DEVELOPING INDIVIDUALISED THERAPY FOR COLISTIN THROUGH APPLICATION OF PHARMACOKINETIC MODEL TAILORED FOR CRITICALLY ILL MALAYSIAN PATIENTS UTILIZING THE HPLC-FLD

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LIST OF SYMBOLS

α	Alpha
β	Beta
γ	Gamma
$t_{1/2}$	Half-life
$t_{1/2\alpha}$	Distribution half-life
$t_{1/2\beta}$	Terminal half-life
µ	Micro
µg	Microgram
µL	Microliter
µm	Micrometer
µg/mL	Microgram per milliliter
%	Percentage
>	More than
-	Negative
+	Positive
^{0}C	Degree of Celsius
pH	Potential of hydrogen
pKa	Acid dissociation constant
Q	Intercompartmental clearance

LIST OF ABBREVIATIONS

ACN	Acetonitrile
AFC	Adaptive feedback control
AJS ESI	Agilent jet stream electrospray ionisation
AKI	Acute kidney injury
ARC	Augmented renal clearance
CE	Collision energy voltage
CI	Confidence interval
CL	Clearance
C_{min}	Minimum concentration
C_{max}	Maximal concentration
C_{ssave}	Average concentration at steady state
CMS	Colistin methanesulfonate sodium
CRE	Carbapenem resistant *enterobacteriaceae*
CrCl	Creatinine clearance
CL_r	Renal clearance
CL_{RCMS}	CMS renal clearance
CRRT	Continuous renal replacement therapy
CVVHDF	Continuous venovenous hemodiafiltration
CV	Coefficient of variation
ESBL	Extended-spectrum beta-lactamase
ESI	Electrospray ionisation
FMOC-CL	9-Flurenylmethyl chloroformate
fm	Fraction of CMS converted to colistin
GFR	Glomerular filtration rate
GNB	Gram negative bacilli
h	hour
HD	Hemodialysis
HDW	High dependency ward
HLB	Hydrophilic-lipophilic balance
HPLC	High performance liquid chromatography
ICU	Intensive care unit

IT2B	Iterative-2-stage Bayesian parametric
IV	Intravenous
IIV	Inter-individual variability
IOV	Inter-occasional variability
L	Litre
L-Dab	L-diaminobutyric acid
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LLOQ	Lower limit of quantitation
LOD	Limit of detection
LPS	Lipopolysaccharide
M	Molar
MDR	Multidrug-resistant
MIC	Minimum inhibitory inhibition
MIU	Million international unit
min	Minute
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
NPAG	Nonparametric adaptive grid
NR	Not reported
OPA	Orthophtalaldehyde
PK	Pharmacokinetic
PD	Pharmacodynamic
QC	Quality control
QTOF-LC/MS	Quadrupole time-of-flight liquid chromatography mass spectrometry
SIRS	Systemic inflammatory response syndrome
SD	Standard deviation
SPE	Solid phase extraction
SRM	Single reaction monitoring
TDM	Therapeutic drug monitoring

THF	Tetrahydrofuran
TLC	Thin layer chromatography
T_{max}	Time to reach maximal plasma concentration
UHPLC	Ultra-high-performance liquid chromatography
UI	Uncertainty interval
UV	Ultraviolet
V_d	Volume of distribution
V	Voltage
v	Volt
v/v	Volume per volume
XDR	Extended drug-resistant

LIST OF APPENDICES

MEMBANGUNKAN TERAPI INDIVIDU COLISTIN MELALUI APLIKASI MODEL FARMAKOKINETIK UNTUK PESAKIT KRITIKAL MALAYSIA MENGGUNAKAN HPLC-FLD

ABSTRAK

Colistin adalah antibiotik yang digunakan sebagai pilihan terakhir untuk merawat jangkitan bakteria. Disebabkan ketoksikannya, colistin diberi dalam bentuk prodrug yang tidak aktif, colistin methanesulfonate sodium (CMS). Penukaran CMS kepada colistin *in vivo*, yang berbeza-beza membawa kepada variasi dalam kepekatan colistin di dalam plasma dan parameter farmakokinetik bagi pesakit kritikal. Ketepatan kaedah analisa kepekatan colistin adalah diperlukan untuk menjayakan kajian farmakokinetik. Colistin mempunyai julat terapeutik yang sempit dan perlu dipantau untuk pengoptimuman dos. Oleh itu, kajian ini bertujuan, untuk membangunkan dos yang diperibadikan untuk colistin dengan menggunakan model farmakokinetik untuk pesakit yang kritikal di Malaysia. Kajian ini telah diluluskan oleh Jawatankuasa Etika Penyelidikan Manusia, Universiti Sains Malaysia, dan Jawatankuasa Penyelidikan and Etika Penyelidikan Perubatan, Kementerian Kesihatan Malaysia. Kaedah kromatografi cecair berprestasi tinggi dengan pengesanan pendarfluor (HPLC-FLD) telah dibangunkan dan divalidasikan untuk pengukuran kepekatan colistin dalam serum manusia. Kaedah yang telah divalidasikan ini kemudiannya digunakan untuk analisis serum pesakit kritikal yang menerima rawatan antibiotik CMS. Farmakokinetik populasi untuk colistin telah dimodelkan dengan pendekatan bukan parametrik menggunakan perisian Pmetrics. Model farmakokinetik yang telah dibangunkan akan digunakan untuk mengoptimumkan dos terapeutik ubat bagi pesakit secara individu. Keluk penentukuran linear diperolehi untuk mengukur colistin dalam kepekatan 0.3

hingga 8 μg/mL dengan kesesuaian yang baik (r^2 = 0.9993). Kaedah analisis ini telah mengukur dengan tepat kepekatan colistin dalam serum tanpa hidrolisis CMS kepada colistin *in vitro* yang ketara diperhatikan semasa prosedur. Ketepatan kaedah analisis ini diperolehi antara 98% hingga 100%. Dalam kebanyakan pesakit, kepekatan palung adalah lebih tinggi daripada purata kepekatan semasa dalam keadaan mantap yang disyorkan (2 μg/mL) dan ianya boleh mengakibatkan nefrotoksisiti. Algoritma Grid Adaptif Bukan Parametrik dalam perisian Pmetrics telah digunakan untuk membangunkan model farmakokinetik colistin menggunakan data meta-analisis daripada 15 kajian farmakokinetik. Pengesahan luaran model akhir telah dilakukan dalam 25 pesakit (data Malaysia dan meta-analisis). Farmakokinetik colistin diterangkan dengan baik oleh model dua bahagian dengan penghapusan tertib pertama. Pengesahan model telah dinilai dengan menggunakan plot terhadap kepekatan yang diukur untuk individu berbanding kepekatan kolistin yang diramalkan dimana R-kuadrat adalah 0.974. Model farmakokinetik colistin kemudiannya digunakan untuk pengoptimuman dos terapeutik ubat pesakit secara individu. Penggunaan pendekatan model-bermaklumat yang memfokuskan pada ubat yang diperibadikan boleh membantu mencapai ketepatan pengindividuan dos.

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ABSTRACT

Colistin is an antibiotic used as a last option to treat bacterial infections. Due to its toxicity, colistin is administered in the form of an inactive prodrug, colistin methanesulfonate sodium (CMS). The conversion of CMS to colistin *in vivo* varies greatly, leading to variations in plasma colistin concentration and pharmacokinetic parameters in critically ill patients. A novel analytical method is necessary for any pharmacokinetic studies to succeed. Colistin has a narrow therapeutic window and needs to be monitored for dose optimisation. Therefore, this study aimed to develop personalised medicine for colistin using a pharmacokinetic model for Malaysian critically ill patients. The Human Research Ethics Committee of Universiti Sains Malaysia and the Malaysian Ministry of Health Research Ethical Committee approved the study. The high-performance liquid chromatography with fluorescence detection (HPLC-FLD) method was developed and validated to measure colistin in human serum. This validated method was then used to analyse serum from critically ill patients receiving CMS. Colistin population pharmacokinetics was modelled with a nonparametric approach using Pmetrics software. The constructed pharmacokinetic model of colistin was then applied to optimise individual patient therapeutic drug doses. Linear calibration curves were obtained for colistin concentrations of 0.3 to 8 μg/mL, with good fit (r^2 = 0.9993). This analytical method accurately measured the amount of colistin in serum, with no significant hydrolysis of CMS into colistin *in*

vitro observed during the procedure. The accuracy ranged from 98% to 100%. In most patients, the trough concentration was higher than the recommended average steady-state concentration (2 μg/mL) and may be associated with nephrotoxicity. The Non-Parametric Adaptive Grid algorithm within Pmetrics software was used to develop a colistin pharmacokinetic model using meta-analysis data from 15 pharmacokinetic studies, and external validation of the final model was performed in 25 subjects (Malaysian and meta-analysis data). A two-compartment model with first-order elimination best describes colistin pharmacokinetics. Model validation was assessed by using a plot of observed versus individual predicted colistin concentration, and an R-squared of 0.974 was obtained in the validation group. The colistin pharmacokinetic model was then implemented for individual patient therapeutic drug dose optimisation. Applying a model-informed approach, focusing on personalised medicine, may help achieve precise dose individualisation.

CHAPTER 1

INTRODUCTION

1.1 Overview

Nosocomial infections cause high mortality and morbidity, particularly in patients who are critically ill (Haque *et al.*, 2018). The widespread nosocomial infections of multidrug-resistant Gram-negative bacteria (MDR GNB), including extended-spectrum β-lactamases (ESBLs), for example, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*, have raised significant concern (Morris & Cerceo, 2020). The effectiveness of many existing antimicrobials against these organisms has significantly decreased, and treatment options have become limited (Tosi *et al.*, 2018). Carbapenems are the first-line therapy in critically ill patients with MDR GNB. Unfortunately, *Enterobacteriaceae* isolates have developed resistance to carbapenems (Meletis, 2016). The number of carbapenem-resistant *enterobacteriaceae* (CRE) isolates is increasing alarmingly (Logan & Weinstein, 2017).

In Malaysia, there has been a noticeable increase in MDR GNB, specifically those demonstrating resistance to carbapenems or colistin due to ESBL or plasmid-mediated colistin resistance (Annual Report IPC, 2022), as shown in Figures 1.1 and 1.2. Polymyxin antibiotics, such as colistin, are increasingly prescribed to combat these pathogens despite their potential to induce nephrotoxicity (Vaara, 2019).

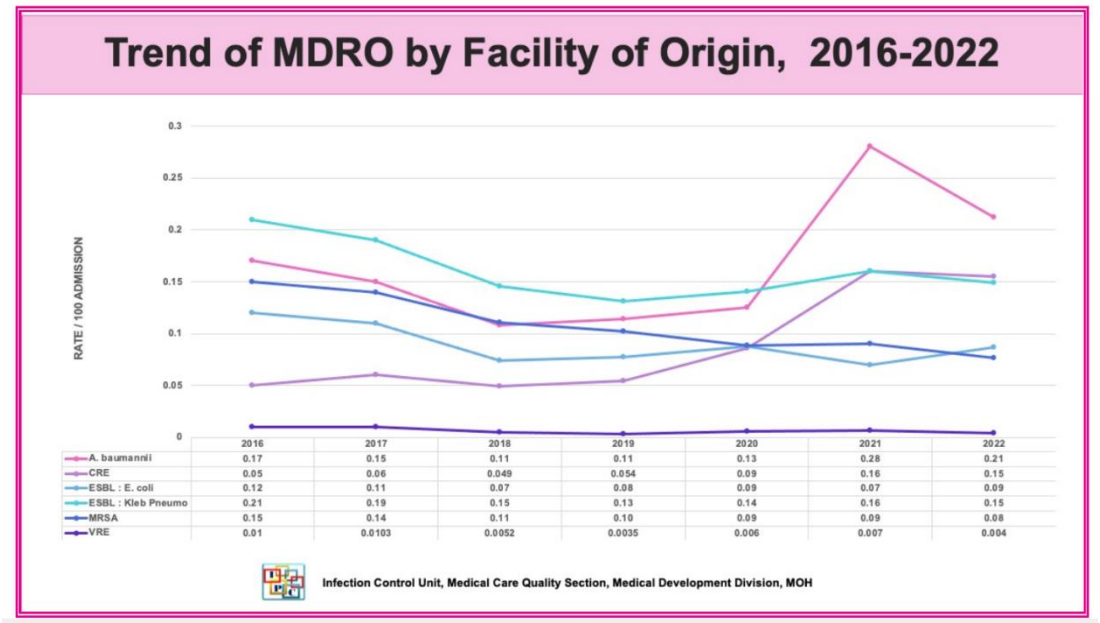

Figure 1.1 The trend of multidrug-resistant organisms has changed over time in Malaysia. (Adapted from Annual Report IPC, (2022))

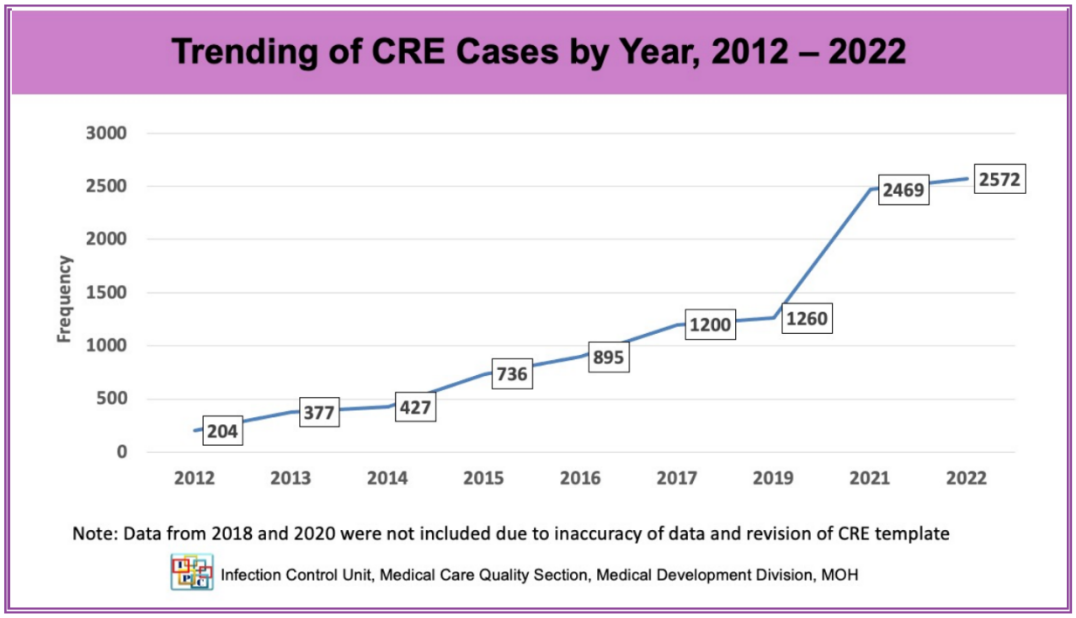

Figure 1.2 The trend of Gram-negative bacteria resistant to carbapenems (CRE) has changed over time in Malaysia. (Adapted from Annual Report IPC, (2022))

Treating infections in critically ill patients is becoming more challenging because of the prevalence of resistant bacteria to multiple drugs (Tosi *et al.*, 2018). Additionally, the antibiotic concentration could be affected by dynamic physiological changes in critically ill patients (Parker, Sime & Roberts, 2015; Nation *et al.*, 2017). The standard antibiotic regimen used to treat critically ill patients may be inadequate (Roberts, Roger & De Waele, 2019).

Critically ill patients exhibit greater pharmacokinetic variability than non-critically ill patients, particularly for hydrophilic antibiotics like aminoglycosides, β-lactam, glycopeptide, and linezolid (Abdul-Aziz *et al.*, 2020). In critically ill patients, the volume of distribution and drug clearance of β-lactam antibiotics can be twice as high as in non-critically ill patients (Abdul-Aziz *et al.*, 2020). Hence, optimising antibiotic therapy for ICU patients poses a significant challenge for managing doctors. The use of therapeutic drug monitoring (TDM) to personalise antibiotic treatment for critically ill patients is now justifiable. This is due to the improved understanding of the relationships between antibiotic dose, pharmacokinetic/pharmacodynamic (PK/PD) exposure, and patient outcomes.

1.2 Problem statement

Colistin use in critically ill patients has increased due to the rise in MDR GNB and the lack of new antibacterial medication classes developed in recent years. When colistin became available, it was known to be toxic. Colistin methanesulfonate sodium, a less toxic prodrug for intravenous injection, was developed to minimise its toxicity. When using CMS, it is important to note that it is converted slowly to colistin in the

body. The initial low concentration at the start of treatment may result in inadequate bacterial killing (Plachouras *et al.*, 2009). Colistin has been found to have a prolonged half-life (14 hours) in individuals who are critically ill (Plachouras *et al.*, 2009) than in patients with cystic fibrosis (10 hours) (Li *et al.*, 2003) and healthy individuals (3 hours) (Couet *et al.*, 2011). These findings indicated delayed attainment of steady-state colistin concentrations in critically ill populations (Plachouras *et al.*, 2009; Mohamed *et al.*, 2012; Karaiskos *et al.*, 2015). Owing to these issues, most current guidelines recommend applying CMS as a loading dosage prior to the maintenance dose (Nation *et al.*, 2016; Tsuji *et al.*, 2019). However, this may not always lead to sufficient colistin plasma levels (Ehrentraut *et al.*, 2020).

Optimising the colistin dosage in critically ill patients to achieve adequate plasma levels has proven difficult. Due to rapid fluctuations in renal function, volume status, and pathophysiology, variability in colistin plasma levels has been observed (Leuppi-Taegtmeyer *et al.*, 2019; Kristoffersson *et al.*, 2020; Ram *et al.*, 2021). Colistin has a narrow therapeutic window (2-4 µg/mL) (Nation *et al.*, 2017). Nephrotoxicity due to colistin is more prevalent when the minimum concentration (C_{min}) of colistin exceeds 2.4 µg/mL (Sorlí *et al.*, 2013; Forrest *et al.*, 2017). Consequently, it may be potentially harmful to use colistin without TDM. It may be helpful that colistin therapy is adjusted based on blood concentration to optimise the balance between the benefits and risks of treatment. High-performance liquid chromatography (HPLC) measures colistin levels in plasma but is not commonly performed in Malaysia. Liquid chromatography-mass spectrometry (LC-MS) methods for colistin assays are expensive and require skilled personnel, making them impractical in smaller settings (Gobin *et al.*, 2010; Gikas *et al.*, 2013). High-

performance liquid chromatography with fluorescence detection (HPLC-FLD) has been extensively used for colistin analysis (Li *et al.*, 2001; Chepyala *et al.*, 2015; Hanai *et al.*, 2018). The colistin levels of critically ill patients receiving CMS treatment were measured using the HPLC-FLD method in this current study.

Recent studies reported by Holford, Ma & Metz (2020) and Wicha *et al.* (2021) that TDM strategies are frequently ineffective or inferior. This is related to the TDM approach that defines therapeutic ranges within which the concentration is expected to be safe and effective. Therapeutic drug monitoring sampling should be conducted when the patient reaches a steady state. Therefore, TDM sampling for drugs with a long half-life will take place on Day 2 or 3 of therapy. In cases of infection, the pharmacokinetic-pharmacodynamic (PK/PD) target should be achieved as early as possible. In TDM, sampling time is crucial; if a sample is taken outside a predefined accepted window time, it cannot be interpreted accurately. Hence, the concept of target concentration intervention has been introduced, where defined targets instead of ranges were used (Holford, Ma & Metz, 2020). The prediction of drug concentration using pharmacometrics models with PK/PD targets might enhance the attainment of individual targets (Neely *et al.*, 2018). The advantage of this approach, Bayesian estimation of the pharmacokinetics (PK) parameter can be done using any timed plasma sample as long as the sampling is accurately documented (Alihodzic *et al.*, 2020).

Our understanding of CMS and colistin population PK has significantly progressed in the past decade (Karaiskos *et al.*, 2015; Kristoffersson *et al.*, 2020). Extensive research, primarily using a parametric approach, has been conducted. The

pharmacokinetics and plasma levels of colistin exhibit substantial variation among critically ill patients, making it challenging to achieve a normal (Gaussian) distribution. Therefore, a nonparametric method may be a more suitable approach to study population pharmacokinetics in this patient population. Although limited, some studies have explored the use of nonparametric methods (Mathew *et al.*, 2022). This study, therefore, employed a nonparametric approach to investigate the population pharmacokinetics of colistin in critically ill Malaysians. The need for further research in this area is urgent to optimise antibiotic dosing using pharmacokinetic modelling combined with software programs such as Pmetrics to calculate individualised doses. This approach has the potential to enhance the accuracy of antibiotic dosing and improve the clinical outcomes of the patients.

1.3 Objectives

1.3.1 General objective

This study aims to develop personalised medicine for colistin using a pharmacokinetic model for Malaysian critically ill patients.

1.3.2 Specific objectives

1. To develop a high-performance liquid chromatography method for quantification of colistin to be used in pharmacokinetics study.
2. To develop a pharmacokinetic model of colistin using meta-analysis data from previous pharmacokinetics studies.
3. To validate the developed pharmacokinetic model using Malaysian critically ill patient data.
4. To develop a personalised dosage optimisation using population pharmacokinetic modelling and simulation.

1.4 Justification of the study

Colistin's therapeutic window is narrow, ranging from 2-4 μg/mL. Improper dosing of colistin can lead to an increased incidence of colistin resistance or toxicity (Nation *et al.*, 2017). Accurate dosage prediction depends on understanding pharmacokinetic variability and measuring it in relation to easily obtainable clinical covariates (Wicha *et al.*, 2021). It is possible to achieve this using population pharmacokinetic models. Population PK models are applied to investigate potential causes of patient exposure variability and determine patients' drug exposure time course. They can be used to simulate different dosage regimens to optimise the achievement of the PK/PD target. Individual PK parameters could be estimated based on available clinical covariates. Personalised dosage optimisation can be determined by using the individual's estimated PK parameters (Holford, Ma & Metz, 2020; Wicha *et al.*, 2021).

There are two pharmacokinetic modelling approaches: parametric and nonparametric, based on the assumption of parameter distribution (Goutelle *et al.*, 2022; Guidi, Csajka & Buclin, 2022). Most pharmacokinetic data for colistin in critically ill patients is derived from a parametric approach. In parametric models, the PK parameter probability distributions are represented by parameters like mean and covariances, describing normal or lognormal distribution assumptions in the population study. Unlike parametric models, non-parametric models have a discrete distribution with no predefined shape. Continuous functions cannot describe the statistical distribution of the model parameter values in the population (Goutelle *et al.*, 2020). They are used to pool and analyse data from groups of patients. The parameter

estimate consists of individual parameters and a probability distribution without pre-existing assumptions about its shape (de Velde *et al.*, 2018).

The nonparametric method utilises an exact likelihood function, whereas the parametric method uses an approximation. The nonparametric approach is chosen for its advantages over parametric methods in identifying unexpected sub-populations or outliers (Jelliffe *et al.*, 2000). Only one study so far used a nonparametric approach, conducted by Mathew *et al.* (2022). Therefore, this current study developed a pharmacokinetic model with a nonparametric approach using data from previous studies on critically ill patients. It incorporates local data and other relevant parameters to help doctors determine the best dosage for individual patients.

1.5 Conceptual framework

Figure 1.3 illustrates the conceptual framework for the population pharmacokinetics model of colistin in critically ill patients, highlighting co-variates that may influence colistin concentration in plasma. The covariates that may also influence outcomes include the patient's comorbidities, hypoalbuminemia, extracorporeal therapies (e.g., renal replacement therapy), fluid management, post-surgical drainage, sepsis, mechanical ventilation, and others investigated in this study.

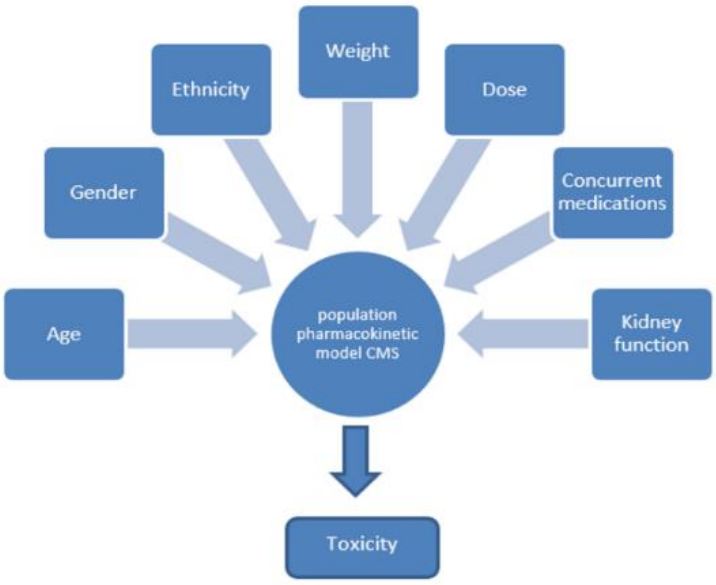

Figure 1.3 Conceptual framework of population PK model of colistin.

1.6 Significance of the study

The current dosing regimens for colistin used in the treatment of GNB infections are unlikely to attain the desired plasma concentration or may potentially increase the risk of toxicity. Since colistin has a narrow therapeutic range and patient severely ill are more prone to have substantial changes in their condition, it is necessary to monitor blood colistin levels to ensure the target concentration is met. Personalised colistin medicine aims to achieve specific PK/PD targets, reduce bacterial resistance, and minimise antibiotic toxicity. Using software to tailor antibiotic concentrations for individual patients can improve dosing accuracy in critically ill patients.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Critically ill patients are prone to infection due to invasive procedures like intubation and mechanical ventilation, as well as vascular access (Blot *et al.*, 2022). They have more chronic comorbid disorders, severe acute physiological derangements, and immunosuppression and are subjected to broad-spectrum antibiotics, causing selection and colonisation pressure in comparison to general ward patients (Jolivet *et al.*, 2020). Because critically ill patients have severe physiological imbalances, they are more vulnerable to MDR GNB infections and may not receive an optimal dosage of antimicrobial therapy (Bonten, 2012; Tängdén *et al.*, 2017; Heffernan *et al.*, 2018; Jolivet *et al.*, 2020).

Antibiotic concentrations may be altered due to extreme physiological derangements, life-saving medical interventions, or the natural course of critical illness (Abdul-Aziz *et al.*, 2015; Cotta, Roberts & Lipman, 2015). Dosing for antibiotics in this population is challenging and not as straightforward as in the general ward (Sime, Roberts & Roberts, 2015). Improving antibiotic therapy in the ICU can be challenging for various reasons, especially when dealing with infections caused by polymyxin-only susceptible GNB.

2.2 Critically ill patients

2.2.1 Definition

Critically ill patients are individuals with life-threatening conditions requiring constant treatment and monitoring. In this current study, critically ill patients are defined as individuals with possibly life-threatening conditions, single or multiple organ failure, who receive medical treatment in a high dependency ward (HDW) or ICU for the purpose of intensive care monitoring and organ support such as haemodynamic, renal, respiratory, neurological and others (Sakr *et al.*, 2018; Evans *et al.*, 2021). Because of the widespread use of invasive devices for diagnosis and treatment, patients in critical condition have a greater chance of acquiring life-threatening infections, leading to sepsis. According to a recent analysis by Rudd *et al.* (2020) an estimated 48.9 million (95% uncertainty interval [UI] 38.9–62.9) cases of sepsis, and there have been 11 million (95% UI 10.1–12.0) sepsis-associated mortality were reported globally, comprising 19.7% (95% UI 18.2–21.4) of all mortality reported to be caused by sepsis.

Sepsis is a life-threatening organ failure caused by a dysregulated immune response to infection, commonly leading to ICU admission (Sakr *et al.*, 2018). Septic shock is a severe form of sepsis that involves significant dysfunction in circulatory, cellular, and metabolic systems, increasing the risk of death compared to sepsis alone (Singer *et al.*, 2016). Controlling the pathogen's source and providing early, appropriate antibiotic therapy is vital for those who are severely ill and have infections (Paul *et al.*, 2010).

2.2.2 Antibiotic pharmacokinetics alteration in the critically ill patients

Dynamic physiological alterations experienced by severely ill patients, drugs (hydrophilicity and lipophilicity of the compound, drug interaction), disease conditions (organ dysfunction, acute phase response), and therapeutic intervention (fluid resuscitation, extracorporeal therapy) can significantly affect antibiotics pharmacokinetics (Parker, Sime & Roberts, 2015; Monogue, Kuti & Nicolau, 2016; Tosi *et al.*, 2018).

Sepsis and septic shock resulting from a severe infection can lead to multiple organ failure and pathophysiological modifications, which may impact the PK/PD characteristics of antibiotics (Roberts, Roger & De Waele, 2019). Bacterial endotoxins trigger systemic inflammatory response syndrome (SIRS), characterised by inflammatory mediators damaging blood vessel lining. Resulting in vasoconstriction or vasodilation, blood flow changes, endothelial injury, and leaky capillaries (Singer *et al.*, 2016; Tosi *et al.*, 2018). Because of vascular endothelial damage and inflammation, capillary leakage occurs due to fluid shifts between blood vessels and tissues (Singer *et al.*, 2016; Tosi *et al.*, 2018).Resulting in an increased volume of distribution (V_d) of hydrophilic drugs.

The volume of distribution determines the relationship between dose and serum concentration. An increase in V_d can decrease the overall concentration of antibiotics in the blood (Roberts, Kumar & Lipman, 2017). Factors that might influence V_d include sepsis, shock, mechanical ventilation, changes in plasma protein binding, hypoalbuminemia (increased capillary leakage), extracorporeal therapy (e.g.,

renal replacement therapy), post-surgical drainage, burn injury, or fluid resuscitation (Blot, Pea & Lipman, 2014). Low plasma antibiotic concentrations can impact the required dosage amount. Severe sepsis is associated with organ failure, while septic shock is characterised by unresponsive hypotension requiring vasopressor support despite adequate fluid administration (Singer *et al.*, 2016). This condition may alter the antibiotic elimination half-life ($t_{1/2}$).

An antibiotic's elimination half-life is the time it takes for its concentration or total quantity to decrease by 50%. Antibiotic elimination half-life is directly related to antibiotic clearance (CL) and $V_{d,}$ as shown by the equation below (Roberts & Lipman, 2009):

$$t1/2 = 0.693\ x\ Vd/CL$$

Antibiotic metabolism occurs predominantly in the liver and kidneys. Renal blood flow directly correlates with the kidneys' capacity to eliminate the medication (Roberts & Hall, 2013). Sepsis or septic shock may occur due to volume depletion caused by capillary leakage and increased vascular capacity by vasodilatation, leading to disruption of the renal blood flow (Jacobi, 2002). This disease process may also increase $V_{d,}$ and reduced CL will likely increase $t_{1/2}$.

Pharmacokinetic parameters may be altered due to interventions by intensivists, such as fluid resuscitation and the use of vasopressors to enhance blood flow and renal perfusion, which help restore blood volume and improve cardiac function (Boucher, Wood & Swanson, 2006; Roberts & Lipman, 2009). The intervention process can impact various PK parameters of antibiotics, such as

decreased V_d and increased CL and is also likely to reduce $t_{1/2}$. Additional interventions, such as mechanical ventilation, extracorporeal therapy, and albumin administration, can potentially modify the PK characteristics of antibiotics (Boucher, Wood & Swanson, 2006; Roberts & Lipman, 2009; Blot, Pea & Lipman, 2014).

Critically ill patients may encounter dynamic physiologic changes that affect their pharmacokinetic parameters, resulting in a change in antibiotic concentration. Multiple factors may influence pharmacokinetics, making predicting an appropriate concentration more challenging.

2.3 Polymyxin antibiotic

In the absence of effective and safe therapy options, polymyxins serve as the primary treatment for carbapenem-resistant infections. Several new antibiotics and combinations have recently been approved to treat certain MDR and extensively drug-resistant (XDR) gram-negative bacteria. Examples of these antibiotics include ceftazidime-avibactam, meropenem-vaborbactam, imipenem-cilastatin-relebactam, plazomicin, and cefiderocol. However, these new antibiotics are not yet available in many countries, especially in developing countries like Malaysia.

2.3.1 Chemical structure

Polymyxins are polypeptide antibiotics that consist of 5 different chemical properties substances (polymyxins A to E). Only Polymyxin B and Polymyxin E (colistin) are available for clinical options (Rhouma *et al.*, 2016; Poirel, Jayol &

Nordmann, 2017). Polymyxins consist of a cyclic structure formed by seven amino acids and a linear chain of three amino acids linked to a fatty acid group (Rhouma *et al.*, 2016; Poirel, Jayol & Nordmann, 2017). Polymyxin E and B differ by a single amino acid, D-leucine or D-phenylalanine, respectively, at position six of the peptide ring (Figure 2.1).

Colistin is an old antibiotic which was not widely used due to its adverse effects, especially nephrotoxicity (Mendes & Burdmann, 2010). To reduce its toxicity, the colistin molecule has undergone modifications and is now administered intravenously as a prodrug known as CMS (Figure 2.2) (Falagas & Kasiakou, 2006).Colistin methanesulfonate sodium is produced by reacting colistin's free γ-amino groups with formaldehyde and sodium bisulfite (Bergen *et al.*, 2006; He *et al.*, 2013). Colistin methanesulfonate sodium is not microbiologically active. After administration, it spontaneously hydrolyses in aqueous media and is converted into colistin and partially sulfomethylated derivatives in biological fluid (Bergen *et al.*, 2006)).

The primary constituents of colistin are colistin A and B (Figure 2.2), available for oral and topical use as colistin sulphate and intravenous formulation as CMS (Orwa *et al.*, 2001). Colistin (colistin sulphate or CMS) can also be administered as an inhalation (Li *et al.*, 2006). Colistin is polycationic, and CMS is a polyanion at a physiological pH of 7.4 (Li *et al.*, 2006; Bergen *et al.*, 2012). The α,γ-diaminobutyric acid residues are ionised at physiological pH. This causes colistin molecules, which carry a net positive charge, to interact with the lipid A's negatively charged phosphate

groups in the bacterial lipopolysaccharide (LPS) membrane (Nation, Velkov & Li, 2014).

Colistin methanesulfonate sodium is replaced by negatively charged methanesulfonate moieties at physiological pH, which are the main amines of the Dab residues. After administration, it must be converted to colistin to become active against bacteria (Bergen *et al.*, 2006). The molecular weight of colistin A is 1169 g/mol, and colistin B is 1155 g/mol. Colistin methanesulfonate sodium has a higher molecular weight due to five extra sulfomethyl groups, and the molecular weights of CMS A is 1635 g/mol, and CMS B is 1621 g/mol (Grégoire *et al.*, 2017). Colistin is a hydrophilic drug (Shah *et al.*, 2014) with basic properties (acid dissociation constant [pKa] of about 10) (Li *et al.*, 2005) and CMS is less basic than colistin (Yapa *et al.*, 2013).

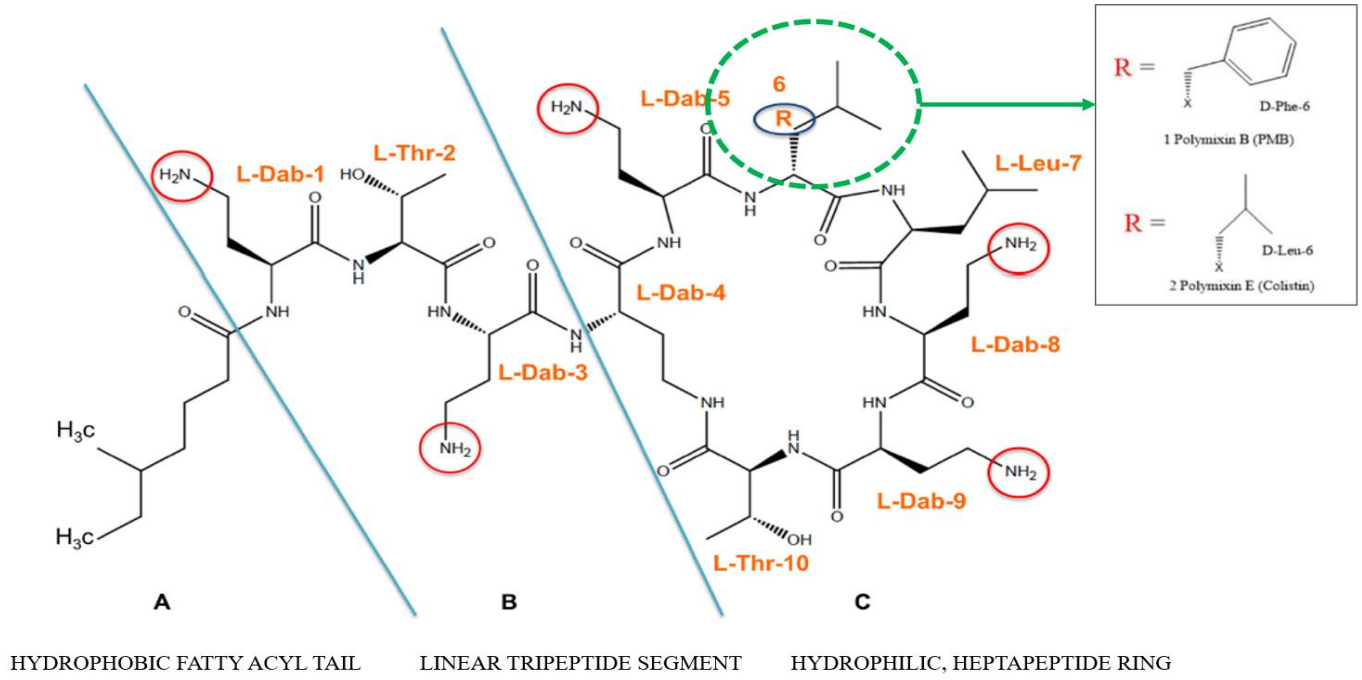

Figure 2.1 Chemical structure of polymyxin.

Number 6 indicates the amino acid position on the structure, D-phenylalanine in polymyxin B, or D-leucine in polymyxin E (colistin) (Adapted from Rhouma *et al.* (2016)).

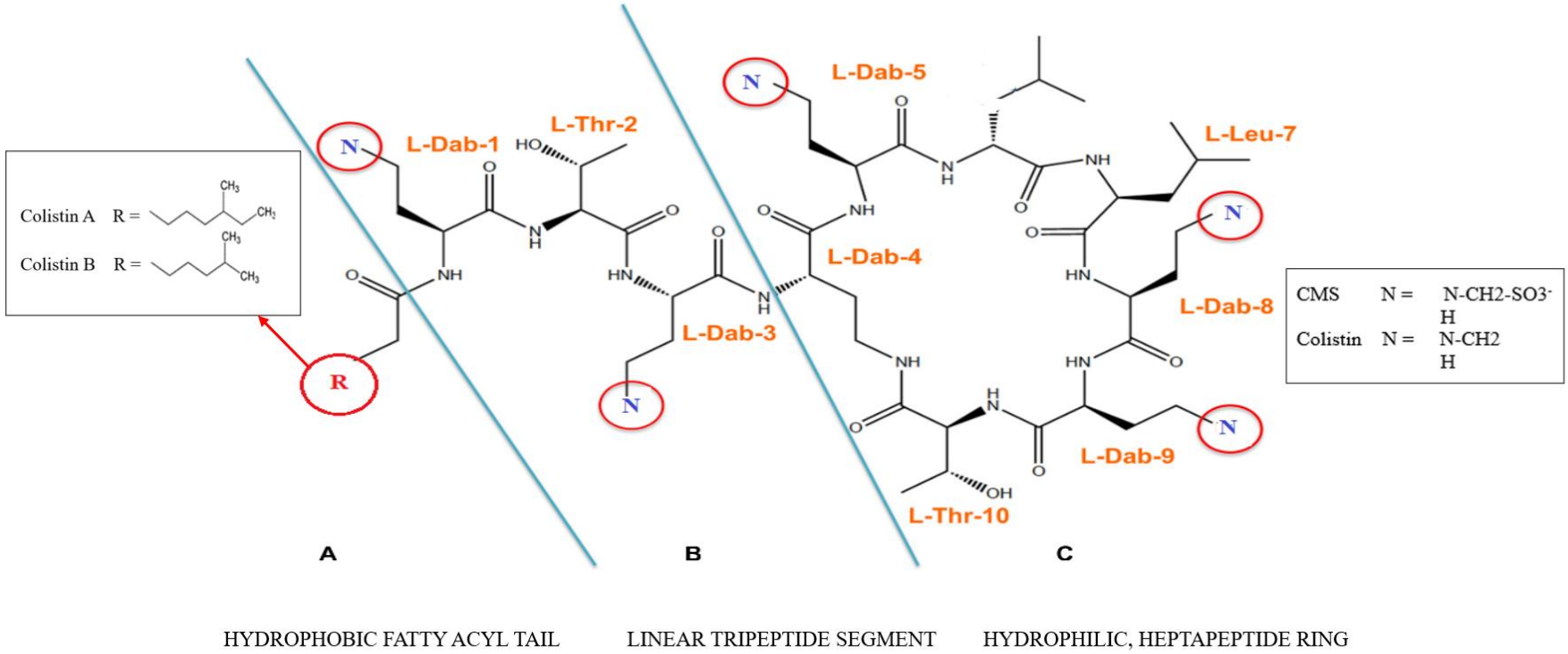

Figure 2.2 Chemical structure of CMS and colistin (polymyxin E). Amino group: α,γ-diaminobutyric acid residues. Fatty acid, R-6-methyloctanoic for CMS A and colistin A, 6-methylheptanoic acid for CMS B and colistin B (Adapted from Rhouma *et al.* (2016)).

2.3.2 Mechanism of antibacterial activity of polymyxins

Colistin's specific antibacterial mode of action in killing bacterial cells is unknown (Kaye *et al.*, 2016). Colistin's potent antibacterial action is driven by its interaction with the LPS molecules in the cytoplasmic membrane of GNB. This interaction destroys the membrane, allowing internal components to leak out and causing cell death (Vaara, 1992; El-Sayed Ahmed *et al.*, 2020).

2.4 Bioassay of colistin methanesulfonate sodium and colistin

A variety of methodologies have been reported for quantifying colistin in biological specimens: capillary electrophoresis (Kjærgaard Kristensen & Honoré Hansen, 1993), microbiological assay (Thomas, Thomas & Holloway, 1980; Leroy *et al.*, 1989), immunological assay (Kitagawa *et al.*, 1985), thin-layer chromatography (TLC)) (Thomas & Holloway, 1978), and HPLC (Dagla *et al.*, 2019).

2.4.1 Microbiological assay

Microbiological assays were previously used in pharmacokinetic research to determine dosage regimens for prescribing information. However, these approaches are inadequate in sensitivity and selectivity, and the processes take considerable time. The microbiological method for assaying CMS and colistin uses a clinical isolate such as *Bordetella bronchiseptica* (Thomas, Thomas & Holloway, 1980) or *Escherichia coli* (Wootton, Holt & Macgowan, 2005) as indicator organism. The indicator strain was evenly distributed on the surface of the agar medium, and then standard antibiotic

concentrations were added to the agar. After that, the plates underwent a 24-hour pre-incubation at 37°C. Li (2005) expressed concerns about the method's inability to differentiate between CMS and colistin during *in vitro* conversion. *In vitro*, the conversion of CMS to colistin was slower than *in vivo*, and the development of colistin from its intermediates was more rapid (Milne *et al.*, 2003). According to Li (2005), samples containing partially methanesulfonate intermediates in the agar plate will exhibit a more rapid colistin formation during incubation than samples containing CMS used for calibration curves. This suggests that previous dosage recommendations based on microbiological assay pharmacokinetic data may have been inaccurate.

2.4.2 High-performance liquid chromatography with fluorimetry detection (HPLC-FLD)

The advancement of HPLC analytical methods has considerably enhanced our comprehension of CMS and colistin pharmacokinetics. These analytical methods can separate and accurately measure colistin and CMS concentration in biological samples. Colistin has low ultraviolet (UV) absorbance and has no native fluorescent properties, so its analysis in biological samples using HPLC requires derivatisation with fluorescent reagents. In the colistin assay, derivative reagents such as orthophthalaldehyde (OPA), 9-flurenylmethyl chloroformate (FMOC-Cl), and dansyl chloride were used. The HPLC method with fluorimetry detection demonstrated high sensitivity and accuracy in quantifying the concentration of colistin within the clinically relevant analytical range of 0.05–20 μg/mL (Nation *et al.*, 2016; Hanai *et al.*, 2018). Liquid chromatography methods require derivatisation and have relatively long run times, typically ranging from 9 to 35 minutes for fluorimetry detection (Le Brun, de Graaf & Vinks, 2000; Li *et al.*, 2001, 2002; Reed *et al.*, 2001). Table 2.1

summarises the bioassay of CMS and colistin in biological fluid using HPLC with a fluorimetry detection method.

2.4.3 Liquid chromatography-tandem mass spectrometry (LC-MS)

Colistin detection via mass spectrometry provides great sensitivity and specificity without requiring a derivatisation procedure. A new and advanced method was developed to measure colistin in human biological fluid using the LC-MS systems (Gobin *et al.*, 2010), liquid chromatography-tandem mass spectrometry (LC-MS/MS) system (Jansson *et al.*, 2009; Dotsikas *et al.*, 2011; Leporati *et al.*, 2014) and Ultra-high-pressure liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) system (Tsai *et al.*, 2013). These newly developed methods were improvised to accelerate the analysis. The introduction of better pre-treatment procedures (Jansson *et al.*, 2009; Gobin *et al.*, 2010; Tsai *et al.*, 2013), using automated robotic liquid-handling workstations (Dotsikas *et al.*, 2011), and an alternative technique utilising hybrid quadrupole time-of-flight mass spectrometry (QTOF MS) combined with ultra-performance liquid chromatography (UPLC) (Gikas *et al.*, 2013), reducing the run time for LC-MS up to two minutes per analysis (Ma *et al.*, 2008; Dotsikas *et al.*, 2011).

The liquid chromatography-tandem mass spectrometry approach shortened the chromatographic method's run times. This method could also detect colistin levels two to seven times lower than those found using the fluorescence method. However, this instrument required trained personnel and mass spectrometry was not widely available. Table 2.2 summarises the bioassay of CMS and colistin in biological fluid using the LC-MS/MS method.

Table 2.1 Bioanalysis of CMS and/or colistin in biological fluid using HPLC with fluorimetry detection method.

Author	Sample	Sample Preparation	Internal Standard	Chromatography method				Total Run Time	Fluorimetry detection method		
		Protein Precipitation		LC	Column	Mobile Phase	Elution		Fluorescent reagent	Detection	Concentration Range
Le Brun, de Graaf & Vinks, (2000) (Colistin)	Human plasma, urine, sputum	MeOH-20% TCA (plasma, sputum), MeOH (urine)	-	HPLC	Novapack C18 (3.9x150 mm)	ACN, 0.01mol phosphate buffer (675:325 v/v)	Isocratic	26min	OPA	excitation 340nm, emission 440 nm	30µg/L-1mg/L
Reed *et al.* (2001) (Colistin)	Human plasma, urine, sputum	Perchloric acid, potassium hydroxide, hydrochloric acid, 9% sodium carbonate, proline, ethyl acetate (plasma, sputum). Water, perchloric acid, sodium phosphate, proline, ethyl acetate. (urine) (170min)	-	HPLC	Eclipse XDB-C8 (150x 4.8mm)	Solvent A: Water-0.1% TFA Solvent B: ACN-0.1% TFA	Gradient	18min	Dansyl chloride	excitation 350nm, emission 500nm	5-7µg/mL
Pinho *et al.* (2018) (Colistin)	Human plasma	0.1M sodium bicarbonate buffer, TCA	Amphetamine sulphate	HPLC	LiChroCART Purospher Star C18 (55mmx4 4mm, 3µm)	Solvent A: ACN Solvent B: Water	Gradient	17min	Dansyl chloride		0.09-9µg/mL

ACN: Acetonitrile; FMOC-CI: 9-flurenylmethyl chloroformate; HPLC: High-performance liquid chromatography; MeOH: Methanol; mg/L: milligram/Liter; mm: millimetre; nm: nanometer; µg/mL: microgram/millilitre; OPA: Orthophthalaldehyde; v/v/v: volume/volume/volume; TCA: Trichloroacetic acid; XDB: eXtra Dense Bonding.

Table 2.1 Continued.

Author	Sample	Sample Preparation			Internal Standard	Chromatography method				Total Run Time	Fluorimetry detection method		
		Protein Precipitation	SPE			LC	Column	Mobile Phase	Elution		Fluorescent reagent	Detection	Concentration Range
			Column	Elution									
Li *et al.* (2001) (Colistin)	Human plasma	MeOH-10% TCA (50:50, v/v)	C18	Acetone 0.2M boric acid solution	Netilmicin	HPLC	Ultrashpere C18 (250x4.6 mm)	ACN-tetrahydrofuran-water (87:4:13 v/v)	Isocratic	35min	FMOC-CI	excitation 260nm, emission 315nm	0.1-4.0mg/L
Li *et al.* (2002) (CMS)	Rat plasma rat urine	MeOH-10% TCA (50:50, v/v)	C18	Acetone 0.2M boric acid solution	Netilmicin	HPLC	Ultrashpere C18 (250x4.6 mm)	ACN-tetrahydrofuran-water (50:30:20 v/v/v)	Isocratic	18min	FMOC-CI	excitation 260nm, emission 315nm	0.33-53.3mg/L (rat plasma), 0.25-40mg/L (rat urine)
Chepyala *et al.* (2015) (Colistin)	Human plasma	MeOH-20% TCA (50:50, v/v)	C18	Acetone	Polymyxin B1	HPLC	Poroshell 120 (100x2.1 mm, 2.7 µm)	ACN, tetrahydrofuran, water (82:2:16, v/v/v)	Isocratic	22min	FMOC-CI	excitation 260nm, emission 315nm	0.3-6.0µg/mL
Hanai *et al.* 2018) (Colistin)	Human plasma	MeOH-10% TCA (50:50, v/v)	C18	Acetone	Netilmicin	HPLC	Hydrospere C18 (50x4.6, 5µm)	ACN/ tetrahydrofuran/ distilled water (50:14:20, v/v/v)	Isocratic	~9min	FMOC-CI	excitation 260nm, emission 315nm	0.1-8µg/mL

ACN: Acetonitrile; FMOC-CI: 9-flurenylmethyl chloroformate; HPLC: High performance liquid chromatography; MeOH: Methanol; mm: milimeter; nm: nanometer; µg/mL: microgram/mililiter; mg/L: milligram/Liter; OPA: Orthophthalaldehyde; v/v/v: volume/volume/volume; TCA: Trichloroacetic acid