Réanimation—SFAR).* Critical Care, 2019. **23**(1): p. 104.
63. Jaruratanasirikul, S., et al., *Pharmacodynamics of imipenem in critically ill patients with ventilator-associated pneumonia.* J Med Assoc Thai, 2013. **96**(5): p. 551-7.
64. Jaruratanasirikul, S., et al., *Population pharmacokinetics and Monte Carlo dosing simulations of meropenem during the early phase of severe sepsis and septic shock in critically ill patients in intensive care units.* Antimicrob Agents Chemother, 2015. **59**(6): p. 2995-3001.
65. Thunyapituk N, et al., *The prediction of Meropenem dosing regimen using Monte Carlo Simulation method for critically ill patients with Gram negative infection at Phramongkutklao Hospital*. 2019.
66. US FDA, *GENTAMICIN INJECTION, USP*. 2013.
67. UK Medicines and Healthcare Products Regulatory Agency (MHRA) and the European Medicines Agency (EMA), *Amikacin 250 mg/ml Injection*. 2015.
68. Zavascki, A.P., B.O. Klee, and J.B. Bulitta, *Aminoglycosides against carbapenem-resistant Enterobacteriaceae in the critically ill: the pitfalls of aminoglycoside susceptibility.* Expert Review of Anti-infective Therapy, 2017. **15**(6): p. 519-526.
69. Kato, H., et al., *Evaluation of Amikacin Pharmacokinetics and Pharmacodynamics for Optimal Initial Dosing Regimen.* Drugs in R&D, 2017. **17**(1): p. 177-187.
70. Rodvold, K.A., et al., *Serum, tissue and body fluid concentrations of tigecycline after a single 100 mg dose.* J Antimicrob Chemother, 2006. **58**(6): p. 1221-9.
71. Hoffmann, M., et al., *Metabolism, excretion, and pharmacokinetics of [14C]tigecycline, a first-in-class glycylcycline antibiotic, after intravenous infusion to healthy male subjects.* Drug Metab Dispos, 2007. **35**(9): p. 1543-53.
72. Wyeth Pharmaceuticals Inc., *TYGACIL® (TIGECYCLINE) FOR INJECTION*. n.d.

73. Bhavnani, S.M., et al., *Pharmacological and patient-specific response determinants in patients with hospital-acquired pneumonia treated with tigecycline.* Antimicrob Agents Chemother, 2012. **56**(2): p. 1065-72.

74. Xie, J., et al., *Population Pharmacokinetics of Tigecycline in Critically Ill Patients with Severe Infections.* Antimicrobial Agents and Chemotherapy, 2017. **61**(8): p. 10.

75. Ni, W., et al., *Use of Monte Carlo simulation to evaluate the efficacy of tigecycline and minocycline for the treatment of pneumonia due to carbapenemase-producing Klebsiella pneumoniae.* Infect Dis (Lond), 2018. **50**(7): p. 507-513.

76. Tsuji, B.T., et al., *International Consensus Guidelines for the Optimal Use of the Polymyxins: Endorsed by the American College of Clinical Pharmacy (ACCP), European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Infectious Diseases Society of America (IDSA), International Society for Anti-infective Pharmacology (ISAP), Society of Critical Care Medicine (SCCM), and Society of Infectious Diseases Pharmacists (SIDP).* Pharmacotherapy, 2019. **39**(1): p. 10-39.

77. Jitaree, K., et al., *Pharmacokinetic/Pharmacodynamic (PK/PD) Simulation for Dosage Optimization of Colistin Against Carbapenem-Resistant Klebsiella pneumoniae and Carbapenem-Resistant Escherichia coli.* Antibiotics (Basel), 2019. **8**(3).

78. Falagas, M.E., et al., *Fosfomycin.* Clinical Microbiology Reviews, 2016. **29**(2): p. 321-347.

79. Candel, F.J., M. Matesanz David, and J. Barberán, *New perspectives for reassessing fosfomycin: applicability in current clinical practice.* Revista espanola de quimioterapia : publicacion oficial de la Sociedad Espanola de Quimioterapia, 2019. **32 Suppl 1**(Suppl 1): p. 1-7.

80. Albiero, J., et al., *Pharmacodynamic Evaluation of the Potential Clinical Utility of Fosfomycin and Meropenem in Combination Therapy against KPC-2-Producing Klebsiella pneumoniae.* Antimicrob Agents Chemother, 2016. **60**(7): p. 4128-39.

81. Rodriguez-Gascon, A. and A. Canut-Blasco, *Deciphering pharmacokinetics and pharmacodynamics of fosfomycin.* Rev Esp Quimioter, 2019. **32 Suppl 1**: p. 19-24.

82. Leelawattanachai, P., et al., *Evaluation of Intravenous Fosfomycin Disodium Dosing Regimens in Critically Ill Patients for Treatment of Carbapenem-Resistant Enterobacterales Infections Using Monte Carlo Simulation.* Antibiotics (Basel), 2020. **9**(9).

83. Rodriguez-Bano, J., et al., *Treatment of Infections Caused by Extended-Spectrum-Beta-Lactamase-, AmpC-, and Carbapenemase-Producing Enterobacteriaceae.* Clin Microbiol Rev, 2018. **31**(2): p. e00079-17.

84. Stein, G.E., et al., *Pharmacokinetic and Pharmacodynamic Analysis of Ceftazidime/Avibactam in Critically Ill Patients.* Surg Infect (Larchmt), 2019. **20**(1): p. 55-61.

85. Martin, A., et al., *Association Between Carbapenem Resistance and Mortality Among Adult, Hospitalized Patients With Serious Infections Due to Enterobacteriaceae: Results of a Systematic Literature Review and Meta-analysis.* Open Forum Infect Dis, 2018. **5**(7): p. ofy150.

86. Lin, Y.T., et al., *Appropriate Treatment for Bloodstream Infections Due to Carbapenem-Resistant Klebsiella pneumoniae and Escherichia coli: A Nationwide Multicenter Study in Taiwan.* Open Forum Infect Dis, 2019. **6**(2): p. ofy336.

87. Chotiprasitsakul, D., et al., *Epidemiology of carbapenem-resistant Enterobacteriaceae: a 5-year experience at a tertiary care hospital.* Infect Drug Resist, 2019. **12**: p. 461-468.

88. Giannella, M., et al., *Effect of combination therapy containing a high-dose carbapenem on mortality in patients with carbapenem-resistant Klebsiella pneumoniae bloodstream infection.* Int J Antimicrob Agents, 2018. **51**(2): p. 244-248.

89. Gutierrez-Gutierrez, B., et al., *Effect of appropriate combination therapy on mortality of patients with bloodstream infections due to carbapenemase-producing Enterobacteriaceae (INCREMENT): a retrospective cohort study.*

Lancet Infect Dis, 2017. **17**(7): p. 726-734.

90. Centers for Disease Control and Prevention, *CDC/NHSN Surveillance Definitions for Specific Types of Infections*. 2019.
91. Naing L, Winn T, and Rusli B.N, *Practical Issues in Calculating the Sample Size for Prevalence Studies.* Archives of Orofacial Sciences, 2006. **1**: p. 9-14.
92. Thongkoom P, et al., *Carbapenem-Resistant Enterobacteriaceae at Rajavithi Hospital: Results of a Microbiology Laboratory Program (2009-2015).* J Med Assoc Thai, 2017. **100**((Suppl. 1)): p. S212-S221.
93. Vanichbuncha K, *Statistical sampling. In: Statistics for research*. 11 ed. 2017, Bangkok: Samlada Co., Ltd.
94. Singer, M., et al., *The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3).* JAMA, 2016. **315**(8): p. 801-10.
95. Kaewkungwal J and Singhasivanon P, *sample size in clinical research*, in *Textbook of clinical research*, Pitisuttithum P and Picheansoonthon C, Editors. 2011, Amarin Printing and Publishing Public Company Limited: Bangkok. p. 107-44.
96. Interlab, *Mueller Hinton II Broth*. n.d.
97. Merck microbiology manual, *MUELLER-HINTON agar CLSI - EMD Millipore*. 2008.
98. Clinical and Laboratory Standards Institute (CLSI), *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Approved Standard M07-A9.* 9 th ed. 2012, Wayne, PA, USA: Clinical and Laboratory Standards Institute.
99. bioMérieux SA, *Etest - Antimicrobial Susceptibility Testing*. 2016.
100. Barus, T., et al., *Genetic Diversity of Klebsiella spp. Isolated from Tempe based on Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction (ERIC-PCR).* HAYATI Journal of Biosciences, 2013. **20**(4): p. 171-176.
101. Poirel, L., et al., *Multiplex PCR for detection of acquired carbapenemase genes.* Diagn Microbiol Infect Dis, 2011. **70**(1): p. 119-23.
102. Mlynarcik, P., M. Roderova, and M. Kolar, *Primer Evaluation for PCR and its Application for Detection of Carbapenemases in Enterobacteriaceae.* Jundishapur J Microbiol, 2016. **9**(1): p. e29314.

103. Preechachuawong, P., et al., *DETECTION OF NEW DELHI METALLO-BETA-LACTAMASE-1-PRODUCING KLEBSIELLA PNEUMONIAE AT A GENERAL HOSPITAL IN THAILAND.* Southeast Asian J Trop Med Public Health, 2015. **46**(6): p. 1031-6.

104. Preechachuawong, P., et al., *Detection of New Delhi Metallo-bata-lactamase-1-producing Klebsiella pneumoniae at a general hospital in Thailand.* Southeast Asian J Trop Med Public Health, 2015. **46**(6): p. 1031-6.

105. Delattre, I.K., et al., *Population pharmacokinetic modeling and optimal sampling strategy for Bayesian estimation of amikacin exposure in critically ill septic patients.* Ther Drug Monit, 2010. **32**(6): p. 749-56.

106. Craig WA, *Optimizing aminoglycoside use.* Crit Care Clin,, 2011. **27**(1): p. 107-21.

107. Drusano, G.L., et al., *Back to the future: using aminoglycosides again and how to dose them optimally.* Clin Infect Dis, 2007. **45**(6): p. 753-60.

108. Goncalves-Pereira, J., A. Martins, and P. Povoa, *Pharmacokinetics of gentamicin in critically ill patients: pilot study evaluating the first dose.* Clin Microbiol Infect, 2010. **16**(8): p. 1258-63.

109. Borsuk-De Moor, A., et al., *Population Pharmacokinetics of High-Dose Tigecycline in Patients with Sepsis or Septic Shock.* Antimicrobial Agents and Chemotherapy, 2018. **62**(4): p. 11.

110. Garonzik, S.M., et al., *Population pharmacokinetics of colistin methanesulfonate and formed colistin in critically ill patients from a multicenter study provide dosing suggestions for various categories of patients.* Antimicrob Agents Chemother, 2011. **55**(7): p. 3284-94.

111. Nation, R.L., et al., *Dosing guidance for intravenous colistin in critically-ill patients.* Clin Infect Dis, 2017. **64**(5): p. 565-571.

112. Tsala, M., et al., *Exploring colistin pharmacodynamics against Klebsiella pneumoniae: a need to revise current susceptibility breakpoints.* Journal of Antimicrobial Chemotherapy, 2018. **73**(4): p. 953-961.

113. Parker, S.L., et al., *Population Pharmacokinetics of Fosfomycin in Critically Ill Patients.* Antimicrob Agents Chemother, 2015. **59**(10): p. 6471-6.

114. Lepak, A.J., et al., *In Vivo Pharmacokinetics and Pharmacodynamics of ZTI-01 (Fosfomycin for Injection) in the Neutropenic Murine Thigh Infection Model*

against Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa. Antimicrobial Agents and Chemotherapy, 2017. **61**(6): p. e00476-17.

115. Dubois A, B.J., and Mentr´F,, *Mathematical Expressions of the Pharmacokinetic and Pharmacodynamic Models implemented in the PFIM software*. 2011.
116. Mouton, J.W., et al., *Standardization of pharmacokinetic/pharmacodynamic (PK/PD) terminology for anti-infective drugs: an update.* Journal of Antimicrobial Chemotherapy, 2005. **55**(5): p. 601-607.
117. Magiorakos, A.P., et al., *Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance.* Clin Microbiol Infect, 2012. **18**(3): p. 268-81.
118. Thongkoom P, et al., *Carbapenem-Resistant Enterobacteriaceae at Rajavithi Hospital: Results of a Microbiology Laboratory Program (2009-2015).* J Med Assoc Thai, 2017. **100**(Suppl. 1): p. S212-21.
119. van Loon, K., A.F. Voor In 't Holt, and M.C. Vos, *A Systematic Review and Meta-analyses of the Clinical Epidemiology of Carbapenem-Resistant Enterobacteriaceae.* Antimicrob Agents Chemother, 2018. **62**(1): p. e01730-17.
120. Netikul, T. and P. Kiratisin, *Genetic Characterization of Carbapenem-Resistant Enterobacteriaceae and the Spread of Carbapenem-Resistant Klebsiella pneumonia ST340 at a University Hospital in Thailand.* PloS one, 2015. **10**(9): p. e0139116-e0139116.
121. Laolerd, W., et al., *Carbapenemase-Producing Carbapenem-Resistant Enterobacteriaceae from Bangkok, Thailand, and Their Detection by the Carba NP and Modified Carbapenem Inactivation Method Tests.* Microb Drug Resist, 2018. **24**(7): p. 1006-1011.
122. Prawang A, *In vitro activity of mono-and combination therapy against colistin-resistant Klebsiella pneumoniae, mechanism of resistance and clinical outcomes among infected patients*. 2019, The College of Pharmacotherapy of Thailand: Bangkok.
123. Paveenkittiporn, W., et al., *Molecular epidemiology of carbapenem-resistant Enterobacterales in Thailand, 2016-2018.* Antimicrob Resist Infect Control, 2021.

10(1): p. 88.

124. Nordmann P and Poirel L, *The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide.* Clin Microbiol Infec, 2014. **20**(9): p. 821-30.

125. Yin, C., et al., *Clonal spread of carbapenemase-producing Enterobacteriaceae in a region, China.* BMC Microbiol, 2022. **22**(1): p. 81.

126. Hansen, G.T., *Continuous Evolution: Perspective on the Epidemiology of Carbapenemase Resistance Among Enterobacterales and Other Gram-Negative Bacteria.* Infect Dis Ther, 2021. **10**(1): p. 75-92.

127. van Duin, D. and Y. Doi, *The global epidemiology of carbapenemase-producing Enterobacteriaceae.* Virulence, 2017. **8**(4): p. 460-469.

128. Tamma, P.D., et al., *Comparing the Outcomes of Patients With Carbapenemase-Producing and Non-Carbapenemase-Producing Carbapenem-Resistant Enterobacteriaceae Bacteremia.* Clin Infect Dis, 2017. **64**(3): p. 257-264.

129. Yu, Z., et al., *Clinical outcomes of prolonged infusion (extended infusion or continuous infusion) versus intermittent bolus of meropenem in severe infection: A meta-analysis.* PLoS One, 2018. **13**(7): p. e0201667.

130. Bassetti, M., et al., *Management of KPC-producing Klebsiella pneumoniae infections.* Clin Microbiol Infec, 2018. **24**(2): p. 133-144.

131. Arabaci, C., et al., *Investigation of carbapenemase and mcr-1 genes in carbapenem-resistant Klebsiella pneumoniae isolates.* J Infect Dev Ctries, 2019. **13**(6): p. 504-509.

132. Mmatli, M., et al., *Emerging Transcriptional and Genomic Mechanisms Mediating Carbapenem and Polymyxin Resistance in Enterobacteriaceae: a Systematic Review of Current Reports.* mSystems, 2020. **5**(6).

133. Bialvaei, A.Z. and H. Samadi Kafil, *Colistin, mechanisms and prevalence of resistance.* Curr Med Res Opin, 2015. **31**(4): p. 707-21.

134. Rojas, L.J., et al., *Colistin Resistance in Carbapenem-Resistant Klebsiella pneumoniae: Laboratory Detection and Impact on Mortality.* Clin Infect Dis, 2017. **64**(6): p. 711-718.

135. Amladi, A.U., et al., *Susceptibility profile, resistance mechanisms & efficacy*

ratios of fosfomycin, nitrofurantoin & colistin for carbapenem-resistant Enterobacteriaceae causing urinary tract infections. Indian J Med Res, 2019. **149**(2): p. 185-191.

136. Santimaleeworagun W, et al., *The prevalence of colistin-resistant Gram-negative bacteria isolated from hospitalized patients with bacteremia.* J. Appl. Pharm. Sci., 2020. **10**(02): p. 056-9.
137. Prawang A, et al., *In vitro antibiotic synergy colistin-resistance Klebsiella Pneumoniae.* Southeast Asian J Trop Med Public Health, 2019. **50**(4): p. 703-07.
138. Tang, H.J., et al., *Colistin-sparing regimens against Klebsiella pneumoniae carbapenemase-producing K. pneumoniae isolates: Combination of tigecycline or doxycycline and gentamicin or amikacin.* J Microbiol Immunol Infect, 2019. **52**(2): p. 273-1.
139. European Committee on Antimicrobial Susceptibility Testing (EUCAST), *Guidance Document on Tigecycline Dosing*. 2018.
140. Cunha, B.A., J. Baron, and C.B. Cunha, *Once daily high dose tigecycline - pharmacokinetic/pharmacodynamic based dosing for optimal clinical effectiveness: dosing matters, revisited.* Expert Rev Anti Infect Ther, 2017. **15**(3): p. 257-267.
141. National Antimicrobial Resistance Surveillance Center (NARST), *Antibiogram 2020 (Jan-Dec)*. 2020.
142. Bercot, B., L. Poirel, and P. Nordmann, *Updated multiplex polymerase chain reaction for detection of 16S rRNA methylases: high prevalence among NDM producers.* Diagn Microbiol Infect Dis, 2011. **71**(4): p. 442-5.
143. Nordmann, P., et al., *Rapid Aminoglycoside NP Test for Rapid Detection of Multiple Aminoglycoside Resistance in Enterobacteriaceae.* J Clin Microbiol, 2017. **55**(4): p. 1074-1079.
144. Aryal, P.U., et al., *Emergence of Carbapenem-Resistant Enterobacteriaceae in a Tertiary Care Hospital in Southern Thailand.* Walailak Journal of Science and Technology (WJST), 2020. **17**(10): p. 1139-1148.
145. Gamal, D., et al., *Carbapenem-resistant Klebsiella pneumoniae isolates from Egypt containing blaNDM on IncR plasmids and its association with rmtF.* Int J

Infect Dis, 2016. **43**: p. 17-20.

146. Kocsis, E., et al., *blaNDM Carriage on IncR Plasmid in Enterobacteriaceae Strains.* Microb Drug Resist, 2016. **22**(2): p. 123-8.

147. Khan, A.U., L. Maryam, and R. Zarrilli, *Structure, Genetics and Worldwide Spread of New Delhi Metallo-beta-lactamase (NDM): a threat to public health.* BMC Microbiol, 2017. **17**(1): p. 101.

148. Rea, R.S., et al., *Suboptimal aminoglycoside dosing in critically ill patients.* Ther Drug Monit, 2008. **30**(6): p. 674-81.

149. Thaden, J.T., J.M. Pogue, and K.S. Kaye, *Role of newer and re-emerging older agents in the treatment of infections caused by carbapenem-resistant Enterobacteriaceae.* Virulence, 2017. **8**(4): p. 403-416.

150. Sader, H.S., et al., *Antimicrobial Activity of Ceftazidime-Avibactam Tested against Multidrug-Resistant Enterobacteriaceae and Pseudomonas aeruginosa Isolates from U.S. Medical Centers, 2013 to 2016.* Antimicrob Agents Chemother, 2017. **61**(11).

151. Sonnevend, Á., et al., *In vitro efficacy of ceftazidime-avibactam, aztreonam-avibactam and other rescue antibiotics against carbapenem-resistant Enterobacterales from the Arabian Peninsula.* Int J Infect Dis, 2020. **99**: p. 253-259.

152. Zou, C., et al., *In vitro Activity of Ceftazidime-Avibactam and Aztreonam-Avibactam Against Carbapenem-resistant Enterobacteriaceae Isolates Collected from Three Secondary Hospitals in Southwest China Between 2018 and 2019.* Infect Drug Resist, 2020. **13**: p. 3563-3568.

153. Kim, T., et al., *In Vitro Activities and Inoculum Effects of Ceftazidime-Avibactam and Aztreonam-Avibactam against Carbapenem-Resistant Enterobacterales Isolates from South Korea.* Antibiotics (Basel), 2020. **9**(12).

154. Wang, Y., et al., *Resistance to ceftazidime-avibactam and underlying mechanisms.* J Glob Antimicrob Resist, 2020. **22**: p. 18-27.

155. Nichols, W.W., et al., *Avibactam Pharmacokinetic/Pharmacodynamic Targets.* Antimicrob Agents Chemother, 2018. **62**(6).

156. Infectious Diseases Society of America (IDSA), *Infectious Diseases Society of*

America Guidance on the Treatment of Antimicrobial Resistant Gram-Negative Infections. 2020.

157. Tamma, P.D., et al., *Infectious Diseases Society of America Guidance on the Treatment of Extended-Spectrum β-lactamase Producing Enterobacterales (ESBL-E), Carbapenem-Resistant Enterobacterales (CRE), and Pseudomonas aeruginosa with Difficult-to-Treat Resistance (DTR-P. aeruginosa).* Clin Infect Dis, 2020. **27**(ciaa1478.).

158. Soukup, P., et al., *Steady-State Ceftazidime-Avibactam Serum Concentrations and Dosing Recommendations in a Critically Ill Patient Being Treated for Pseudomonas aeruginosa Pneumonia and Undergoing Continuous Venovenous Hemodiafiltration.* Pharmacotherapy, 2019. **39**(12): p. 1216-1222.

159. Zusman, O., et al., *Systematic review and meta-analysis of in vitro synergy of polymyxins and carbapenems.* Antimicrob Agents Chemother, 2013. **57**(10): p. 5104-11.

160. Doern, C.D., *When does 2 plus 2 equal 5? A review of antimicrobial synergy testing.* J Clin Microbiol, 2014. **52**(12): p. 4124-4128.

161. Garneau-Tsodikova, S. and K.J. Labby, *Mechanisms of Resistance to Aminoglycoside Antibiotics: Overview and Perspectives.* Medchemcomm, 2016. **7**(1): p. 11-27.

162. Zusman, O., et al., *Polymyxin monotherapy or in combination against carbapenem-resistant bacteria: Systematic review and meta-analysis.* J Antimicrob Chemother 2017. **72**(1): p. 29-39.

163. Andrade, F.F., et al., *Colistin Update on Its Mechanism of Action and Resistance, Present and Future Challenges.* Microorganisms, 2020. **8**(11).

164. Van Bambeke, F., et al., *Alterations in membrane permeability induced by aminoglycoside antibiotics: studies on liposomes and cultured cells.* Eur J Pharmacol, 1993. **247**(2): p. 155-68.

165. Li, C., et al., *Treatment options and clinical outcomes for carbapenem-resistant Enterobacteriaceae bloodstream infection in a Chinese university hospital.* J Infect Public Health, 2019. **12**(1): p. 26-31.

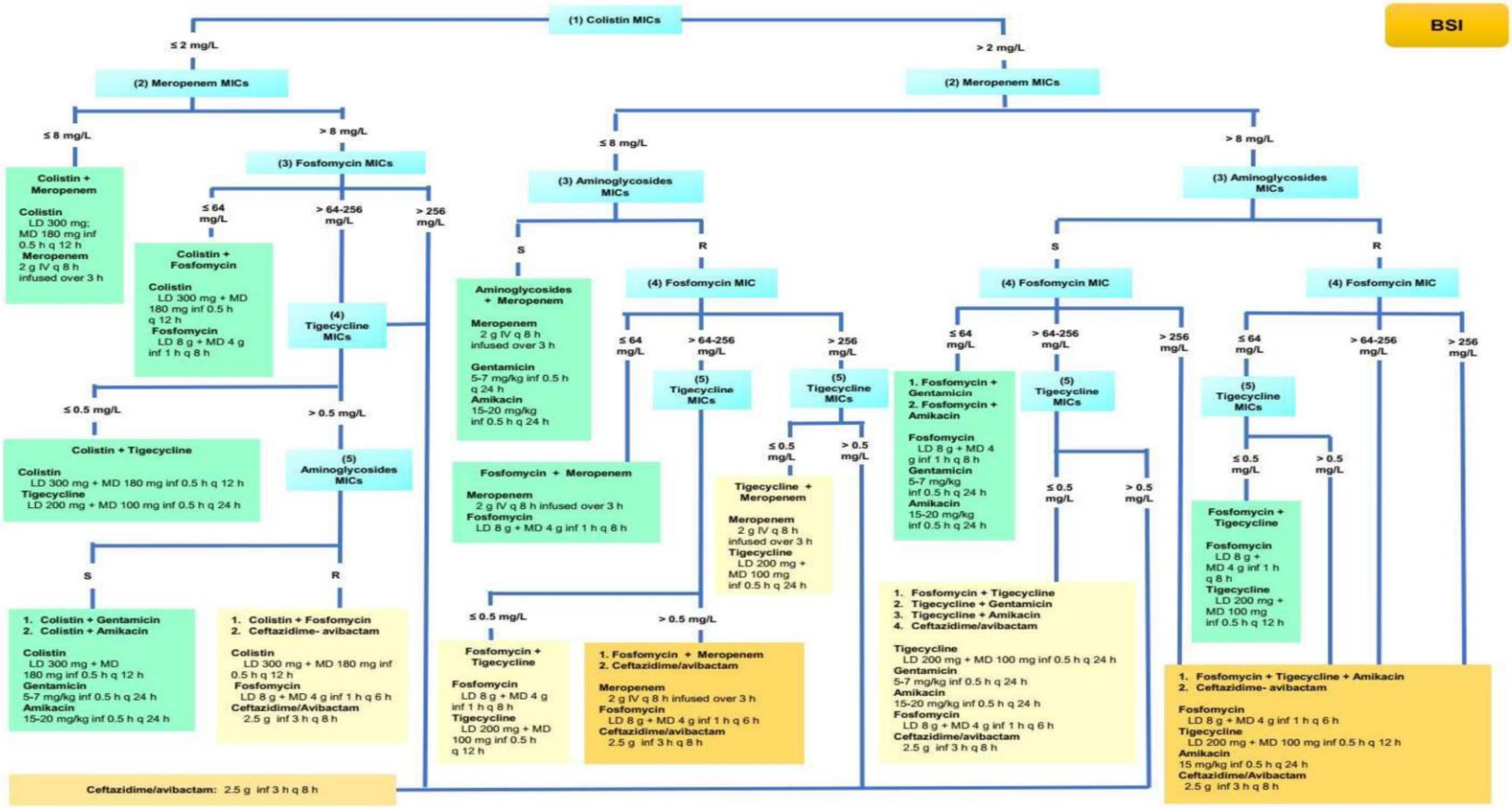
166. Gradel, K.O., et al., *Impact of appropriate empirical antibiotic treatment on recurrence and mortality in patients with bacteraemia: a population-based cohort study.* BMC Infect Dis, 2017. **17**(1): p. 122.

167. Lin, T.C., et al., *Risk factors and clinical impact of bacteremia due to carbapenem-nonsusceptible Enterobacteriaceae: A multicenter study in southern Taiwan.* J Microbiol Immunol Infect, 2021. **54**(6): p. 1122-1129.

168. Soontaros, S. and N. Leelakanok, *Association between carbapenem-resistant Enterobacteriaceae and death: A systematic review and meta-analysis.* Am J Infect, 2019. **47**(10): p. 1200-1212.

APPENDIX

Appendix i: The suggested optimal antibiotic dosing regimens for bloodstream infections (BSIs) caused by CRE



Appendix ii: Ethical approval from the Ethics Committee for Human Research of Silpakorn University

(Ethics number: REC 63.0429-033-1871 issued on 13 August 2020)

มหาวิทยาลัยศิลปากร

หนังสือฉบับนี้ให้ไว้เพื่อแสดงว่า

รหัสโครงการ: REC 63.0429-033-1871

ชื่อโครงการ (ภาษาไทย): แบบแผนกำหนดการใช้ยาที่เหมาะสม สำหรับการรักษาการติดเชื้อที่มีสาเหตุจาก Enterobacteriaceae ที่ดื้อต่อยา carbapenem ในประเทศไทย: การศึกษากลไกการดื้อยาระดับอณูวิทยา ฤทธิ์ของยาต้านจุลชีพชนิดเดี่ยวและคู่ผสม และผลลัพธ์ทางการรักษา

ชื่อโครงการ (ภาษาอังกฤษ): Optimizing antibiotic dosing regimens for the treatment of infection caused by Carbapenem Resistant Enterobacteriaceae in Thailand: the study of molecular resistance mechanisms, in vitro activity of monotherapy and combination therapy, and treatment outcomes

ผู้วิจัยหลัก: ร้อยเอกหญิง ปานรดา นวลโสภาภณ **สังกัด:** คณะเภสัชศาสตร์

เอกสารที่รับรอง:

1. แบบเสนอเพื่อขอรับการพิจารณาจริยธรรมการวิจัยในมนุษย์ เวอร์ชั่น 03 ฉบับลงวันที่ 11 สิงหาคม 2563
2. แบบเสนอโครงการวิจัยเพื่อการพิจารณาจริยธรรมการวิจัยในมนุษย์ (ฉบับภาษาไทย) เวอร์ชั่น 03 ฉบับลงวันที่ 11 สิงหาคม 2563

ได้ผ่านการรับรองจากคณะกรรมการจริยธรรมการวิจัยในมนุษย์ มหาวิทยาลัยศิลปากร โดยยึดหลักเกณฑ์ตามคำประกาศ เฮลซิงกิ (Declaration of Helsinki) และมีความสอดคล้องกับหลักจริยธรรมสากล ตลอดจนกฎหมายข้อบังคับ และข้อกำหนดภายในประเทศ โดยขอให้รายงานความก้าวหน้าของโครงการวิจัยทุก 6 เดือน และรายงานฉบับสมบูรณ์เมื่อโครงการเสร็จสิ้น

(ศาสตราจารย์ ดร.พรศักดิ์ ศรีอมรศักดิ์)
ประธานกรรมการจริยธรรมการวิจัยในมนุษย์
มหาวิทยาลัยศิลปากร

หมายเลขใบรับรอง COE 63.0813-062
วันที่รับรอง: 13 สิงหาคม พ.ศ.2563
วันหมดอายุ: 12 สิงหาคม พ.ศ.2564

สำนักงานบริหารการวิจัย นวัตกรรมและการสร้างสรรค์
6 ถนนราชมรรคาใน ตำบลพระปฐมเจดีย์ อำเภอเมืองนครปฐม จังหวัดนครปฐม 73000
โทร 0-3425-5808 โทรสาร (Fax) : 0-3425-5808
email : su.ethicshuman@gmail.com

Appendix iii: Ethical approval from the ethics review committee of the Royal Thai Army Medical Department and Phramongkutklao Hospital (Ethics number: Q011h/63 issued on 13 July 2020)

RL 01_2560

คณะอนุกรรมการพิจารณาโครงการวิจัย กรมแพทย์ทหารบก

317/5 ถนนราชวิถี เขตราชเทวี กรุงเทพฯ 10400

ที่ IRBRTA....947... /2563 **รหัสโครงการ: Q011h/63**

ชื่อโครงการวิจัย : แบบแผนกำหนดการใช้ยาที่เหมาะสม สำหรับการรักษาการติดเชื้อที่มีสาเหตุจากเอนเทอโรแบคทีเรียซีอี (Enterobacteriaceae) ที่ดื้อต่อยาคาร์บาพิเนม (carbapenem) ณ โรงพยาบาลพระมงกุฎเกล้า: การศึกษากลไกการดื้อยาระดับอณูวิทยา ฤทธิ์ของยาต้านจุลชีพชนิดเดี่ยวและคู่ผสม และ ผลลัพธ์ทางการรักษา
[Optimizing antibiotic dosing regimens for the treatment of infection caused by Carbapenem Resistant Enterobacteriaceae at Phramongkutklao hospital: the study of molecular resistance mechanisms, *in vitro* activity of monotherapy and combination therapy, and treatment outcomes]

เลขที่โครงการวิจัย : -

ชื่อผู้วิจัยหลัก: ร้อยเอกหญิง ปานรดา นวลโสภาภณ

สังกัดหน่วยงาน : คณะเภสัชศาสตร์ มหาวิทยาลัยศิลปากร

สถานที่ทำการวิจัย: โรงพยาบาลพระมงกุฎเกล้า

เอกสารรับรอง :

(1) แบบรายงานการส่งโครงร่างการวิจัยเพื่อพิจารณา ฉบับที่ 3 ลงวันที่ 2 กรกฎาคม 2563
(2) โครงร่างการวิจัย ฉบับที่ 3 ลงวันที่ 2 กรกฎาคม 2563
(3) เอกสารชี้แจงข้อมูลแก่ผู้เข้าร่วมโครงการวิจัย และหนังสือแสดงเจตนายินยอมเข้าร่วมการวิจัย ฉบับที่ 3 ลงวันที่ 2 กรกฎาคม 2563
(4) แบบฟอร์มการเก็บข้อมูล ฉบับที่ 3 ลงวันที่ 2 กรกฎาคม 2563
(5) ประวัติผู้วิจัย ร.อ.หญิง ปานรดา นวลโสภาภณ ฉบับที่ 3 ลงวันที่ 2 กรกฎาคม 2563
(6) ประวัติผู้ร่วมวิจัย พ.ต.วรพงศ์ นาสมทรง ฉบับที่ 3 ลงวันที่ 2 กรกฎาคม 2563
(7) ประที่ปรึกษา รศ.ดร.ภก.วิชัย สันติมาลีวรกุล ฉบับที่ 3 ลงวันที่ 2 กรกฎาคม 2563
(8) ประที่ปรึกษา รศ.ดร.ภก.มนัส พงศ์ชัยเดชา ฉบับที่ 3 ลงวันที่ 2 กรกฎาคม 2563

ขอรับรองว่าโครงการดังกล่าวข้างต้นได้ผ่านการพิจารณารับรองจากคณะอนุกรรมการพิจารณาโครงการวิจัยกรมแพทย์ทหารบกว่าสอดคล้องกับแนวทางจริยธรรมสากล ได้แก่ ปฏิญญาเฮลซิงกิ รายงานเบลมองต์แนวทางจริยธรรมสากลสำหรับการวิจัยในมนุษย์ของสภาองค์การสากลด้านวิทยาศาสตร์การแพทย์ (CIOMS) และแนวทางการปฏิบัติการวิจัยที่ดี (ICH GCP)

วันที่รับรองด้านจริยธรรมของโครงร่างการวิจัย: 12 กรกฎาคม 2563
วันสิ้นสุดการรับรอง: 11 กรกฎาคม 2564
ความถี่ของการส่งรายงานความก้าวหน้าของการวิจัย: 1 ปี

สำนักงานคณะอนุกรรมการพิจารณาโครงการวิจัย พบ.

RL 05_2563

คณะอนุกรรมการพิจารณาโครงการวิจัย กรมแพทย์ทหารบก
ชั้น 5 อาคารพระมงกุฎเกล้าเวชวิทยา วิทยาลัยแพทยศาสตร์พระมงกุฎเกล้า
317/5 ถนน ราชวิถี เขตราชเทวี กรุงเทพฯ 10400 โทรศัพท์, (662) 763-4297, (662) 763-4270 โทรสาร (662) 354-9011
www.irbrta.pmk.ac.th, www.amed.go.th/rtamed/irbrta/ E-mail; irbrta@yahoo.com, irbrta@amed.go.th

ที่ IRBRTA 1684/2564 18 พฤศจิกายน 2564

เรื่อง ตอบรับรายงานความก้าวหน้าของการวิจัย และการขอต่ออายุการรับรองโครงร่างการวิจัย
เรียน ร้อยเอกหญิง ปานรดา นวลโสภาภณ
อ้างถึง บันทึกข้อความ ที่ พิเศษ ลง 9 ตุลาคม 2564

ตามที่ ท่านได้ส่งรายงานความก้าวหน้าของการวิจัย และขอต่ออายุการรับรองโครงร่างการวิจัยเรื่อง “แบบแผนกำหนดการใช้ยาที่เหมาะสม สำหรับการรักษาการติดเชื้อที่มีสาเหตุจากเอนเทอโรแบคทีเรียซีอี (Enterobacteriaceae) ที่ดื้อต่อยาคาร์บาพิเนม (carbapenem) ณ โรงพยาบาลพระมงกุฎเกล้า: การศึกษากลไกการดื้อยาระดับอณูวิทยา ฤทธิ์ของยาต้านจุลชีพชนิดเดี่ยวและคู่ผสม และ ผลลัพธ์ทางการรักษา” [Optimizing antibiotic dosing regimens for the treatment of infection caused by Carbapenem Resistant Enterobacteriaceae at Phramongkutklao hospital: the study of molecular resistance mechanisms, in vitro activity of monotherapy and combination therapy, and treatment outcomes] (Q011h/63)

คณะอนุกรรมการพิจารณาโครงการวิจัย กรมแพทย์ทหารบก ได้ทบทวนแล้ว จึงขอตอบรับรายงานดังกล่าว

- **รับรอง (approval)**

วันที่รับรองต่อเนื่องโครงร่างการวิจัย: 18 พฤศจิกายน 2564
วันสิ้นสุดการรับรอง: 17 พฤศจิกายน 2565
ความถี่ในการส่งรายงานความก้าวหน้า: 1 ปี

จึงเรียนมาเพื่อกรุณาทราบ

ขอแสดงความนับถือ

พันเอก .....
(สุธี พานิชกุล)
ประธานคณะอนุกรรมการพิจารณาโครงการวิจัย
กรมแพทย์ทหารบก

สำนักงานคณะอนุกรรมการพิจารณาโครงการวิจัย พบ.

มหาวิทยาลัยศิลปากร

VITA

NAME	Parnrada Nulsopapon
DATE OF BIRTH	18 December 1985
PLACE OF BIRTH	Bangkok
INSTITUTIONS ATTENDED	- Bachelor of Science in Pharmacy (2nd Class Honours), Faculty of Pharmaceutical Sciences, Chulalongkorn University in 2010 - Master of Pharmacy Program in Community Pharmacy, Faculty of Pharmaceutical Sciences, Naresuan university
HOME ADDRESS	90/105 Moo 5 Kunasiri Buathong 4 village Thanon Ban Kluai - Sai Noi Bang Khu Rat, Bang Bua Thong, Nonthaburi
PUBLICATION	1. Wichai Santimaleeworagun and Parnrada Nulsopapon. A review of Metrics in Antimicrobial Stewardship Program. TJPP 2020; 12(2): 411-20. 2. Pornwalai Boonmuang, Wichai Santimaleeworagun and Parnrada Nualsopapon. Doxazosin Induced Angioedema: A Case Report. RTA Med J. 2018; 71:285-9. 3. Wichai Santimaleeworagun, Pornwalai Boonmuang, Nitchanun Ueapanjasin, Duangkamon Janmitsakun, Thananat Saengungkanawin, Teerachod Leerungsrithong, Parnrada Nulsopapon. The effect of rifampicin on international normalized ratio in patients taking warfarin: A retrospective study at A University hospital in Thailand. The Thai Journal of Pharmaceutical Sciences 42 (supplement). 2018: 80-83. 4. Pornwalai Boonmuang, Wichai Santimaleeworagun, Parnrada Nulsopapon. Pomegranate Juice Elevated International Normalized Ratio in a Patient on Warfarin: A Case Report. Journal of Health Science 2016 Vol. 25 No. 3. 529-533. 5. Napon Temeesak, Nutnicha Kheokasem, Natnicha Phatcharawongsagorn, Parada Nontakulwiwat, Pornwalai, Boonmuang, Wichai Santimaleeworagun and Parnrada Nulsopapon. The Effects of Herbs or Dietary Supplements on International

Normalized ratio in Warfarin Users: A Retrospective Study at Phramongkutklao Hospital. Thai Pharm Health Sci J Vol. 10 No. 4, Oct. – Dec. 2015. 139-146

6. Parnrada Nulsopapon and Paveena Sonthisombat. Efficacy and Safety of Low Dose of Fondaparinux with Rivaroxaban and Enoxaparin after Total Knee and Total Hip Replacement Surgery,Phramongkutklao Hospital. RTA Med J 2014; 67:11-20.