THE UNIVERSITY OF QUEENSLAND

AUSTRALIA

Improving Antimicrobial Use In Critically Ill Patients

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A thesis submitted for the degree of Doctor of Philosophy at

The University of Queensland in 2014

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Abstract

Although delivery of prompt and appropriate antimicrobial therapy is of paramount importance for critically ill patients, few data exist to guide what doses of antimicrobials should be used in critically ill patients. Presently available generic guidelines are derived from healthy young adults and are unlikely to be appropriate for critically ill patients due to the major physiological changes that occur. Altered physiology can result in significant changes in antimicrobial pharmacokinetics and greatly affect dosing requirements. There is limited guidance to determine therapeutic antimicrobial doses due to only limited pharmacokinetic data being available in these patients. Additionally, there will be great variance between patients because of differences in co-morbidities, the invasiveness of procedures and the disease status. Thus, it is very unlikely that a dose found to be effective in non-critically ill patients will be optimal for the majority of critically ill patients. Since available data do not appear to suitably guide dosing in these patient subpopulations, directed pharmacokinetic studies should be considered fundamental. Therefore, the principal aims of this thesis were to evaluate the appropriateness of standard doses in achieving pharmacokinetic/pharmacodynamic targets and clinical scenarios affecting this target in critically ill patients.

Dynamic renal function is commonly seen in critically ill patients. For drugs cleared through the renal route; while decreasing renal function trigger reduced dosing, elevated renal function or augmented renal clearance should also trigger dosing increases. This is to avoid sub-therapeutic concentrations. However, due to scarce data available, this phenomenon has not been clearly described. This led to a study in this thesis, conducted in Malaysian intensive care units to describe this clinical scenario. It was found that almost half of the subjects recruited have augmented renal clearance. Significant bias and imprecision was demonstrated when comparing estimated Cockcroft-Gault creatinine clearance and measured urinary creatinine clearance with the bias being larger in augmented renal clearance patients and significant difference were found between this two methods. This study supports previous data that equation-based estimates of creatinine clearance are unreliable for use in critically ill patients.

As mentioned above, invasive procedures are one of the factors that could lead to physiological changes in critically ill patients. The insertion of surgical drains is an invasive procedure commonly undertaken for critically ill patients, particularly for those with abdominal disease. The complicated physiology of abdominal disease will therefore have a great influence on the pharmacokinetics of antimicrobials. In doing this, structured reviews were carried out in this thesis to review all published literature available on beta-lactam antimicrobials in this context. Our analysis has described significant pharmacokinetic variability for different beta-lactam antimicrobials in patients with intra-abdominal disease and therefore, standard dosing may not be optimal when dealing with less susceptible pathogens. Additionally, possible antimicrobial clearance through surgical drains suggests a need for studies to be conducted in this area. Given this, a study to describe the pharmacokinetics of two commonly prescribed antimicrobials; meropenem and piperacillin, was conducted in critically ill patients with surgical drains. These two antimicrobials have shown altered pharmacokinetics and dosing modifications may be necessary if less susceptible pathogens are suspected. A linear correlation was found between antimicrobial clearance and the volume output of surgical drain fluid suggesting the additional doses should be used when the presence of high volume output of surgical drains is encountered.

Resistant pathogens are one of the challenges in these patients and one of the increasingly important pathogens is multi-drug resistant *A. baumanni*. Treatment options are very limited, to either colistin or sulbactam combination antimicrobials. Therefore, it is vital to optimize all therapeutic options and ampicillin/sulbactam is one of those limited options available. A review on the potential use of ampicillin/sulbactam in treating critically ill patients is described in this thesis. It was found that sulbactam has strong intrinsic activity against multi-drug resistant *A. baumanni* and current therapeutic challenges result partly from bacterial susceptibility and also from pharmacokinetic alterations. Administration of 4 hour infusion and combination therapy was found to be more likely to be effective in treating serious bacterial infection. Nevertheless, since this dosing was derived from pharmacokinetic studies conducted in non-critically ill patients, there is still a strong need of similar study to be carried out in critically ill patients. This led to a study in this thesis, conducted in a Malaysian intensive care unit to describe pharmacokinetics of ampicillin/sulbactam in critically ill patients at risk of multi-drug resistant *A. baumanni* infections. We found that the pharmacokinetics of ampicillin/sulbactam differs significantly

from that in healthy volunteers. Significant correlation was observed between ampicillin and sulbactam clearance and creatinine clearance. A significantly increased V_d was observed, which implies extensive distribution. These pharmacokinetic changes also manifested as inadequate trough concentrations which were observed in some of the studied subjects, which did not exceed the susceptibility breakpoint for *A. baumannii*. Findings from this study support the use of higher initial doses of ampicillin/sulbactam for those patients with a high V_d with adjustment of subsequent doses performed according to renal function.

Keywords : antimicrobial; critically ill; pharmacokinetics; ampicillin/sulbactam; piperacillin; meropenem; surgical drains; augmented renal clearance.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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Publications during candidature

Adnan S, Paterson DL, Lipman J, Kumar S, Li J, Rudd M, Roberts JA ; Pharmacokinetics of beta-lactam antibiotics in patients with intra-abdominal disease: A structured review. Surg Infect (Larchmt). 2012 Feb;13(1):9-17.

Adnan S, Li JX, Wallis SC, Rudd M, Jarrett P, Paterson DL, Lipman J, Udy AA, Roberts JA; Pharmacokinetics of meropenem and piperacillin in critically ill patients with indwelling surgical drains. Int J Antimicrob Agents. 2013 Jul;42(1):90-3.

Adnan S, Paterson DL, Lipman J, Roberts JA ; Ampicillin/sulbactam: Its potential use in treating infections in critically ill patients. Int J Antimicrob Agents. 2013 Nov;42(5):384-9.

Adnan S, Ratnam S, Kumar S, Udy A, Paterson DL, Lipman J, Roberts JA; Critically ill patients at risk of augmented renal clearance : Experience in a Malaysian intensive care unit. Anaesthesia and Intensive Care. Volume 42, Issue 6

Wallis SC, Adnan S, Meija JLO, Parker S, Paterson S, Lipman J, Roberts JA; Simultaneous determination of ampicillin and sulbactam in human plasma and urine by UHPLC-MS/MS. Submitted to Journal of Chromatography B

Adnan S, Ratnam S, Kumar S, Paterson DL, Lipman J, Roberts JA; Pharmacokinetics of ampicillin/sulbactam in critically ill patients at risk of multi-drug resistant *Acinetobacter baumannii* infections. Submitted to Antimicrobial Agents and Chemotherapy

Publications included in this thesis

Adnan S, Ratnam S, Kumar S, Udy A, Paterson DL, Lipman J, Roberts JA; Critically ill patients at risk of augmented renal clearance : Experience in a Malaysian intensive care unit. Anaesthesia and Intensive Care. Volume 42, Issue 6. Incorporated as part of Chapter 3

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Adnan S, Paterson DL, Lipman J, Kumar S, Li J, Rudd M, Roberts JA ; Pharmacokinetics of beta-lactam antibiotics in patients with intra-abdominal disease: A structured review. Surg Infect (Larchmt). 2012 Feb;13(1):9-17. Incorporated as part of Chapter 4

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Adnan S, Li JX, Wallis SC, Rudd M, Jarrett P, Paterson DL, Lipman J, Udy AA, Roberts JA; Pharmacokinetics of meropenem and piperacillin in critically ill patients with indwelling surgical drains. Int J Antimicrob Agents. 2013 Jul;42(1):90-3. Incorporated as part of Chapter 4

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Adnan S, Paterson DL, Lipman J, Roberts JA ; Ampicillin/sulbactam: Its potential use in treating infections in critically ill patients. Int J Antimicrob Agents. 2013 Nov;42(5):384-9. Incorporated as part of Chapter 5

Contributor	Statement of contribution
Prof David Paterson	Reviewing draft
Prof Jeffrey Lipman	Reviewing draft
Prof Jason Roberts	Data collection, interpretation and writing draft

Wallis SC, Adnan S, Meija JLO, Parker S, Paterson S, Lipman J, Roberts JA; Simultaneous determination of ampicillin and sulbactam in human plasma and urine by UHPLC-MS/MS. Incorporated as Chapter 5

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Prof Jeffrey Lipman	Reviewing draft
Prof Jason Roberts	Data interpretation and writing draft

Adnan S, Ratnam S, Kumar S, Paterson DL, Lipman J, Roberts JA; Pharmacokinetics of ampicillin/sulbactam in critically ill patients at risk of multi-drug resistant *Acinetobacter baumannii* infections. Incorporated as Chapter 5.

Contributor	Statement of contribution
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Prof Jason Roberts	Data interpretation and writing draft

Contributions by others to the thesis

All data collection and analysis for chapters taken from the PhD was undertaken by the PhD candidate, Syamhanin Adnan. All chapters and papers of this thesis is drafted and written by Syamhanin Adnan under the guidance of the other co-authors.

Study on prevalence of augmented renal clearance in critically ill patients has led to an published article entitled “Critically ill patients at risk of augmented renal clearance: Experience in Malaysian intensive care unit” in Chapter 3. Sample collection and analysis were undertaken by the PhD candidate, assisted by Dr Shanthi Ratnam and Dr Suresh Kumar. Prof Jason Roberts and Dr Andrew Udy assisted with the data analysis and interpretation. Prof David Paterson and Prof Jeffrey Lipman oversaw all aspects of this chapter.

The structured review described in Chapter 4 has led to a published article, entitled : “Pharmacokinetics of beta-lactam antibiotics in intra-abdominal disease : A structured review”. All data collection, data interpretation and drafting of the paper were undertaken by the PhD candidate, Syamhanin Adnan, assisted by Ms Janice Li. The names and roles of co-authors are: Prof Jason Roberts; who assisted with the data collection and data interpretation, Dr Suresh Kumar, Dr Michael Rudd and Janice Lee assisted with the draft. As PhD supervisors, Prof David Paterson, Prof Jason Roberts and Prof Jeffrey Lipman oversaw all aspects of this chapter. The study on critically ill patients with surgical drains has led to a published article entitled “Pharmacokinetics of meropenem and piperacillin in critically ill patients with indwelling surgical drains”. For this manuscript, patient recruitment and consent were assisted by Prof Jason Roberts. Sample collection and analysis were undertaken by the PhD candidate and Ms Janice Li, with assistance from research nurses and Dr Steven Wallis. Prof Jason Roberts assisted with the data analysis and interpretation. Dr Michael Rudd assisted with the data interpretation. Prof David Paterson, Prof Jason Roberts and Prof Jeffrey Lipman oversaw all aspects of this chapter.

The review written in Chapter 5 has led to a published article, entitled : “Ampicillin/Sulbactam : Its potential use in treating infections in critically ill patients”. All data collection, data interpretation and drafting of the paper were undertaken by the PhD candidate, Syamhanin Adnan. The names and roles of co-authors are: Prof Jason Roberts; who assisted with the data collection, data interpretation and manuscript drafting.

Prof David Paterson, Prof Jason Roberts and Prof Jeffrey Lipman oversaw all aspects of this chapter.

The development of ultra high performance liquid chromatography analysis - mass spectrometry assay for ampicillin/sulbactam described in Chapter 4 led to an article, entitled “Simultaneous determination of ampicillin and sulbactam in human plasma and urine by UHPLC-MS/MS”, with Dr Steven Wallis as the main author of this manuscript. All data collection, data interpretation and drafting of the paper were undertaken by Dr Steven Wallis, assisted by the PhD candidate, Syamhanin Adnan, Jenny Lisette Ordóñez Meija and Suzanne Parker. Prof Jason Roberts, Prof David Paterson and Prof Jeffrey Lipman oversaw all aspects of this chapter.

The study on critically ill patients treated with ampicillin/sulbactam led to an article entitled “Pharmacokinetics of ampicillin/sulbactam in critically ill patients at risk of multi-drug resistant *Acinetobacter baumannii* infections”. Sample collection and analysis were undertaken by the PhD candidate, assisted by Dr Shanthi Ratnam and Dr Suresh Kumar. Dr Steven Wallis has assisted with the bioanalysis of the samples. Prof Jason Roberts assisted with the data analysis and interpretation. Prof David Paterson, Prof Jason Roberts and Prof Jeffrey Lipman oversaw all aspects of this chapter.

Statement of parts of the thesis submitted to qualify for the award of another degree

None

Acknowledgements

First of all, I would like to thank Almighty God, ALLAH, Who has blessed and guided me so that I am able to accomplish this Thesis as a partial fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Queensland, Queensland, Australia.

There are a number of people I am greatly indebted, people that I believe were chosen by HIM to assist me, and without them, this Thesis might not have been completed on time.

To my advisory team, led by Professor David Paterson, I would like to express my deepest appreciation for guiding me all the way and without their persistent help; this Thesis would not have been possible.

To Professor Jason Roberts, no words can truly describe my heartfelt gratitude of having him as one of my supervisor, he has the attitude of a genius, and he continually and convincingly conveyed a spirit of adventure in regard to research. I am truly indebted to him.

I also wish to express my gratitude to Professor Jeffrey Lipman for his kind guidance and words of encouragement, he reminds me of my own father. Indeed, it is blessing from HIM to have him in the advisory team.

I would also like to express my warm thanks to Dr Andrew Udy for his support and guidance for the study on augmented renal clearance; Chapter 3 of this Thesis. I am sincerely grateful to his truthful and illuminating views on a number of issues related to the project.

I would also like to thank Dr Steven Wallis and his great team, Jenny Lisette Ordonez Meija and Suzanne Parker, who provided me the technical support for the laboratory work of Chapter 4 and 5. I thank Ms Janice Li, who contributes in data collection of the study in Chapter 4. To all team of Clinical Research Centre Unit, Intensive Care Unit and Pathology Department of Hospital Sungai Buloh, thank you for providing me the facilities that I need while conducting my research in Malaysia.

To my mother and father, Normah Abd Kadir and Adnan Ahmad Sharif, thank you for nurturing me throughout my life with unconditional love. To my sisters, Elaramzah Adnan

and Fadhillah Adnan, warmest thank for both of you who has constantly, give me the encouragement that I need.

To my dear friends, who remains willing to engage with my struggle, a very special thank you for your practical and emotional support as I go through the difficult stage of preparing and writing this Thesis.

I would like to thank my team member; the Paterson group from The University of Queensland, Centre for Clinical Research and also the great team of Burns, Trauma and Critical Care Research Centre, your encouragement have been extremely helpful.

Last but not least, I dedicated this Thesis all my Muslim friends throughout the world, especially those who are struggling and striving in obtaining HIS pleasure.

Keywords

Antimicrobial, critically ill, augmented renal clearance, ampicillin/sulbactam, meropenem, piperacillin, pharmacokinetics, pharmacodynamics

Australian and New Zealand Standard Research Classifications (ANZSRC)

ANZSRC code: 111502, Clinical Pharmacology and Therapeutics, 60%

ANZSRC code: 030101, Analytical Spectrometry, 20%

ANZSRC code: 060502, Infectious Agent, 20%

Fields of Research (FoR) Classification

FoR code: 1115, Pharmacology and Pharmaceutical Sciences 60%

FoR code: 030101, Analytical Spectrometry, 20%

FoR code : 0605, Microbiology,20%

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List of Abbreviations used in the thesis

ADH	Anti-diuretic hormone
AKI	Acute kidney injury
APACHE II	Acute Physiology and Chronic Health Evaluation
ARC	Augmented renal clearance
AUC_{0-24}	Area under the concentration time curve during a 24 hour time period
AUC_{0-24}/MIC	Ratio of the area under the concentration time curve during a 24 hour time period to minimum inhibitory concentration
$AUC_{0-\infty}$	Area under the concentration time curve extrapolated to infinity
C_{drain}	Antimicrobial concentration in drain fluid
CG	Cockcroft -Gault equation for creatinine clearance
CI	Continuous infusion
CKD	Chronic Kidney Disease
CL	Clearance
C_{max}	Peak serum concentration achieved by a single dose
C_{max}/MIC	Ratio of the maximum serum antibiotic concentration to minimum inhibitory concentration
C_{min}	Lowest concentration during a dosing period
Cr_{CL}	Creatinine clearance
CSF	Cerebrospinal fluid
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FDA	Food and Drug Administration
$fT_{>MIC}$	Time the free concentration is maintained above the minimum inhibitory concentration during a dosing interval

GFR	Glomerular filtration rate
HPLC	High performance liquid chromatography
HPLC-UV	High performance liquid chromatography-ultra-violet
IAH	Intra-abdominal hypertension
IAI	Intra-abdominal infection
ICU	Intensive care unit
IDC	Indwelling urinary catheter
II	Intermittent infusion
IQR	Inter quartile range
k_{el}	Elimination rate constant
LLOQ	Lower limit of quantification
MDR	Multidrug-resistant
MDRD	The Modification of Diet in Renal Disease
MIC	Minimum inhibitory concentrations
MODS	Multiple organ dysfunction syndrome
MPC	Mutant Prevention Concentrations
MSV	Mutant Selection Window
MRM	Multiple reaction monitoring
MRSA	Methicillin-resistant *Staphylococcus aureus*
OR	Odds ratio
PD	Pharmacodynamics
PK	Pharmacokinetics

QC	Quality control
RRT	Renal replacement therapy
SD	Standard deviation
SDF	Surgical drains fluid
SIRS	Systemic inflammatory response syndrome
SOFA	Sequential organ failure assessment
UHPLC	Ultra high performance liquid chromatography
UHPLC-MS/MS	Ultra high performance liquid chromatography-mass spectrometry/mass spectrometry
VAP	Ventilator-associated pneumonia
V_d	Volume of distribution

1. Chapter 1 : Overview

Management of severe infections in critically ill patients remains an ongoing challenge for physicians. Early and effective antimicrobial therapy is essential as critically ill patients are at a high risk of dying [1]. Antimicrobial therapy is defined to be inadequate if the empirical choices, doses and method of administration are not effective against the causative pathogen [1].

Critically ill patients are also at risk of infection by resistant pathogens. Data from the worldwide bacterial surveillance program has shown higher resistance rates in intensive care unit isolates compared to non- intensive care unit isolates [2]. This surveillance reports a 4 – 16 fold higher minimum inhibitory concentrations $(MIC)_{90}$ of gram negative bacilli against the studied antimicrobials [2]. The presence of resistant pathogens increases the likelihood of inappropriate antimicrobial therapy [3] both in terms of antimicrobial agent and dose, thereby exposing the patient to a higher risk of dying [4].

Most critically ill patients have significant co-morbidities prior to admission with the majority of admissions being non-elective; secondary to surgical procedures, cardiovascular complications and trauma [5]. These chronic illnesses; in combination with the acute pathology causing admission to the intensive care unit (e.g. surgical procedures, sepsis and/or trauma), cause a significantly unstable clinical status. The physiological changes that occur will also impact the pharmacokinetics (PK) of antimicrobials [6].

PK describes the fate of a drug from the administration time up to elimination from the body. It is also used to describe the relationship between the dose and drug concentrations in plasma and at the target site. PK is also often studied in conjunction with pharmacodynamic (PD)s, the study of relationship between drug concentration and its therapeutic effect. Since PK describes how the body affects the fate of a drug; physiological changes of the critically ill patients will greatly cause significant changes in its PK properties. Therefore, standard dosing, which derives from PK data from healthy volunteers may not be optimal for critically ill patients. It follows that an individualized approach is required to ensure adequate therapy to increase the likelihood of clinical success [7]. However, this individualized approach requires dosing guidance for this specific group of patients. Since limited data is readily available, this approach can only be achieved by conducting research that describes the PK of the antimicrobial of interest in these patients. The antimicrobial use in critically ill patients will greatly improve by characterizing its PK and PD profile.

There are a number of clinical scenarios and problems encountered during clinical practice that are often left unanswered. Three clinical scenarios were chosen to be studied in this Thesis, as follows:

Project 1 – Critically Ill Patients at Risk of Augmented Renal Clearance

Antimicrobial clearance will greatly depend on the organ involved in the elimination process. For renally excreted drugs, renal function will therefore determine its clearance. Often, prompt dose adjustment is required to avoid either toxicity; in decreasing renal function or sub-therapeutic concentrations; in elevated renal function. However, dosing guidance is readily more available for dose adjustment in decreasing renal function rather than elevated renal function. The primary purpose of this study is to establish the prevalence of augmented renal clearance in critically ill patients. A comparison between Cockcrof-Gault equations with measured urinary creatinine clearance will also be undertaken.

Project 2 – Dosing of Meropenem and Piperacillin in Critically Ill Patients with Indwelling Surgical drains.

Meropenem and piperacillin/tazobactam are commonly prescribed as empiric therapy in critically ill patients. It has been observed that those patients with indwelling surgical drains often had lower antimicrobial concentrations. This observation has raised concern as there are few data available to suggest that these surgical drains are associated with sub-therapeutic concentrations, which may lead to impaired antimicrobial efficacy. Therefore, this study was conducted to characterize the PK profile of meropenem and piperacillin in patients with indwellling surgical drains and to determine the magnitude and relative importance of clearance through this route.

Project 3 – Dosing of Ampicillin/sulbactam in Critically Ill Patients at risk of Multi-drug resistant Acinetobacter baumannii infections

Ampicillin/sulbactam is one of those limited antimicrobial options available to treat patients with multi-drug resistant *A. baumannii* infections, a particularly important pathogen in intensive care settings. Ampicillin is a time-dependent antimicrobial with its bacterial killing largely dependent on the time the free concentration is maintained above the minimum inhibitory concentrations during a dosing interval ($fT>_{MIC}$). Sulbactam is probably best

described as an area under the concentration—time curve dependent drug where bacterial killing is associated with the area under the concentration—time curve, however, recent data have also suggested $fT_{>MIC}$ may be important for sulbactam. Most of the dosing recommendations of ampicillin/sulbactam for this particular pathogen were not derived from PK studies and for those available PK studies; it was not conducted in critically ill patients. Therefore, the optimal use of this antimicrobial in critically ill patients is still unknown. This study was conducted to characterize the PK profile of ampicillin/sulbactam in critically ill patients who are at risk of multi-drug resistant pathogens.

Chapter 1 and 2 of this thesis will review the physiological changes in critically ill patients and its implication to PK of antimicrobials.

Chapter 3 will discuss the prevalence and implications of augmented renal clearance infections in critically ill patients. This chapter will include a published manuscript on a study describing the prevalence of augmented renal clearance in a Malaysian intensive care unit.

Chapter 4 will discuss the effect of intra-abdominal disease on PK of beta-lactam antimicrobials in critically ill patients. This chapter will include a published structured review of PK of beta-lactam antimicrobials in intra-abdominal disease, followed by a published manuscript on a study describing PK of meropenem and piperacillin in critically ill patients with indwelling surgical drains.

Chapter 5 will discuss the potential use of ampicillin/sulbactam in treating infections in critically ill patients. This chapter will include a published manuscript on a review of ampicillin/sulbactam and its potential use in critically ill patients, development of methods used for analysing ampicillin/sulbactam in PK samples, followed by the study describing PK of ampicillin/sulbactam in critically ill patients at risk of multi-drug resistant *A. baumannii* infections.

Chapter 6 of the thesis summarises the general discussion on the findings of this Thesis and directions for further research.

All of these studies will help define appropriate dosing regimens for commonly used antimicrobials in patients at risk of developing multi-drug resistant infections (patients with

indwelling surgical drains and/or with augmented renal clearance), as well as in patients that already have such infections. Such dosing regimens can be used to optimize antimicrobial use and improve the likelihood of positive clinical outcomes in these challenging critically ill patients.

1.1 Aims

The overall aim of this thesis is to improve the use of antimicrobials in critically ill patients. To achieve this, we firstly identify clinical scenarios that pose challenges in the use of antimicrobials in critically ill patients. Three clinical scenarios were identified:

1. Critically ill patients at risk of augmented renal clearance
2. Dosing of meropenem and piperacillin in critically ill patients with indwelling surgical drains
3. Dosing of ampicillin/sulbactam in critically ill patients with multi-drug resistant *A. baumannii* infections

Each clinical scenario has its specific aims; as follows:

1. Critically ill patients at risk of augmented renal clearance
   1.1. To describe the incidence of augmented renal clearance in patients admitted to the intensive care unit of Hospital Sungai Buloh, Malaysia
   1.2. To compare the prevalence of augmented renal clearance in Malaysian intensive care unit patients with other intensive care unit around the world
   1.3. To compare the estimated Cockcroft-Gault equation with measured urinary creatinine clearance to determine whether these two methods can be used interchangeably

2. Dosing of meropenem and piperacillin in critically ill patients with indwelling surgical drains:
   2.1. To describe the pharmacokinetics of meropenem and piperacillin in critically ill patients with indwelling surgical drains with a focus on the comparative drug clearance through the drains and the renal system
   2.2. To use the results from the primary aim to develop dosing recommendations for these drugs in these patients

3. Dosing of ampicillin/sulbactam in critically ill patients at risk of multi-drug resistant *A. baumannii* infections:
   3.1. To study the pharmacokinetic of ampicillin/sulbactam in critically ill patients with multi-drug resistant *A. baumannii* infections
   3.2. To use the results of the above study to develop dosing recommendations of ampicillin/sulbactam in these patients

2. Chapter 2: The Effect of The Altered Physiology of Critically Ill Patients on Pharmacokinetics

In critically ill patients, the determination of an optimal dose of an antimicrobial is a complex process. The factors contributing to this challenge are described in Figure 2.1. Therefore, an understanding of drug properties and pathophysiological changes and their effects on antimicrobial dosing is essential. For the purposes of this review, the pathophysiological changes in critical illness are divided into four main categories; fluid shifts, organ dysfunction, changes in protein binding and circulatory failure.

2.1 Fluid shifts

Accumulation of fluid in extravascular compartments such as the interstitial space, is a common phenomenon seen in critically ill patients. Since interstitial fluid is the site of most infections, it is an important site for the therapeutic activity of antimicrobials. Further to this, accumulation of fluid in the interstitial space may affect the therapeutic activity of antimicrobials. Increased capillary permeability, fluid retention, decreased oncotic pressure and endothelial damage [8] are among several mechanisms resulting in the accumulation of interstitial fluid. Several pathologies that may lead to fluid shift are discussed below.

Factors causing fluid shifts

Sepsis

Sepsis causes an excessive production of inflammatory cytokines, which increase membrane permeability, resulting in vasodilatation and oedema. The end result is capillary leakage, leading to fluid movement from intravascular compartment into the extravascular compartment [9].

Acute kidney injury

The incidence of acute kidney injury (AKI) among critically ill patients with sepsis is high [10]. Fluid therapy may be prescribed in the presence of AKI to provide adequate hydration and to prevent further kidney injury. However, if it is given too aggressively, it may lead to fluid overload, causing an increase in total body water [11].

Hepatic failure

Patients with hepatic dysfunction may develop ascites and oedema. These patients frequently have a reduced ability to excrete sodium, which leads to accumulation of sodium and water in the abdominal cavity. The end result is a larger volume of extracellular fluid [12].

Fluid resuscitation

Aggressive fluid resuscitation following septic shock can cause expansion of the extracellular fluid. Fluid resuscitation is rightly listed as one of the urgent treatment strategies in the Surviving Sepsis Campaign [13] which outlines appropriate sepsis treatments. Thus, it is likely that many septic shock patients will develop fluid expansion.

Burns

Physiological changes in burns patients are well known; with extensive capillary leakage together with intensive fluid resuscitation to compensate for the development of hypovolaemia, causing significant movement of fluid from the intravascular to the extravascular compartment [14].

Others

Other pathologies that could cause an increase in extracellular fluid include pleural effusions, the presence of surgical drains and extracorporeal circuits although the effects of both are poorly described [15]. Pregnancy is also another condition which causes an increase in total body fluid [16].

2.2 Organ dysfunction

Severe infection may cause organ dysfunction. In the context of the eliminating organs, decreased functionality may impair elimination of waste products from the body as well as decreased drug elimination [17].

Renal failure

There are three mechanisms of drug elimination through the kidney; filtration, secretion and metabolism [18]. For many renally cleared drugs, filtration is considered to be the main mechanism. Thus, reduced renal blood flow in AKI patients may lead to reduction in glomerular filtration causing drug accumulation and potential drug toxicities.

Liver failure

Liver dysfunction in critically ill patients may result from infection, organ hypoperfusion and administration of hepatotoxic drugs [19]. As the liver is the major site of drug metabolism in the body, hepatic dysfunction can lead to accumulation of hepatically cleared antimicrobials [18].

It has been reported that, for drugs subject to phase I metabolism (e.g. flucloxacillin and fluoroquinolones), which involves transformation of parent drug to a more hydrophilic metabolite, metabolism is only significantly affected when the liver's metabolic capacity is reduced by more than 90%. For drugs with phase II metabolism (e.g. tetracycline), which includes conjugation, this process is not capacity limited and will still occur in the presence of severe liver dysfunction [20].

Cardiovascular dysfunction

Reduced vascular resistance due to peripheral vasodilatation mediated by inflammation caused by infection can lead to decrease in cardiac after load and a consequent increase in cardiac output. In the presence of fluid loading, this increase in cardiac output can lead to augmented systemic perfusion [9]. However, as cardiac output approaches its limit, in line with the Frank Starling law, cardiac contractility will lessen, resulting in a decrease in cardiac output. Compensatory peripheral vasoconstriction will follow and reduce peripheral tissue perfusion and potentially drug distribution to those sites [8].

2.3 Changes in protein binding

The incidence of hypoalbuminaemia is high among critically ill patients; reported as 40-50% [21]. There are several mechanisms that can lead to hypoalbuminaemia; either due to decreased production, capillary leakage or increased elimination [19]. Sepsis is one of the physiological conditions that could cause albumin loss via capillary leakage.

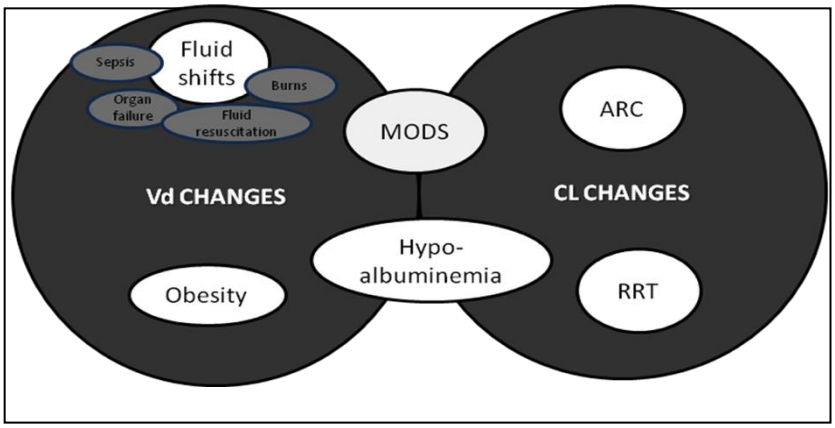
2.4 Circulatory failure

Excessive production of inflammatory mediators in sepsis can be harmful and cause tissue injury, leading to the development of microcirculatory lesions and decreased tissue oxygenation. Diminished microcirculatory function may affect the delivery of antimicrobials to the interstitial fluid of tissue, which is the site of infection.

Additionally, formation of an abscess as a result of activation of the coagulation and complement cascade, secondary to the inflammatory process will also be another important factor to be considered in this context. Antimicrobial penetration is impaired as a result of limited perfusion in the presence of fibrin clots and the abscess wall [22].

Volume of distribution (V_d) and clearance (Cl) are the two most important PK parameters for drug dosing. These two PK parameters represent the distribution and elimination phases of drug disposition respectively. Physiological changes in critically ill patients can cause changes in these PK parameters, which will require different doses to achieve the same drug concentrations seen in non-critically ill patients. The physiological changes affecting specific PK parameters are described in Figure 2.1.

Figure 2.1 : Diagram illustrating pharmacokinetic parameters affected by physiological changes.



Legend :
Vd : Volume of Distribution
Cl : Clearance
MODS : Multiple Organ Dysfunction Syndrome
ARC : Augmented Renal Clearance
RRT : Renal Replacement Therapy

2.5 Effect on Volume of Distribution

The apparent volume of distribution (V_d) is a pharmacological term used to quantify the distribution of a drug between plasma and other body compartments after administration. It is defined as the theoretical volume in which the total amount of drug would need to be uniformly distributed to produce the observed blood concentration of a drug [23].

Hydrophilic antimicrobials (e.g. beta-lactams and aminoglycosides) are typically distributed into plasma and the interstitial fluid of tissues with little intracellular distribution whereas more lipophilic antimicrobials (e.g. quinolones) will have a larger V_d because of more extensive adipose tissue and intracellular distribution. Increments in V_d suggest more extensive drug distribution throughout the body. Physiological changes that could alter V_d are fluid shifts, hypoalbuminemia and obesity.

Fluid shifts

Accumulation of fluid in extravascular compartments such as the interstitial fluid, is a common phenomenon seen in critically ill patients. Increased capillary permeability, fluid retention, decreased oncotic pressure and endothelial damage [24] are among several mechanisms resulting in the accumulation of fluid at interstitial space.

Hypoalbuminemia

The reduced number of circulating albumin molecules in the presence of hypoalbuminaemia, results in a reduced number of drug binding sites. For highly protein-bound drugs (e.g. cefoperazone 90%), this initially leads to a larger proportion of unbound drug in the plasma. However, the unbound drug then distributes into the extravascular compartment, eventually leading to decreased unbound drug concentrations in plasma. Since only the unbound or free drug is able to exert a pharmacological effect, a reduced unbound drug concentration may result in less effective concentrations in plasma over time [19].

Obesity

Lipophilic antimicrobials can cross the bi-lipid cell membrane and distribute intracellularly and these drugs are more likely to penetrate adipose tissue and therefore drug V_d is heavily dependent on the amount of adipose tissue present. Therefore, in the presence of excessive adipose tissue stores, such as in obesity, a significant increase in V_d of the

lipophilic antimicrobials is expected [25]. Examples of lipophilic antimicrobials include macrolides, fluoroquinolones and tigecycline.

2.6 Effect on Clearance

CL is defined as the volume of blood cleared of drug per unit time. Where drug elimination is performed by multiple organ systems, CL refers to sum of CL contributed by each organ system[26]. Pathologies that can lead to changes in CL are discussed below. Pathologies that can lead to changes in CL are augmented renal clearance, organ dysfunction and renal replacement therapy.

Augmented renal clearance

An important phenomenon common to critically ill patients is that of enhanced renal elimination, also known as augmented renal clearance (ARC) [27]. Aggressive fluid loading that increases cardiac output, leads to increased systemic perfusion including renal blood flow and subsequent glomerular filtration[28]. This phenomenon can result in sub-therapeutic concentrations of drugs and put the patient at risk of treatment failure. Sub-therapeutic concentrations of antimicrobials could later lead to treatment failure and development of colonisation or infection by multi-drug resistant organisms [29]. Patients considered at risk of ARC include the young, trauma, postoperative, low Acute Physiology and Chronic Health Evaluation (APACHE II) scores, burns injury, pancreatitis, autoimmune disorders and ischemia.

Organ dysfunction

A decreased rate of elimination of antimicrobials due to organ dysfunction often leads to reduced CL, leading to drug accumulation and potential toxicity as discussed above.

Renal replacement therapy

Renal replacement therapy (RRT) is used to eliminate accumulated waste products, or fluid from patients with kidney dysfunction. There are numerous types and settings used for RRT and the implications of this has been reviewed in detail by Choi et al [30]. Suffice to say, different drug clearances occur in patients receiving different RRT and as such a guideline approach to dosing is problematic. It is suggested that individualized drug dosing, using sound PK and pharmacodynamic (PD) principles is essential for this particular group of patients [30].

The dynamic nature of the clinical status of critically ill patients can result in frequent fluctuations in renal function and will require continuous evaluation for potential dose adjustment.

2.7 Implications of altered pharmacokinetic of antimicrobials on bacterial killing

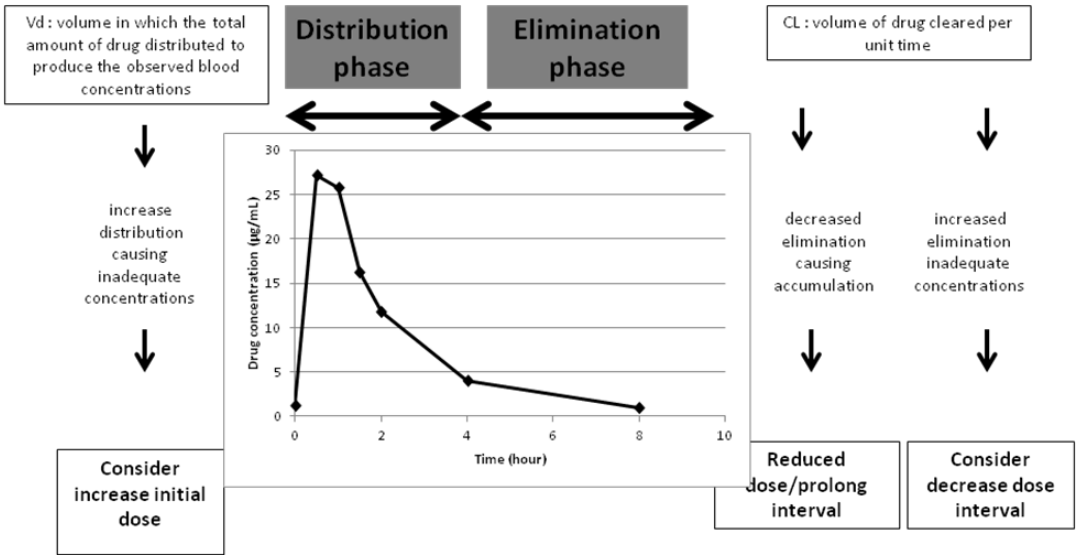
The effectiveness of antimicrobial therapy is not straightforward due to the interrelationship between all the parameters involved. Correlation of the drug profile, PK with antimicrobial effect, PD, also known as PK/PD indices, has shown to be a predictive index in differentiating outcome with its magnitude.

Pathophysiology changes can alter drug concentrations resulting in different antimicrobial efficacy to that anticipated based on PK from non-critically ill patients' studies. However, it will not change the antimicrobial activity measured by the minimum inhibition concentration (MIC), as it only measures growth inhibition in an artificial media [6]. However, for this reason, describing a PK profile can enable a rational dose change that can ensure optimal concentrations are achieved to enable maximal bacterial killing. Thus, the combination and integration of the PK profile of an antimicrobial with its MIC provides a robust description of its killing activity.

Antimicrobials can be divided into three major classes on the basis of their PK/PD indices [31]. Concentration dependent antimicrobials (e.g. aminoglycosides) exhibit bacterial killing which increases as drug concentration increases and is closely associated with the peak concentration (C_{max})/MIC ratio and achieving a higher ratio has been advocated to maximize bacterial killing [32]. Administration of higher doses should be clinically useful when a bacteria with a higher MIC is present [33]. On the contrary, the killing of bacteria by time dependent antimicrobials (e.g. beta-lactams) increases within a relative narrow range of drug concentration and will depend largely on the time that the antimicrobial concentration is maintained above the MIC. Some *in vitro* data of the antimicrobial killing of *Pseudomonas spp* suggest increased activity [34] when concentrations are maintained at 4–5×MIC . It follows therefore that the goal of a dosing regimen for time-dependent antimicrobials would be to optimize duration of exposure. The third group has properties consistent with both concentration and time dependent antimicrobials, and bacterial killing is associated with the ratio of the area under the concentration time curve (AUC), to MIC (AUC/MIC; e.g. fluoroquinolones and glycopeptides). The strong relationship between PK and PD means that a change in PK will affect the PK/PD index; therefore it may affect the

treatment outcome of the infection. Substantial evidence has shown that wide array of PKs variable could result in inadequate drug concentrations leading to different clinical responses for the same standard dosing. Since the drug concentration is a direct result of the dosage; this variation can be accounted for by modification of the dosing regimen as described in Figure 2.2.

Figure 2.2 : Diagram illustrating the implications of pharmacokinetic parameters on dosing



3. Chapter 3 : Critically Ill Patients at Risk of Augmented Renal Clearance

3.1 Sypnosis

The aim of this chapter is to describe the incidence of augmented renal clearance among critically ill patients and to compare two different methods of assessment of renal function, measured and calculated creatinine clearance. This chapter include the study of the prevalence of augmented renal clearance among critically ill patients in Malaysian intensive care, which led to a published manuscript.

3.2 Published manuscript `Selected Critically Ill Patients at Risk of Augmented Renal Clearance : Experience in Malaysian Intensive Care Unit'

The manuscript entitled, `Selected Criticaly Ill Patients at Risk of Augmented Renal Clearance : Experience in Malaysian Intensive Care Unit' has been accepted for publication by Anaesthesiology and Intensive Care, official journal of the Australian Society of Anaesthetists, Australian and New Zealand Society of Anaesthetists (2014, Volume 42, Issue 6).

All data collection, data interpretation and drafting of the paper were undertaken by the PhD candidate, assisted by Dr Shanthi Ratnam and Dr Suresh Kumar. Dr Andrew Udy and Prof Jason Roberts assisted with the data analysis and data interpretation. Prof David Paterson and Prof Jeffrey Lipman oversaw all aspects of this chapter.

The manuscript is presented a published; except figures and tables have been inserted into the text at slightly different positions. Also, the numbering of pages, figures and tables has been adjusted to fit the overall style of the Thesis. The references are found alongside the other reference of the Thesis, in the section `Bibliography'.

`Selected Critically Ill Patients at Risk of Augmented Renal Clearance : Experience in a Malaysian Intensive Care Unit'

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Summary

Augmented renal clearance (ARC) refers to increased solute elimination by the kidneys. ARC has considerable implications for altered drug concentrations. We aimed to describe the prevalence of ARC in a selected cohort of patients admitted to a Malaysian intensive care unit (ICU), and to compare measured urinary creatinine clearance with calculated Cockcroft-Gault creatinine clearances in this group. Patients with an expected ICU stay of > 24 hours plus an admission serum creatinine concentration < 120 µmol/L, were enrolled from May to July 2013. 24 hour urinary collections and serum creatinine concentrations were used to measure creatinine clearance. A total of 49 patients were included with a median age of 34 years. Most study participants were male and admitted after trauma and 39% were found to have ARC. These patients were more commonly admitted emergently (P=0.03), although no other covariates were identified as predicting ARC, likely due to the inclusion criteria and the study being underpowered. Significant imprecision was demonstrated when comparing calculated Cockcroft-Gault creatinine clearance (CG Cr_{cl}) and measured creatinine clearance (Cr_{cl}). Bias was larger in ARC patients, with CG Cr_{cl} being significantly lower than measured Cr_{cl} (P<0.01), and demonstrating poor correlation (r_s=-0.04). In conclusion, critically ill patients with 'normal' serum creatinine concentrations have varied $Cr_{cl.}$ They are at risk of ARC, which may necessitate the need for individualized drug dosing. Furthermore, significant bias and imprecision between calculated and measured creatinine clearance exists, suggesting clinicians should carefully consider which method they employ in assessing renal function.

Introduction

The kidney is essential for maintaining water and electrolyte homeostasis in the body. It acts as a filter, allowing the 'recycling' of extracellular fluid and excretion of waste products, through a combination of glomerular filtration, tubular reabsorption and tubular secretion [35]. The glomerular filtration rate (GFR) is the most widely accepted measure of renal function in both health and disease. It defines the rate at which plasma water is filtered by the kidney. In critical illness, determination of renal function informs several issues, including overall mortality and morbidity, optimization of drug dosing, and the initiation of renal replacement therapy. It is widely accepted that many critically ill patients will develop acute kidney injury (AKI) due to many factors including sepsis, use of nephrotoxic agents and obstruction to urinary flow [36]. However, perhaps just as frequently [37], certain patients will manifest elevated renal function, or augmented renal

clearance (ARC) [38]. ARC often occurs in those who do not have renal impairment and have achieved adequate resuscitation during their intensive care unit (ICU) admission [39].

Mostly for convenience, ARC is defined by an elevated creatinine clearance (Cr_{cl}), which is used as a surrogate of GFR. Values ≥ 130 ml/min/1.73m^2 have been proposed as a useful threshold, given the association with low antibiotic concentrations when using standard doses [40], and inferior clinical outcomes [41]. However, this requires further validation, as the prevalence of ARC in ICU patients varies significantly (17.9% - 51.6%), depending on the definition employed, and case-mix studied [27, 37, 42, 43]. Patients considered to be at risk of ARC include young, admitted post -trauma, and postoperative patients, with low illness severity scores [44, 45]. Systemic inflammation (SIRS)[46], coupled with peripheral vasodilatation, increased cardiac output, and greater renal blood flow[44] are thought to be important mechanisms. Traumatic brain injured (TBI) patients receiving vasopressor therapy have also been noted to have an elevated Cr_{cl} [47].

The most accurate method of identifying ARC among critically ill patients is still controversial [39]. Commonly employed parameters, such as serum creatinine concentrations, may be misleading in the critically ill; as low values may be a reflection of reduced protein stores and malnourishment, rather than altered renal function. Therefore, equations that only use serum creatinine concentrations to estimate glomerular filtration (such as The Modification of Diet in Renal Disease (MDRD) and Cockcroft-Gault (CG) Equation) have been demonstrated to be inaccurate in this setting [39, 48, 49]. Consequently, measuring a timed urinary Cr_{cl} is probably the most pragmatic and reliable method, to identify ARC in the critical care setting [42, 43].

The primary aim of this study was to describe the prevalence of ARC in a selected cohort of critically ill patients admitted to the ICU of Hospital Sungai Buloh (HSB), Malaysia, over a two-month period. We also aimed to compare the prevalence of ARC in our cohort with previous reports from other ICUs, while attempting to identify clinical characteristics that may help identify these patients in a timely fashion. In addition, we also compared CG calculated creatinine clearance (CG Cr_{cl}) with measured urinary Cr_{cl} to determine whether these two methods can be used interchangeably.

Materials and Methods

This prospective observational study was conducted in a 36 bed tertiary level, adult ICU of a 620-bed public hospital in Malaysia. Ethics approval was obtained from Malaysian Medical Research Ethics Committee (NMRR ID NMRR-12-137-11118 S4RO). The requirement for individual informed consent was waived for this study.

Patients were enrolled according to the following inclusion criteria, 1) admission to the ICU with expected length of stay >24 hours, 2) admission serum creatinine concentration <120 µmol/L), and 3) no history of chronic kidney disease (CKD), or renal replacement therapy (RRT). Patients were excluded from the study if one or more of the following criteria were met; 1) absence of invasive haemodynamic monitoring as part of routine management, 2) absence of an indwelling urinary catheter (IDC) as part of routine management and 3) "Risk" stage of AKI (> 1.5 fold increase in serum creatinine from baseline or urine output <0.5 ml/kg/hr for >6 hrs prior to enrolment [50]. Our study cohort therefore represents a selected group of ICU patients; those without AKI, requiring invasive monitoring, and with an expected length of stay >24 hrs.

Admission type was classified as "Elective" when a routine ICU bed was requested post-operatively. All other cases were treated as "Emergency admissions". Independently, all cases were also categorised as being trauma or non-trauma related. Additional demographic, therapeutic, and outcome data were collected while the patient was in the ICU, via the institutional computerized medical record system. Sequential organ failure assessment (SOFA) [51] scores were obtained from a national database (Malaysian Registry of Intensive Care).

Cr_{cl} measurement

A 24hr Cr_{cl} study was commenced within 24 hours of ICU admission. Serum creatinine concentrations obtained from routine morning blood samples were used to calculate Cr_{cl}. ARC was defined as >130 ml/min. A CG Cr_{cl} was also calculated for comparison (CG = [(140-Age) x Weight(kg)/(Serum creatinine(mmol/L)x0.814] x 0.85 if female).

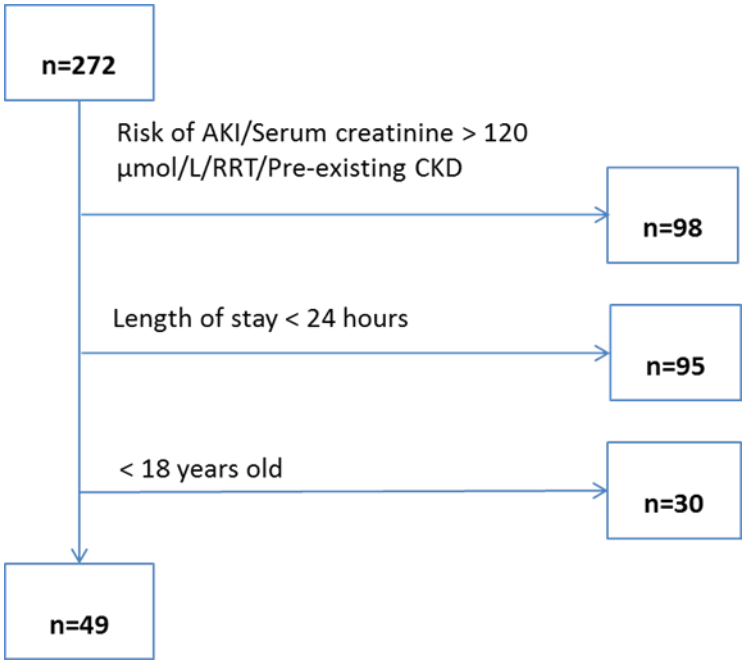
Statistical analysis

For continuous variables, data are presented as the median and interquartile range [IQR]. Qualitative variables are presented as frequencies and percentages. A Mann-Whitney U or Chi-square test was used to compare independent sub-groups, for continuous and categorical variables respectively. Comparisons between measured and estimated clearances utilised the Wilcoxon Sign Rank test, Spearman correlation (r_s), linear regression, and Bland-Altman analysis. A *P*-value < 0.05 was considered statistically significant. All statistical analyses employed SPSS v.21.0 (SPSS Inc., Chicago, IL).

Results

A total of 49 eligible patients were included in this study, from 272 patients admitted to the ICU during the study period. Figure 3.1 illustrates the common reasons for patients excluded from this study. Demographic and clinical data of the 49 patients included in this study are presented in Table 3.1. The majority of participants were young (median age 34 years), male, trauma patients. All trauma patients suffered TBI, and also underwent emergency surgery prior to ICU admission. Overall SOFA scores were found to be moderate in this cohort of patients (median [IQR] = 9 [6.0-10.0]).

Figure 3.1: Study Enrolment and Patient Exclusion



Descriptive data analysis identified that 39% of patients in this study manifested ARC. Data were later separated into two categories, patients with ARC and without ARC, as displayed in Table 3.2. No significant differences were identified between the two groups, other than in admission type (elective versus emergency admission). Although patients manifesting ARC were more frequently trauma victims, this did not reach statistical significance (P=0.06). Data were also separated into four quartiles, based on the Cr_{cl} result. The age of patients in each quartile is presented in Figure 3.2. The first and second quartiles show a wider distribution, as compared to the third and fourth quartiles.

Table 3.1 : Demographic, laboratory and illness severity data of all patients (n=49)

Demographic data	Category	n (%)	Median	IQR
Age (years)			34	24-47
Gender	Male	37(75.5)		
	Female	12(24.5)		
Weight (Kg)			61	56-67
ICU LOS (days)			9	5-12
Hospital LOS (days)			12	6-22
Trauma Admission	No	21(42.8)		
	Yes	28 (57.2)		
Admission Type	Elective	14 (28.6)		
	Emergency	35 (71.4)		
ICU outcome	Discharge	42(85.7)		
	Death	7(14.3)		
SOFA score			9	6-11
Serum creatinine (µmol/L)			67	61-74
Urinary creatinine (mmol/L)			6	4-9
Cr_{cl} (ml/min)			116	86-155
ARC Status	ARC	19(38.8)		
	Non-ARC	30(61.2)		
Vasopressor requirement (at the time of Cr_{cl} collection)	Yes	32(65.3)		
	No	17(34.7)		

Table 3.2 : Comparison of patients with and without augmented renal clearance (ARC)

Demographic data	**Category**	**ARC (n=19)**	**Non-ARC(n=30)**	***P value***
		Median (IQR)/ N (%)	**Median (IQR)/ N (%)**	
Age (years)		35 (25-45)	34 (24-50)	0.51
Gender	Male	17 (89.5)	20 (66.7)	0.10
	Female	2 (10.5)	10 (33.3)	
ICU LOS (days)		6 (3-19)	9 (6-12)	0.83
Hospital LOS (days)		17 (5-25)	11 (7-21)	0.44
SOFA Score		9 (7-10)	9 (6-11)	0.66
Trauma Admission	No	5 (26.3)	16 (53.3)	0.06
	Yes	14 (73.7)	14 (46.7)	
Admission Type	Elective	2 (10.5)	12 (40.0)	0.03
	Emergency	17 (89.5)	18 (60.0)	
ICU outcome	Discharge	16 (84.2)	26 (86.7)	1.00
	Death	3 (15.8)	4 (13.3)	
Serum creatinine (µmol/L)		66 (59-79)	66 (61-72)	0.33
Cr_{cl} (ml/min)		173 (141-223)	91 (64-112)	<0.01
Vasopressors (during Cr_{cl} collection)	Yes	11 (57.9)	21 (70.0)	0.39
	No	8 (42.1)	9 (30.0)	

Figure 3.2 : Distribution of age between quartiles which illustrate lower ages in those with higher creatinine clearance

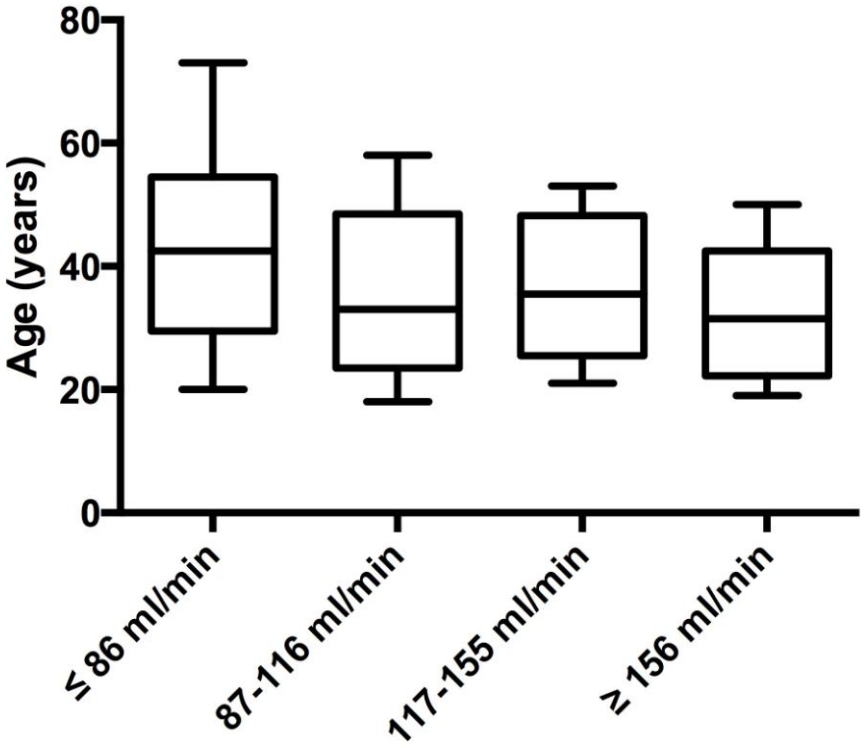


Figure 3.3 : Linear regression of measured urinary creatinine clearance (urinary Cr_{CL}) and estimated glomerular filtration rate by Cockcrof-Gault equation for a) ARC patients (n=19) and b)non-ARC patients (n=30)

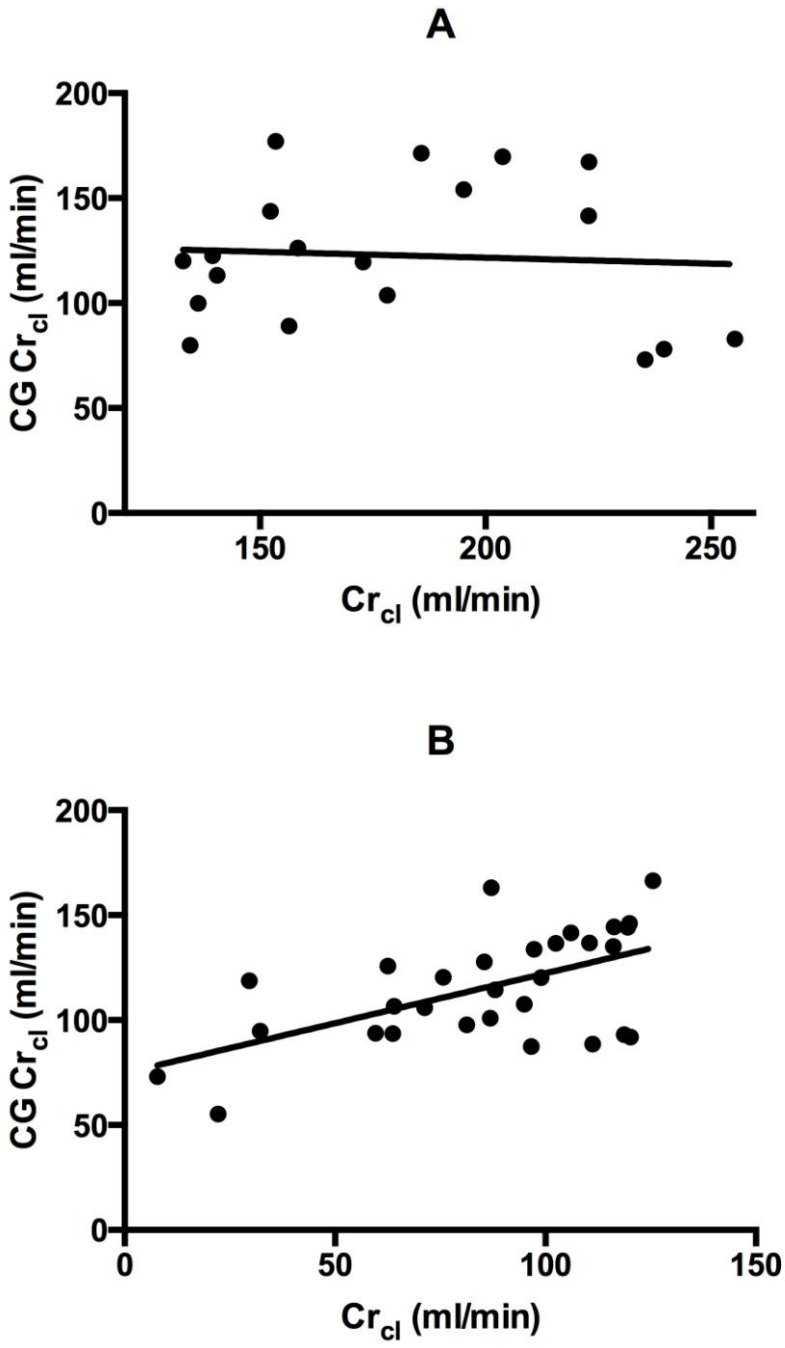


Figure 3.4 : Comparison of measured urinary creatinine clearance (urinary Cr_{CL}) and estimated glomerular filtration rate by Cockcroft-Gault (CG Cr_{CL}) equation and for a) all studied cohort (n=49) and b) ARC patients (n=19)

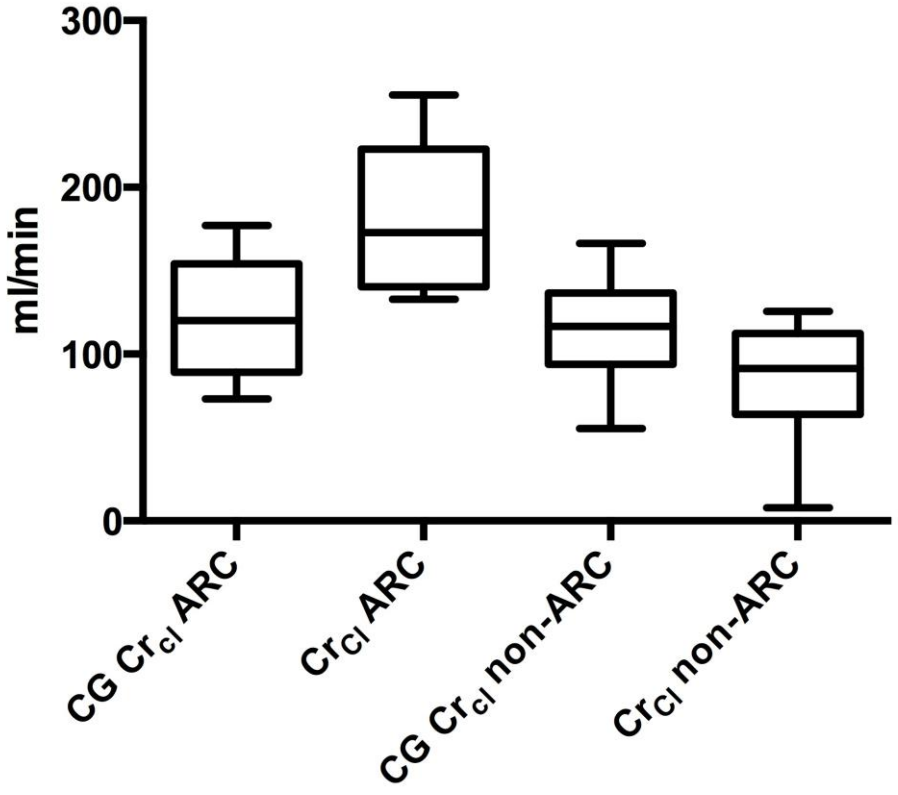


Figure 3.5: Bland-Altman plot of Cockcroft-Gault (CG Cr_{CL}) equation and measured urinary creatinine clearance (urinary Cr_{CL}) for A) ARC patients (n=19) and B) Non-ARC patients (n=30)

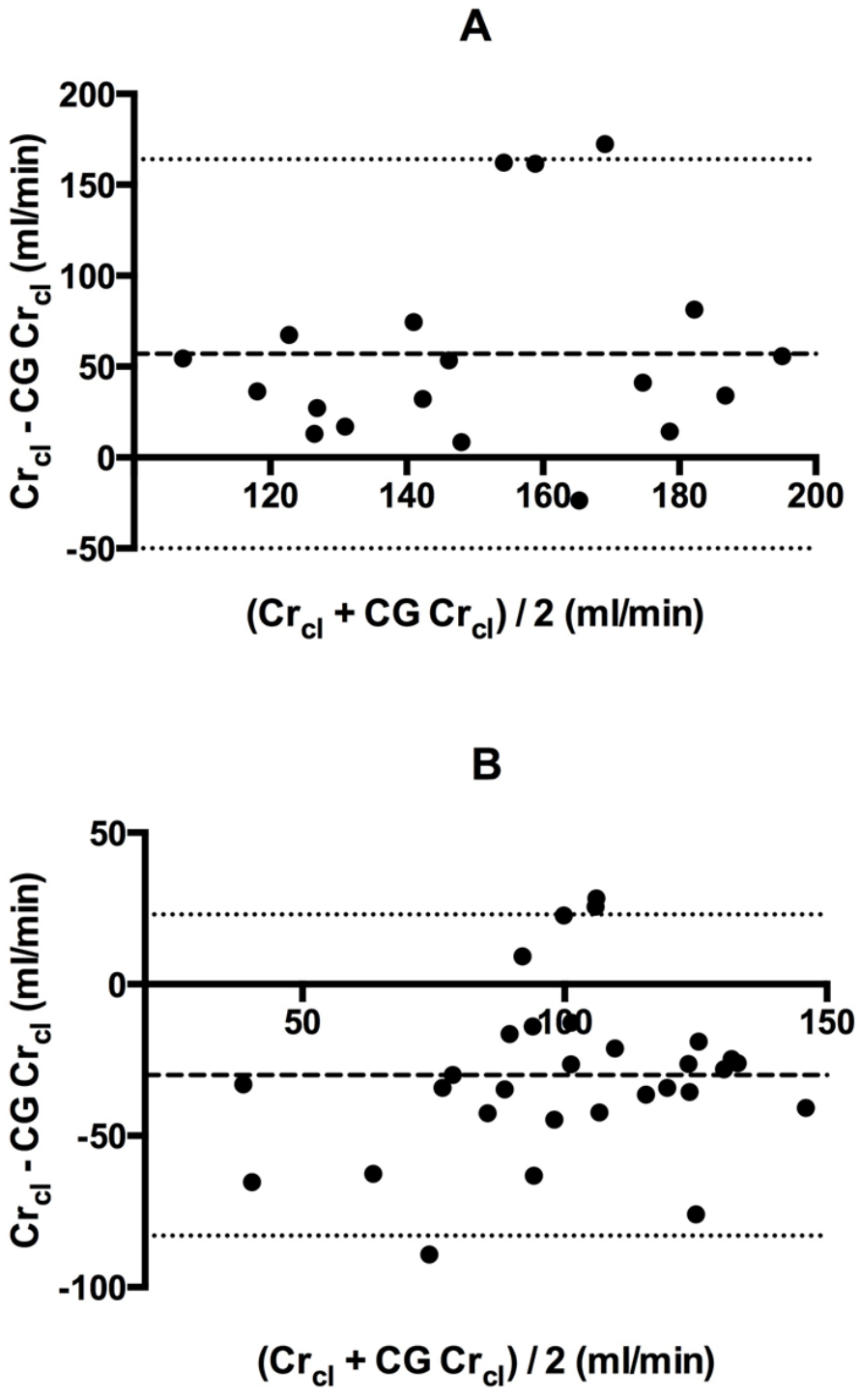


Table 3.3 : Comparison of Cockcroft-Gault (CG Cr_{CL}) equation and measured urinary creatinine clearance (urinary Cr_{CL}) for all patients vs ARC patients

	All		ARC		Non ARC	
	CG Cl_{cr}	**Cl_{cr}**	**CG Cl_{cr}**	**Cl_{cr}**	**CG Cl_{cr}**	**Cl_{cr}**
Median	120	116	120	173	117	91
IQR	94-142	86-155	89-154	141-223	94-137	64-112
r_s (*P*-value)	0.24 (0.05)		-0.04 (0.44)		0.48 (<0.01)	
Bias (SD)	3.9 (58)		57 (54)		-30 (27)	
95% LOA	-110-118		-50-164		-83-23	

Linear regression between CG Cr_{cl} and measured Cr_{cl}, for non-ARC and ARC patients, is presented in Figure 3.3. As illustrated, significantly worse correlation was observed in ARC patients (r_s=-0.04, *P*=0.44) as compared to non-ARC patients (r_s=0.48, *P*<0.01). Furthermore, in those patients with ARC, measured Cr_{cl} values were significantly higher (173 [141-223] ml/min) compared to CG Cr_{cl} (120 [89-154] ml/min), *P*<0.01) as illustrated in Figure 3.4. Figure 3.5 compares the different methods using Bland-Altman analysis. For all patients, it yielded an average bias of 3.9 ml/min (58 ml/min) with broad limits of agreement, -110 ml/min to 118 ml/min. A larger bias was observed in the ARC sub-group; 57 ml/min (54 ml/min), with similar limits of agreement; -50 ml/min to 164 ml/min. All data are summarized in Table 3.

Discussion

To our knowledge, this is the first study examining the epidemiology of ARC in a selected cohort of Malaysian critical care patients. Although 39% of study subjects manifested ARC on admission, the overall prevalence of this phenomenon in the wider Malaysian critical care population is likely to be lower, as only 18% of all admissions to the ICU were included over the study period (Figure 3.1). ARC was more likely to occur in emergent ICU admissions (*P*=0.03) and possibly also in trauma patients (*P*=0.06). Perhaps most significantly, poor agreement was noted between CG Cr_{cl} and measured Cr_{cl} in patients manifesting ARC, suggesting that clinicians should be cautious when using mathematical estimates of renal function in this setting.

The reported prevalence of ARC from other studies range from 17.9% to 41.1% [27, 42, 43], albeit with varying definitions employed. Unlike this prior work; no significant differences were noted in demographic data between ARC and non-ARC patients in the

current study. Specifically, previous authors have observed ARC as a frequent finding in younger, male patients predominantly [27, 39, 45]. In our study cohort, this demographic was over represented, such that age and gender were not identified as discriminatory variables. Although this is likely a reflection of our inclusion criteria, and subsequent small sample size, significant differences in critical care case mix in Malaysia may have also contributed. Of note, stratification of data into quartiles did illustrate a trend towards younger age in those with higher Cr_{cl} (Figure 3.2).

The association between ARC and emergent admission to the ICU, often in the setting of trauma, represents an observation consistent with previous data [36, 45, 52, 53]. It is also interesting to note that all trauma victims in our study suffered TBI, a previously reported risk factor for ARC [36, 47]. No significant differences were found for length of stay and ICU outcome between groups, although this study is significantly underpowered for such an observation.

An additional major finding from this work is the poor relationship between CG Cr_{cl} and measured urinary Cr_{cl} in ARC patients, as illustrated in Figures 3.3 and 3.4. Bland-Altman analysis confirms significant bias and imprecision in this sub-group (Figure 3.5). A similar positive bias (between 17 ml/min to 39 ml/min), has been reported by others in this setting [42, 48, 54], while some have identified a negative bias [39, 43]. Importantly, the bias becomes significantly larger in patients exhibiting ARC, such that dose adjustment of renally excreted drugs is unlikely to be accurate on the basis of CG $Cr_{cl.}$ In this respect, it has been suggested that a measured Cr_{cl} represents a more accurate surrogate of GFR in the ICU setting [43].

Although our study did not attempt to quantify the clinical implications of ARC, the observation that approximately two out of five participants in this study manifested this phenomenon, represents an important consideration in drug dose selection, particularly antibiotics. While decreasing renal function is a common trigger for dose reduction of renally cleared agents, ARC should also trigger increased dosing to avoid sub-therapeutic concentrations [55]. Failure to consider this in dose selection may increase the likelihood of treatment failure [56], or promote colonisation and infection by multi-drug resistant organisms. In this context, several studies have demonstrated that beta-lactam

antibiotics are more rapidly cleared in septic patients without organ dysfunction, resulting in sub-therapeutic levels, and associated poor clinical outcomes [37, 40, 57, 58].
As a consequence of sub-therapeutic level, a selection pressure may occur leading to emergence of resistance pathogens as suggested by pre-clinical data [59]. Indeed, this might be one of the reasons for why there is a high prevalence of resistant pathogens in the intensive care unit. Available evidence of the relationship between antibiotic resistance and under-dosing been extensively reviewed by Roberts et al [29]. The main parameter related to this emerging area of research, include :

- Mutant Prevention Concentrations (MPC); drug concentrations require to prevent emergence of mutations;
- Mutant Selection Window (MSW); selection of resistant mutants if antibiotic concentrations falls between MIC and MPC

Since ARC is commonly seen among ICU patients, the likelihood of standard antibiotic doses not exceeding the MPC and remaining within the MSW is high. This result in a mutant selection pressure; leading to the emergence of antibiotic resistant pathogens. It is highly plausible that underdosing of antibiotics in patients with ARC could be one of the possible causes of high prevalence of resistant pathogen in these settings.

Applying the results of our study suggests that significantly altered dosing may be required for patients with ARC. Our data describing the range of Cr_{cl} that can be observed in the critically ill serves as an important reminder that a simple 'one dose fits all' approach for renally cleared drugs in patients without renal dysfunction; is likely to be grossly flawed. Such variation in Cr_{cl} between critically ill patients supports an individualized drug dosing approach that may not be necessary in a ward environment. To this end, therapeutic drug monitoring has been strongly advocated as a tool for optimised dosing of antibiotics in critically ill patients [27].

We wish to acknowledge the following limitations of this work. Only 18% of patients admitted to the ICU over the study period were enrolled (Figure 1). Rapid clinical turnover (resulting in a shorter expected length of stay), and a high percentage of patients with co-morbid disease pre-disposing to the development of AKI appeared to be the key factors contributing to the low rate of patient enrolment. As such, a post-hoc power analysis

suggests that this study has only 80% power to detect a difference in incidence in risk factors between 20% and 60% or 40% and 80% (with a higher prevalence in the ARC group), if we assume $P<0.05$ is statistically significant. In addition, even though a measured Cr_{cl} is considered a reliable method of assessing renal function, it is not a gold standard measurement of GFR[28]. Furthermore, we have only examined a single Cr_{cl} per patient, and as such, we cannot confirm how common ARC would be in our patients in the whole ICU stay.

In conclusion, in a selected population of critically ill patients without AKI requiring invasive monitoring, admitted to a Malaysian intensive care unit with an expected length of stay greater than 24 hrs, ARC was identified in a significant proportion of patients. This represents an important finding, as ARC is a key predictor of sub-therapeutic drug concentrations, such that these patients are at risk of inadequate drug exposure.

Patients admitted emergently appeared to be at particular risk, although unlike prior studies, there was no significant difference in age and gender between ARC and non-ARC patients. Significant bias and imprecision was also noted between CG Cr_{cl} and measured urinary $Cr_{cl,}$ in this setting, suggesting that clinicians should be cautious in modifying dosing on the basis of mathematical estimates of creatinine clearance from plasma creatinine concentrations alone.

Acknowledgement

We would also like to thank all the personnel of Department of Anaesthesiology/Critical Care and Clinical Research Centre of Hospital Sungai Buloh, Malaysia for their kind co-operation and assistance throughout the study period. Jason Roberts is funded in part by a National Health and Medical Research Council of Australia Research Fellowship [NHMRC APP1048652].

Conflict of Interest

Nil

3.3 Conclusion

This chapter has described the risk of augmented renal clearance amongst critically ill patients in a Malaysian intensive care unit. Nearly half of the studied cohort had ARC, demonstrating that ARC is a common phenomenon in critically ill patients. The presence of ARC appeared to be more prominent in post-severe trauma and those that had undergone emergency surgery and so these could both be considered predictors of the presence of ARC. Significant bias resulted from comparison between measured and calculated creatinine clearance in patients with ARC. This result suggests that measured creatinine clearance should be considered for routine use in the ICU setting to detect the presence of ARC. The implications of this study in context of previous PK studies demonstrating the relationship between antimicrobial clearance and creatinine clearance is that many ICU patients are likely to require non-standard doses to ensure achievement of target concentrations. Without dose modification, a poor outcome from treatment is likely. This effect has been reported recently in the discontinuation of clinical trials due to inferior outcomes for younger patients with higher renal clearances for whom dose modification was not carried out. Identifying patients at risk of ARC should be considered important for improving antimicrobial use in critically ill patients.

4. Chapter 4 : Pharmacokinetics of Beta-Lactam Antibiotics in Intra-Abdominal Disease in Critically Ill Patients

4.1 Synopsis

The aim of this chapter is to explore available PK studies of beta lactam antimicrobials in intra-abdominal disease. The consequent effect on antimicrobial PK and dose adjustment will be discussed in this review to serve as a guide on therapeutic prescription to the physicians. From this review, possible antimicrobial clearance through surgical drains suggests a need for further studies on this topic. Therefore, this chapter will also include the prospective study of the PK of meropenem and piperacillin in critically ill patients with surgical drains. The methods used in analysing meropenem and piperacillin in PK samples will be incorporated in the published manuscript of findings of this study. Additionally, the appropriateness of standard doses in achieving PK/PD targets in critically ill patients will be evaluated in this chapter.

4.2 Published manuscript entitled `Pharmacokinetics of Beta-Lactam Antibiotics in Intra-Abdominal Disease : A Structured Review'

The manuscript entitled, "Pharmacokinetics of Beta-Lactam Antibiotics in Intra-Abdominal Disease: A Structured Review" has been accepted for publication by Surgical Infections (2012; 13(1) 9-17).

The co-authors contributed to the manuscript as follows: All literature reviews, data collection and interpretation was performed by the PhD candidate, Syamhanin Adnan with assistance from Prof Jason Roberts under the supervision of Prof David Paterson and Prof Jeffrey Lipman. The PhD candidate, Syamhanin Adnan, took the leading role in manuscript preparation and writing. Other co-authors, Dr Michael Rudd, Dr Suresh Kumar and Janice Li had also assisted with the draft.

The manuscript is presented as published, except figures and tables have been inserted into the text at slightly different positions. Also, the numbering of pages, figures and tables has been adjusted to fit the overall style of the Thesis. The references could be found in the section of `Bilibiography'.

"Pharmacokinetics of Beta-Lactam Antibiotics in Patients with Intra-abdominal Disease: A Structured Review"

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Keywords: antibiotics, critically ill, intra-abdominal infection, pharmacokinetics, pharmacodynamic

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Abstract

Background and Purpose : The objective of this structured review is to critically analyze the findings of pharmacokinetic studies of antibiotics in patients with intra-abdominal disease, that is, intra-abdominal infection (IAI) or previous abdominal surgery and determine the requirements for dosage modification in this patient population.

Methods : Data were identified by structured review of PubMed from February 1983 to February 2011. All 14 articles reviewed described antibiotic pharmacokinetics in patients with intra-abdominal disease.

Results : Antibiotic classes included carbapenems, penicillins, cephalosporins and monobactams. Possible physiological changes that can occur in these patients include development of abscesses, perforation, or ischemia of the bowel as well as intra-abdominal hypertension. These disorders may cause changes in antibiotic pharmacokinetics including increased volume of distribution and faster drug clearance, both resulting in lower antibiotic concentrations. High inter-individual pharmacokinetic variability was common to each of the studies.

Conclusion: Most of the available data demonstrates that drug volume of distribution can be increased significantly in the presence of intra-abdominal disease. Drug clearance is likely to vary in line with renal or hepatic function. Thus, dose optimization is important to prevent development of antibiotic resistance or therapeutic failure. However, further research is necessary to determine the clinical outcome of individualized dosing on the basis of pharmacokinetic/pharmacodynamic studies.

Introduction

Managing sepsis in critically ill patients remains a great challenge for clinicians. The incidence of sepsis in such patients has been reported as approximately 10% with a population incidence of 1/ 1000 [60]. Complicated intra-abdominal infection (IAI) is a frequent cause of sepsis and is the second most frequent cause of infectious mortality in the intensive care unit (ICU) [61]. The treatment of complicated IAI consists of both source control and antibiotic therapy, as outlined by both the Surgical Infection Society and the Infectious Disease Society of America [62]. This guideline recommends the use of antibiotics at optimal doses so as to maximize efficacy, minimize toxicity and reduce antibiotic resistance. Patients with complicated IAI are also at risk of infections by resistant

pathogens [63] and therefore the importance of appropriate selection, timing of administration and dose adjustment of the antibiotic is paramount [64, 65]. Multiple factors may contribute to the selection of a resistant pathogen, but achieving optimal drug exposure is likely to be a key factor for minimizing its occurrence [66].

Determination of optimal antibiotic doses based on the basis of pharmacokinetic/ pharmacodynamic (PK/PD) principles correlates the antibiotic concentration in the body (i.e. PK) with its ability to kill, or inhibit growth, of the pathogen (i.e. PD) [67]. Optimal antibiotic doses will result in therapeutic concentrations at the focus of infection to ensure organism eradication. This approach is likely to improve clinical outcomes, especially in critically ill patients, who are at high risk of developing severe infections and dying [7, 68, 69]. However, penetration of the antibiotic to the site of infection can be hindered by various factors, including the patient's underlying co-morbidities, immune function status, renal and liver function status and concomitant drug use [70]. These factors can be more profound in critically ill patients due to pathophysiological changes, as described in detail in a review article by Roberts and Lipman [32]. Numerous studies have been conducted to describe the pharmacokinetics of antibiotics in IAI, although we are unaware of any structured reviews on this topic. The aim of this structured review is to critically analyze the PK studies on beta-lactam antibiotics used in patients with intra-abdominal disease including patients with IAI and after abdominal surgery.

Search Strategy and Selection Criteria

Data for this review were identified by structured review of PubMed (February 1983 to February 2011). Keywords used included `pharmacokinetic', `pharmacodynamic' `beta-lactam antibiotics', `critically ill', `intra-abdominal' and 'infection'. Twenty-four papers were identified, ten articles were reviews and were excluded because they were guideline documents only, discussing antibiotic choice and general management of IAI. Articles discussing PK studies of patients undergoing peritoneal dialysis were excluded, as this group is likely to have significantly different values. All papers reviewed were written in the English language and described antibiotic pharmacokinetics in patients with intra-abdominal disease. The studies used for this systematic review are summarized in Table 3.1 and are listed according to antibiotic class. This review also includes a paper by Wittman and Schassan [71] which studied eight beta-lactam antibiotics in patients post-abdominal surgery although this paper is not listed in Table 3.1 because it only reports

matching serum and peritoneal fluid concentrations but does not describe relevant PK parameters. The PK data available from healthy volunteers were also included for each antibiotic for comparison.

General Concept

Antibiotics can be divided into three major classes on the basis of their bacterial-killing properties [72]. Concentration dependent antibiotics (e.g. aminoglycosides) exhibit bacterial killing that correlates with the peak concentration during a dosing interval (C_{max}). Achieving a higher ratio of C_{max} to the minimum inhibitory concentration (MIC) of the infecting pathogen (C_{max}/MIC ratio) has been advocated to maximize killing. Administration of higher doses should result in a higher ratio. This may be clinically useful when a higher MIC is present [33]. On the contrary, the killing of bacteria by time dependent antibiotics (e.g. beta-lactams) will depend largely on the time that the antibiotic concentration is maintained above the MIC, preferably at least 40 – 60% of the dosing interval. Some data on the killing of *Pseudomonas spp* suggest greater activity [34] when concentrations are maintained at 4–5×MIC [72]. The third group of antibiotic has PK/PD properties consistent with both concentration and time dependent antibiotics, and bacterial killing is associated with the ratio of the area under the concentration time curve (AUC), to MIC (AUC/MIC; e.g. fluoroquinolones and glycopeptides). The strong relationship between PK and PK/PD means that a change in PK may affect the treatment outcome. It follows that an understanding of the pathophysiology of IAI and its effect on PK is necessary to determine the need for altered dosing.

Relation between physiological alterations in patients with intra-abdominal disease and pharmacokinetics

Intra-abdominal infections (IAIs) are categorized as either complicated or uncomplicated, depending on the extent of infection [73]. From clinical point of view, the infectious process involved in complicated IAI extends beyond the organ that is the source of infection and causes localised or diffuse peritonitis, depending on the ability of the host to contain the process within a part of the abdominal cavity [73]. Pathologic changes that could occur during IAI include the development of abscesses, perforation and ischemia of the bowel. These may lead to intra-abdominal hypertension as well as significant physiologic changes such as hypotension and tachycardia. IAI could also develop as a complication of an invasive procedure (i.e. abdominal surgery). Varied IAI rates have been reported, but in the presence of intra-abdominal surgical packing, infection rates can be as high as 70% [74]. Similar physiologic changes could also occur in these patients secondary to higher level of anti-diuretic hormone (ADH) as response to the trauma of surgery. Higher concentrations of ADH eventually lead to fluid retention in the intra-abdominal cavity [75].

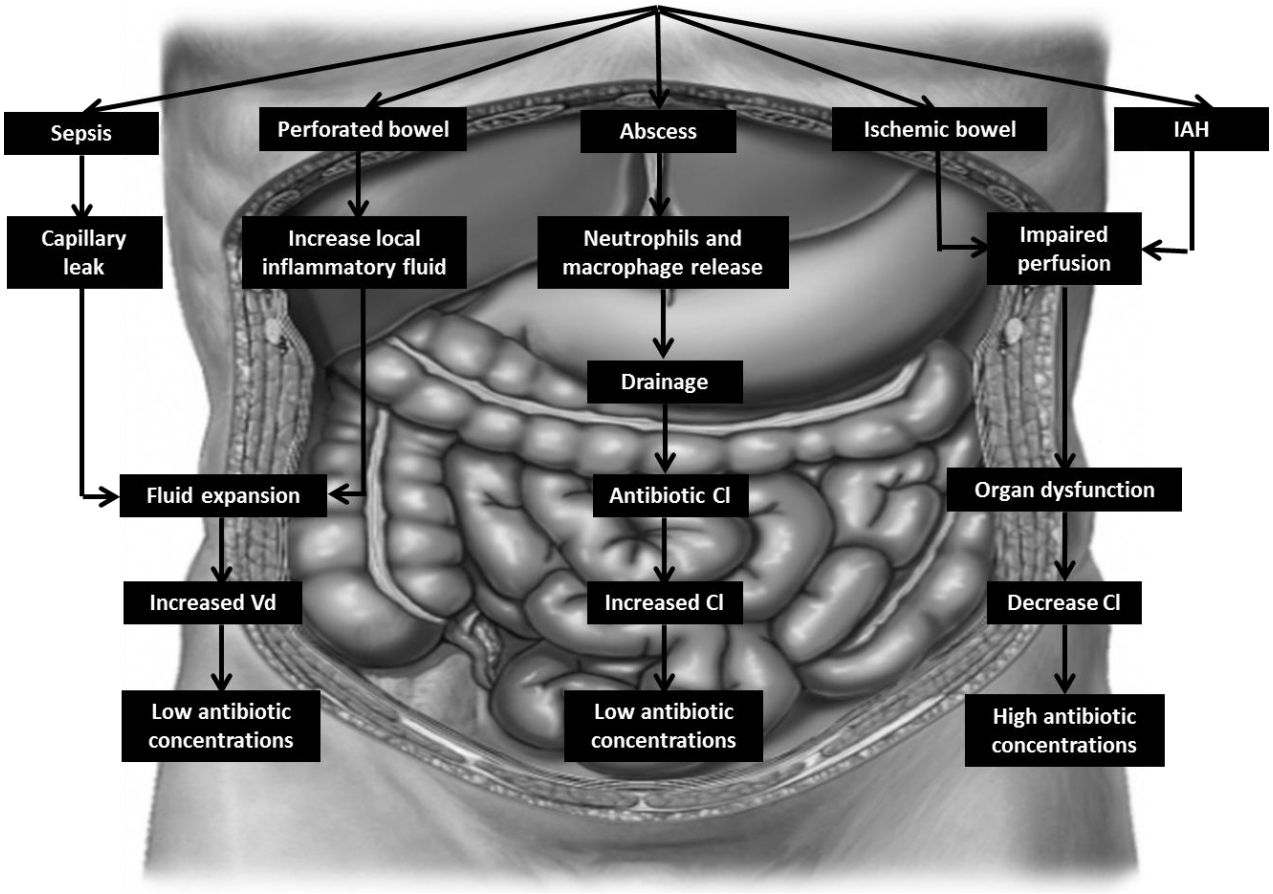
Pharmacokinetics - Changes in distribution

Fluid expansion

Antibiotic transfer from intravascular to interstitial space is diffusion driven; either through pores or transcellularly, depending on the chemical properties of the drug. Small polar hydrophilic molecules will move through fenestrated capillary pores and more lipophilic substances pass directly through the endothelial wall [76]. For hydrophilic antibiotics (e.g. aminoglycosides, glycopeptides and beta-lactams), distribution is typically limited to the plasma and the interstitial fluid of tissues due to limited extravascular permeability, and the concentrations are in equilibrium during steady state. Thus, any changes in interstitial fluid will alter the distribution of hydrophilic antibiotics. This is commonly seen when systemic pathologies such as the systemic inflammatory response syndrome (SIRS) or sepsis (defined as SIRS in the presence of infection) are present. In IAI, local increases in fluid volumes within the peritoneal cavity will occur secondary to fluid shifts from the intravascular space to the interstitial space as a result of capillary leak associate with sepsis [13]; fluid therapy for restoration of intravascular volume [62] and inflammatory fluid collections can lead to increased fluid volumes within the peritoneal cavity. Furthermore, gut pathology such as ileus can develop from the development of increased intra-luminal

pressure, . This local inflammation contributes to systemic `third spacing' effects. Each of these factors will effectively increase the volume of distribution (V_d) of a hydrophilic antibiotic, leading to a dilutional effect, as illustrated schematically in Figure 4.1. For lipophilic antibiotics (e.g. fluoroquinolones), which have a large V_d because of their intracellular penetration, fluid expansion is unlikely to cause significant changes in plasma or interstitial fluid concentrations [77].

Figure 4. 1 : The effect of IAI on antibiotic concentrations in plasma and ISF fluid of tissues



Impaired penetration - abdominal abscess

Another challenge in the management of IAIs is the formation of an abscess. This may result from an immune defence mechanism causing the release of macrophages and neutrophils into the peritoneal cavity, as well as activation of the coagulation and complement cascades. Bacteria remain viable, as the abscess environment is not conducive to antibiotic cavity. Antibiotic penetration is impaired as a result of limited perfusion in the presence of fibrin clots and the abscess wall [22]. The acidic environment of the abscess [78] may cause therapeutic failure of aminoglycosides, with in vitro studies demonstrating a reduced bactericidal effect. In vitro studies have shown that at pH 5.5, amikacin and netilmicin had practically no bactericidal effect on *P. aeruginosa*[79].

Even though an abscess may be managed with antibiotic therapy alone, it has been reported that patients with both larger intra-abdominal abscesses (>6.5cm) and pyrexia, have an increased likelihood of antibiotic failure and therefore drainage, or other source control is required [80].

Pharmacokinetics - Changes in clearance

Organ dysfunction

As discussed above, patients with a large intra-abdominal fluid volume are at risk of intra-abdominal hypertension (IAH) [81]. The pressure within the abdomen is normally atmospheric (<7 mm Hg) or sub-atmospheric (i.e., negative), but the presence of some form of intra-abdominal pathology may increase the pressure in the abdominal compartment. Hypoperfusion of the gastrointestinal tract is reported at or above an intra-abdominal pressure (IAP) of 12 mmHg [82]. IAH reduces abdominal wall blood flow because of the higher arteriolar and venous resistance, which will lead to intestinal mucosal hypoperfusion and ischemia. Persistent IAH could later result in development of multiple organ dysfunction; for example, it could cause hepatic and gastrointestinal effects by impairing lymphatic flow and produce renal dysfunction as a result of decreased glomerular filtration rate [83]. Thus, IAH can lead to development of multiple organ dysfunction syndrome (MODS) and it has been reported that the incidence of MODS is as high as 90% as compared to 31.5% in patients without acute compartment syndrome secondary to IAH [81]. In the presence of MODS, antibiotic doses may need to be adjusted

to minimize the likelihood of drug accumulation and toxicity if the affected organ is responsible for antibiotic elimination [17, 84].

Clearance by abdominal surgical drains

It has been suggested that the presence of indwelling surgical drains are a frequently overlooked cause of antibiotic loss in critically ill patients [7, 85]. However, most studies documenting presence of antibiotics in drain fluid were for the main purpose of understanding drug penetration to specific sites. Thus, these studies provide little information about the extent of drug loss from these drains or the PK characteristics of drugs in patients with indwelling surgical drains or if the PK differs from patients without drains.

Specific antibiotic classes

The studies are grouped together according to antibiotic classes and are discussed below. Data were available from patients with IAI and also from patients who had undergone abdominal surgery; where possible, the results are compared (see Table 4.1).

Table 4.1 : Studies describing pharmacokinetic of beta-lactam antibiotics in patients with intra-abdominal disease.

Antibiotic class	Reference	Patient population	Samples	CL (L/hour)	V_d (L)	AUC plasma (mg.h/L)	AUC PF (mg.h/L)	AUC ratio
Carbapenem								
Meropenem	Bedikian et al (1994)[86]	Intra-abdominal infection (n=12)	Plasma samples at steady state	18. 9 ± 4.3	V_{ss}: 26.7 ± 6.9	57.5 ± 20.1	Not available	Not available
	Karjagin et al (2008)[87]	Severe peritonitis and septic shock (n=6)	Plasma and peritoneal fluid. AUC from simulate profile of 3g/day	6.7 ± 4.2	V_{ss}: 23.8 ± 4.9	625	491	0.78
	Nilsson-Ehle et al(1991)[88]	Healthy volunteers (n=8)	Plasma samples after single dose	11.3 ± 1.7	V_{ss}: 12.5 ± 1.5	77.5 ± 11.5	Not available	Not available
Imipenem	Ikawa et al (2008)[a] [89]	Patients post laparatomy (n=10)	Plasma and peritoneal fluid after single dose	Total :46.8	Total:14.3	59.0 ± 15.7	41.01 ± 6.13	0.82 ± 0.06
	Nilsson-Ehle et al(1991)[88]	Healthy volunteers (n=8)	Plasma samples after single dose	11.0 ± 1.5	V_{ss}: 14.4 ± 1.2	94.4 ± 12.0	Not available	Not available
Doripenem	Ikawa et al (2007)[90]	Patients post abdominal surgery (n=10)	Plasma samples after single dose	8.6 ± 1.1	V_{ss}: 8.56 ± 1.14	59.3 ± 7.2	49.3 ± 6.5	0.4 ± 0.3
	Cirillo et al (2009)[91]	Healthy volunteers (n=48)	Plasma samples after single dose	13.0 – 14.6	V_{ss}:14.4 – 18.0	35.6 – 78.8	Not available	Not available

Antibiotic class	Reference	Patient population	Samples	CL (L/hour)	V_d (L)	AUC plasma (mg.h/L)	AUC PF (mg.h/L)	AUC ratio
Biapenem	Ikawa et al (2008)[92]	19 patients post abdominal surgery	Plasma and peritoneal fluid after single dose	8.11 ± 5.7[b]	Total:16.4	Not available	Not available	Not available
Ertapenem[c]	Arrigucci et al, (2009)[93]	Patients post abdominal surgery (n=21)	Plasma and peritoneal samples taken at different times from three different groups	Not available	Not available	Not available	Not available	Not available
	Majumdar et al (2002)[94]	Healthy volunteers (n=16)	Plasma samples after single dose of 1gm	1.7 ± 0.2	V_{ss} :8.2± 1.5	572.1 ± 68.6	Not available	Not available
Amino-penicilln								
Piperacillin/ tazobactam	Li et al (2005)[95]	Patients with complicated intra-abdominal infection (n=56)	26 in continuous infusion (CI) 30 in intermittent infusion (IT)	CI 15.9 ±5.7 IT 13.7±4.3	CI : 22.2±4.5 IT 22.4±6.2	Not available	Not available	Not available
	Sorgel and Kinzig (1994)[96]	Healthy volunteers (n=33)	Plasma samples after single dose	13.1 – 16.7	V_{ss} :11.9-18.8	121 – 294	Not available	Not available

Antibiotic class	Reference	Patient population	Samples	CL (L/hour)	V_d (L)	AUC plasma (mg.h/L)	AUC PF (mg.h/L)	AUC ratio
Cephalosporins								
Ceftazidime	Bujik et al (2002)[97]	Patients with severe intra-abdominal infections (n=13). 9 received continuous infusion (CI) and 4 received intermittent bolus (IB)	Plasma and peritoneal exudates	CI : 4.1(Day 2) 4.2 (Day 4) IB 5.1 (Day 2) 4.0 (Day 4)	IB 21 (Day2) 14 (Day 4)	CI 1131(Day 2) 1098(Day 4) IB : 1064 (Day 2) 1166 (Day 4)	CI 522 (Day 2) 637 (Day 4) IB : 316 (Day 2) 346 (Day 4)	CI : 0.56 (Day 2) 0.35 (Day 4) IB : 0.64 (Day 2) 0.35 (Day 4)
	Heim-Duthoy et al (1988) [98]	Patient post abdominal surgery (n=11)	Plasma samples on D2 of ceftazidime	7.0 ± 2.8	V_{ss} :21.0 ± 7.0	340.4 ± 277.0	Not available	Not available
	Sommers DK (1983) [99]	Healthy volunteers (n=24)	Plasma samples after single dose	5.8 – 6.9	11.0 – 13.3	153 - 178	Not available	Not available
Cefotiam	Ikawa et al[a] (2008)[100]	Patient post abdominal surgery (n= 8)	Plasma and peritoneal fluid after single dose	Total :16.5	Total :11.6	101.1 ± 26.7	86.5 ± 22.6	0.9 ± 0.2
Cefozopran	Ikawa et al[a] (2007)[101]	Post abdominal surgery patient (n=10)	Plasma and peritoneal samples obtained after first dose.	Total :20.5	Total :15.1	189.9 ± 32.0	174.1 ± 36.0	0.9 ± 0.1

[b]This study reports CL as 0.036 x creatinine clearance (CL_{cr}) + 4.88 with Cl_{cr} reported as 89.9 ± 22.3.[c]This study only reports C_{max} or random concentration and cannot be contrasted against the other studies. The authors reported tissue: plasma ratio of 46.7 ± 25.3 at 1h ± 15 min, 56.4 ± 24.1 at 2h± 15min and 83.1± 46.6 at 3h ± 15 min. All the PK studies identified report PK parameter from either non-compartmental or compartmental analysis. All PK data are presented with standardized unit of measurement and whenever necessary, conversion was carried out. For PK data reported by body weight, body weight of 70 kg was used in the re-calculating.

Carbapenems

Meropenem is the only carbapenem that has had its PK studied in patients with IAI, with data on intra-abdominal penetration of other carbapenems coming from abdominal surgery patients. Bedikian et al [86] compared the pharmacokinetics of meropenem in patients with IAI and healthy volunteers [88], and observed that the V_d in these patients had doubled, the AUC was 25% lower, and the clearance (CL) twice as high as in healthy volunteers. However, since patients with organ impairment, life-threatening infection, septic shock and immunodeficiency and long term therapy were excluded, this study may not reflect the likely spectrum of critically ill patients that may be encountered. The V_d is likely to remain augmented, although CL values may be significantly lower in some patients. Unfortunately, this study did not confirm antibiotic penetration into the peritoneal fluid (the site of infection). Karjagin et al [87] also studied meropenem but described a reduced CL with a similar V_d to that reported by Bedikian et al. Further PD analysis using PK simulations, suggest that a dose of 3g/day produces an AUC ratio of peritoneal fluid to plasma of 0.78 and achieves concentrations that exceed an MIC of 4 µg/mL, for both plasma and peritoneal fluid for 87% of the dosing interval. Since the majority of infections are localized in extracellular fluid [76], doses that can achieve a high ratio of peritoneal fluid to plasma ratio are most likely to attain therapeutic antibiotic concentrations in treatment of IAI.

Other studies on carbapenems which include imipenem, doripenem, biapenem and ertapenem were performed in patients who had undergone abdominal surgery. Imipenem-cilastin [89] showed peritoneal : plasma AUC similar to that of doripenem [90], 0.82 and 0.84 respectively. No ratio is available for biapenem[92] and ertapenem [93] (see Table 1).

The PK data from compartmental analysis for carbapenems which have been studied have shown that imipenem had the highest total CL compared with doripenem and biapenem. Both imipenem-cilastin and doripenem have higher total CLs in these patients than in healthy volunteers [102, 103]. Compartmental analysis also revealed elevated distribution clearances from the central to the peritoneal compartment which may indicate good peritoneal penetration or high fluid volumes in the peritoneal compartment. Available imipenem-cilastin data suggest that the minimum dosage required to attain at least 80% probability of bactericidal activity at MICs of 1, 2, 4, 8 and 16 µg/mL are 500mg IV q12h, 1000mg q12h, 1000mg q 8h and 1000mg q6h, respectively [89]. For doripenem, the

percentage of drug concentration that exceeds the MIC ($fT>_{MIC}$) is slightly greater in peritoneal exudate than in serum [90], however, these differences are unlikely to mandate different dosing. Confounding the interpretation of the ertapenem PK study is the sampling times, with only random serum concentrations reported [93]. As such comparison with other studies is not possible. The great extent of protein binding of ertapenem (~95%), would also necessitate the measurement of unbound (pharmacologically active) concentrations, which was not done [93].

Penicillins

Piperacillin and mezlocillin are the only two penicillins that have been studied. The first study on piperacillin was as part of a randomized clinical trial comparing continuous infusion (CI) and intermittent infusion (II) of piperacillin (co-formulated with tazobactam) in hospitalized patients with complicated IAI [95]. No difference in relevant PK parameters was found between the two infusion methods. When compared with healthy volunteer data [104], the V_d was two times greater, with CL largely unchanged. However, no samples were obtained from the peritoneal fluid and therefore, this study did not confirm antibiotic penetration into that fluid. In another study by Wittman and Schassan [71] in post abdominal surgery patients, serial serum and peritoneal fluid concentrations were reported at different time intervals for eight beta lactams. No C_{max} and AUC data were provided. However, the peritoneal fluid concentrations were reported at 2 h and the ratio of the peritoneal fluid: plasma concentration 2 h after administration was 0.83 and 0.74 for piperacillin and mezlocilin, respectively.

Cephalosporins

Five cephalosporin antibiotics have been studied, although only two studies were conducted in patients with IAI [97, 105]. The first study [97] was a non-randomized study and compared the PK of CI with II administration of ceftazidime. The differences in PK parameters of V_d and CL within the two groups were not statistically significant , but the AUC value in peritoneal exudates was higher in the CI group, and this difference was statistically significant on Day 2. The peritoneal exudate: plasma AUC ratio was higher in CI group (56% - 64%) than in the II group (33-35%) but this difference was not statistically significant. Other data have shown that the AUC ratio of peritoneal exudates to plasma decreases over time (~0.6 on Day 2 and ~0.3 day 4). The low ratio may have resulted from

dilution of the peritoneal fluid as a result of continuous lavage, which resulted in rapid peritoneal ceftazidime clearance. However, drainage clearance was reported to be minor at 1% on Day 2 and 0.6% on Day 5, whereas the total ceftazidime CL was almost half than that reported in healthy volunteers [99] (see Table 1).

A study with cefoperazone (co-formulated with sulbactam) [105] described a half life 3.9 times that observed in healthy volunteers [106] because of a 50% larger V_d and a 43% lower CL. However, analysis of unbound concentrations was not undertaken in this study, which would have been significantly more relevant, as cefoperazone is highly protein bound (95%). Further, no samples were obtained from peritoneal fluid, which would have been indicative of antibiotic effects at the site of infection. Other studies [71, 100, 101, 107] have been performed in patients after abdominal surgery. In these studies, the AUC ratio of peritoneal exudate: plasma was ~90% for each of cefepime, cefozopran and cefotiam respectively (cefozopran and cefotiam are only available at certain countries, e.g. China and Japan). Very high CL values of these drugs were observed.

For cefepime, 3-hour infusion of 1g q8h and 2g q12h achieved $fT>_{MIC}$ above 85% for an MIC of 4µg/mL in peritoneal fluid [107]. The MIC data were derived from susceptibility surveillances of surgical infections from 4 major types of bacteria that cause post-operative IAIs: *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter cloacae*. Higher CL and lower AUC values were noted than in healthy volunteers [108]. A regimen of 1g every 8 hour for cefotiam achieved a $fT>_{MIC}$ above 80%, based on MIC data derived from local susceptibility surveillances of surgical infections. No pharmacodynamic analysis is available for cefozopran. In a study by Wittman and Schassan [71], the concentration ratio of peritoneal fluid to plasma at 2 hours is 0.86 for cefuroxime, 0.85 for ceftazidime, 0.74 for cefoperazone, 0.71 for moxalactam and 1.40 for both cefoxitin and cefotaxime. Both cefoxitin and cefotaxime had an unusual ratio that probably reflects rapid achievement of therapeutic concentrations in peritoneal fluid although the duration of persistence of these concentrations remains unknown.

Summary of PK data

The PK studies including IAI patients consistently show higher V_d values than in healthy volunteers except for cefoperazone [105]. The increment of V_d, was twice as high for both meropenem [86, 87] and piperacillin [95] and 20% higher for ceftazidime [97]. Despite these PK differences in healthy volunteers, because of the high susceptibility of the majority of pathogens causing IAI, a loading dose in response to this increased V_d would be required only on suspicion of a less susceptible bacterium (e.g. *Pseudomonas aeruginosa*)[63].

On the contrary, PK studies including post abdominal surgery patients have shown lower V_d values, indicating more rapid clearance than in healthy volunteers. Specifically, CL was four times greater for imipenem-cilastin [89], three times higher for cefepime [107] and two times greater for doripenem [90]. The increased CL associated with post-operative state suggests the need for more frequent dosing of beta-lactam antibiotics.

Approaches to dosing antibiotic in IAI

The importance of dose optimization of antibiotics in treating infection has been highlighted by various studies [33, 109-112]. For critically ill patients with complicated IAI, optimizing antibiotic therapy is challenging. Dosing guidance from the product insert is obtained from well-controlled clinical trials in patients who are not critically ill and so does not account for the complex pathophysiology of these patients [113, 114]. As is evidenced above, antibiotic concentrations at the infection site and drug clearance can be variable between antibiotics. Major changes of these parameters will affect antibiotic concentrations and therefore bacterial killing. Changes in distribution through volume expansion may cause a dilution effect to hydrophilic antibiotics, thereby necessitating larger doses, as shown in those PK studies involved patients with IAI; higher V_d values were noted than in healthy volunteers [86, 87, 95]. There is a lack of data on the presence and effect of impaired penetration and possible antibiotic clearance through surgical drains, suggesting that further studies be should be conducted. As discussed above, the prescription of antibiotics that attain a higher peritoneal fluid: plasma ratio is suggested for this patient population, although such data are not readily available from all the studies reviewed. Where data suggest that antibiotic penetration into the peritoneum may be low, higher empiric doses to increase peritoneal fluid concentrations should be considered. Nevertheless, as studies describing antibiotic PK at the site of IAI are limited, more

research should be undertaken to determine whether individualized dosing based on PK/PD does in fact improve clinical outcome. However, when susceptibility data is available, standard doses are likely to be sufficient because many bacteria causing IAI are highly susceptible, and PD targets are still achieved in the peritoneal fluid. Other factors, such as the presence of hypoalbuminemia [17, 19] and impaired or augmented renal function [115-117], phenomena commonly seen in critically ill patients, also may lead to changes in plasma concentrations for antibiotics [118].

Conclusion

There are marked changes in PK parameters of antibiotics in patients with intra-abdominal disease. The majority of these alterations may lead to decreases in antibiotic concentrations and therefore, dosage individualization or the development of revised evidence-based dosing guidelines is required for these patients. The variability and unpredictability of PK in patients with IAI disease may support monitoring of blood antibiotic concentrations to guide dosing. Such dose optimization should be considered important, as absence of appropriate dosing adjustment can lead to antibiotic resistance, therapeutic failure or both. In this context, the data from PK studies in this review can enable clinician to tailor dosing in this patient population. We suggest further research to determine the clinical outcomes of patient-specific dosing.

4.3 Published manuscript "Pharmacokinetics of Meropenem and Piperacillin/Tazobactam in Critically Ill Patients with Indwelling Surgical drains"

The manuscript entitled, "Pharmacokinetics of Meropenem and Piperacillin/Tazobactam in Critically Ill Patients with Indwelling Surgical drains" has been accepted for publication by International Journal of Antimicrobial Agents (2013; 42(1):90-3).

Patient recruitment and consent were assisted by Prof Jason Roberts. Sample collection and analysis were undertaken by the PhD candidate and Janice Li, with assistance from research staff of Burns, Trauma and Critical Care Research Centre. Dr Steven Wallis assisted with the bio-analysis of the samples. Prof Jason Roberts assisted with the data analysis and interpretation. Dr Michael Rudd assisted with the data interpretation, Prof David Paterson and Prof Jeffrey Lipman oversaw all aspects of this chapter.

The manuscript is presented as published; except figures and tables have been inserted into the text at slightly different positions. Also, the numbering of pages, figures and tables has been adjusted to fit the overall style of the Thesis. The references are found alongside the other reference of the Thesis, in the section `Bibliography'.

"Pharmacokinetics of Meropenem and Piperacillin in Critically Ill Patients with Indwelling Surgical drains"

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Keywords: pharmacokinetic, meropenem, piperacillin, critically ill, surgical drains

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Abstract

Meropenem and piperacillin are two commonly prescribed antibiotics in critically ill surgical patients. To date the pharmacokinetics of these antibiotics in the presence of indwelling abdominal surgical drains is poorly defined. This was a prospective pharmacokinetic study of meropenem and piperacillin. Serial plasma, urine and surgical drains fluid samples were collected over one dosing interval of antibiotic treatment in ten patients, meropenem (n=5) and piperacillin (n=5). Drug concentrations were measured using a validated high performance liquid chromatography assay. Median (inter quartile range) pharmacokinetic parameters estimates for meropenem were as follows: area under concentration-time curve (AUC): 128.7 mg.h/L (95.3-176.7mg h/L), clearance (CL): 5.7 L/h (5.1-10.5L/h), volume of distribution (V_d): 0.41L/kg (0.35-0.56l/kg), AUC ratio (drain: plasma): 0.2 (0.1-0.2) and calculated antibiotic clearance via surgical drains: 3.8% (2.8-5.4%). For piperacillin, unbound pharmacokinetics results are as follows; AUC: 344.3 mg.h/L (341.1-348.4 mg.h/L), CL: 13.1 L/h (12.9-13.9 L/h), V_d: 0.63 L/kg (0.38-1.28 L/kg), AUC ratio (drain: plasma) : 0.2 (0.2-0.3) and calculated antibiotic clearance via surgical drains for piperacillin is 8.2 % (3.3-14.0 %). A linear correlation was present between the percentage of antibiotic cleared through the drain and the volume of surgical drains fluid output for meropenem (r^2=0.89, P=0.05) and piperacillin (r^2=0.63, P=0.20). Meropenem and piperacillin have altered pharmacokinetics in critically ill patients with indwelling surgical drains. We propose that only when very high drain fluid output is present (> 1000 mL/day) would an additional dose of antibiotic be necessary.

Introduction

Meropenem and piperacillin/tazobactam (TZP) are commonly prescribed for postoperative infections in critically ill patients. Surgical drains may be inserted for either therapeutic, prophylactic or decompressive drainage of excess air or fluid, or to monitor production of wound exudate post-surgery [119].

During clinical practice at our tertiary referral Intensive Care Unit (ICU), we have observed that critically ill patients with indwelling surgical drains have lower plasma concentrations of antibiotics than other comparable patients [7]. There are few data available to suggest whether these surgical drains are associated with sub-therapeutic concentrations, which may lead to impaired antibiotic efficacy. Most of the studies documenting the concentrations of antibiotics in intra-abdominal and pleural fluid, primarily described

antibiotic penetration and do not examine whether these surgical drains are a mechanism for increased drug clearance. There are also limited data on the time-course profile of both meropenem and piperacillin in patients with surgical drains. This lack of data limits the ability to predict dosing requirements for such patients [120-122].

The importance of achieving adequate antibiotic concentrations at the site of infection is well recognised, with sub-therapeutic concentrations hypothesized to be associated with therapeutic failure [123]. However, measurement of drug concentrations at the site of infection is often not feasible, and plasma drug concentrations remain an important surrogate.

The target exposure for antibiotics is guided by the minimum inhibitory concentration (MIC) of the target bacterial pathogen. For beta-lactam antibiotics, bacterial killing depends largely on the time the free (or unbound) antibiotic concentration remains above the MIC, i.e. $fT_{>MIC}$. The specific percentage of the dosing interval differs between beta-lactam classes; 40% for carbapenems, 50% for cephalosporins and 60-70% for penicillins [124]. The primary aim of this project was to describe the pharmacokinetics (PK) of both meropenem and piperacillin in critically ill patients with indwelling surgical drains with a focus on describing drug clearance through the drains.

Materials and methods

This was a prospective open labelled PK study conducted at Intensive Care Unit, Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia. Critically ill patients who met the following criteria were eligible for inclusion: a) written informed consent had been obtained from the patient or his/her substitute decision maker; b) presence of at least one indwelling surgical drains actively producing fluid (defined as >10mL in the preceding 6 hours); c) clinical indication for meropenem or piperacillin/TZP therapy; and d) an intra-arterial catheter in situ (for the purposes of blood sampling). Patients were excluded from the study if one or more of the following criteria were met: a) renal impairment (defined as plasma creatinine concentration > 170 □ mol/L), b) pregnancy or c) admission following burns injury.

Antibiotic administration and sample collection

All samples were collected over a single dosing interval. Standard doses were administered (1 gm intravenous every 8 h for meropenem and 4.5 gm intravenous every 6 h for piperacillin/TZP). Blood samples were drawn at seven time points. For meropenem, this was at 0 (pre-dose), 0.5 (end of infusion), 1, 1.5, 2, 4, and 8 hours post dose. For piperacillin/TZP, this was at 0 (pre-dose), 0.5 (end of infusion), 1, 1.5, 2, 4 and 6 hours post dose. Surgical drains fluid was collected from the indwelling surgical drains every hour during the dosing interval. Urine samples were collected from indwelling urinary catheters every hour during the dosing interval.

All samples were immediately placed in polypropylene tubes on ice and were centrifuged at 3000 rpm for 10 minutes within 4 hours of collection. The plasma and supernatant were removed; aliquots of the plasma were placed into labelled polypropylene screw-cap cryo-vials and stored at -80ºC until assay.

Assay

Meropenem and piperacillin in plasma, surgical drains fluid and urine were measured by high performance liquid chromatography with ultraviolet detection (HPLC-UV) on a Shimadzu Prominence instrument. All samples were assayed alongside calibration standards and quality controls prepared by spiking drug into matching drug-free biological matrix (surgical drains fluid samples were treated as plasma samples as they are both proteinaceous matrices and no drug-free drain fluid was available). Assays were validated and conducted using criteria from the FDA guidance on bioanalysis [125].

To measure unbound piperacillin concentrations, the unbound fraction was obtained by ultrafiltration of plasma at 37°C using Merck Millipore Centrifree® 30 KDa MWCO centrifugal filter units for 5 minutes at 1410 ×*g*, so that only 15-40% of the plasma volume was filtered to prevent perturbation of the binding equilibrium. Unbound concentrations were not measured for meropenem as plasma protein binding is only 2%, which we considered not significant.

The precision and accuracy of the methods were validated to be within 6% (total meropenem in plasma/surgical drains fluid from 0.2 to 50 mg/L), 3% (meropenem in urine from 10-2000 mg/L), 10% (total piperacillin in plasma/surgical drains fluid from 0.5-500

µg/mL), 6% (unbound piperacillin in plasma from 1-500 mg/L), and 6% (piperacillin in urine from 100 to 40,000 mg/L) at low, medium and high concentrations of the calibration range.

Pharmacokinetic Analysis

A non-compartmental PK analysis was performed to describe the disposition of meropenem and piperacillin in critically ill patients with indwelling surgical drains. The C_{max} was the observed maximum concentration at the end of infusion and trough concentration(C_{min}) was the observed minimum concentration prior to drug administration. The area under the concentration-time curve (AUC) from 0 to 8 hour for meropenem and from 0 to 6 hour for piperacillin was calculated using the trapezoidal rule. The AUC extrapolated to infinity ($AUC_{0-\infty}$) was calculated using AUC and the elimination rate constant (k_{el}). The k_{el} was calculated as the negative slope of the non-weighted squares curve fit of the final 3 sampling points during the elimination phase. The percentage of antibiotic cleared through the surgical drains was calculated with the following equation: = ($C_{drain\ (total)}$/Volume $_{drain\ (total)}$)/dose where C_{drain} is the concentration in the drain. Clearance (CL) was calculated as dose/$AUC_{0-\infty}$. The volume of distribution (V_d) was calculated as CL/ k_{el}. Half-life was calculated at 0.693/ k_{el}.

Statistical Analysis

Statistical analysis was performed using Graphpad Prism version 5.0 (GraphPad Software Inc., La Jolla, CA). Linear regression on the percentage of antibiotic clearance through the surgical drains and the volume of surgical drains fluid output was performed. *P*-values <0.05 were considered significant.

Results

Ten patients were included in this study; meropenem (*n*=5) and piperacillin/TZP (*n*=5). The mean (SD) age was 69 (± 15) years, weight 75 (± 23) kg, APACHE II Score 11 (± 2), SOFA Score 3 ± 2. Five (50%) of the patients were male; nine patients had intra-abdominal drains whilst the other patient had a left leg drain because of severe lower limb trauma.

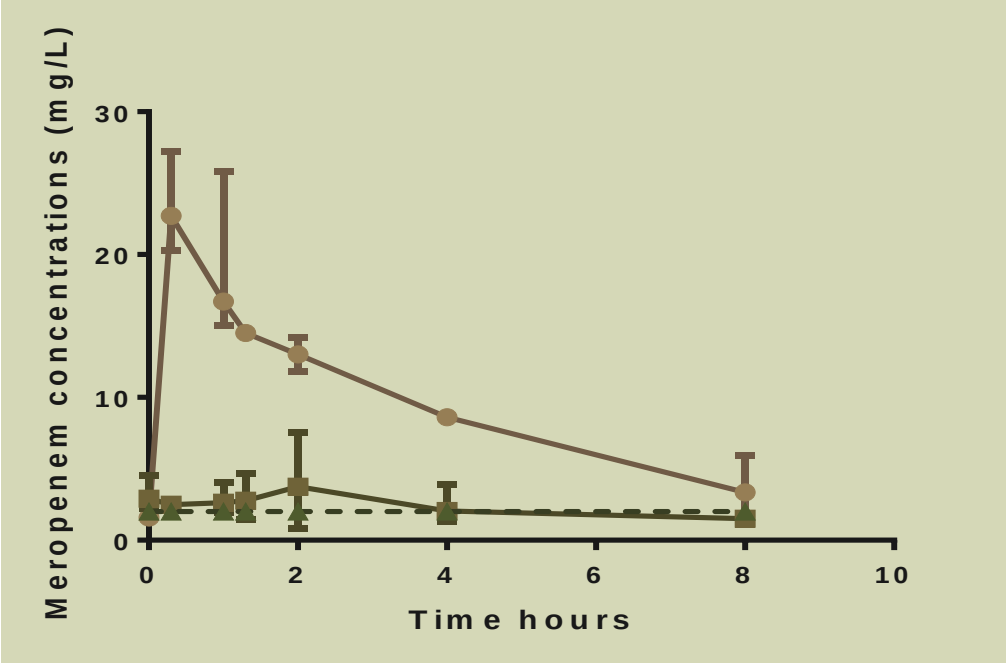
Figure 4.2 displays the concentration-time profile for meropenem and piperacillin both in plasma and drain fluid. Both plasma and drain concentrations of meropenem were above the European Committee on Antimicrobial Susceptibility Testing (EUCAST) MIC breakpoint of *Pseudomonas aeruginosa*, 2mg/L, but this was not achieved for the

piperacillin breakpoint, (16mg/L) [126]. However, the observed concentrations were above the EUCAST MIC breakpoint for *Enterobacteriaceae spp*, for both drugs (2mg/L for meropenem and 8 mg/L for piperacillin) [126].

Table 4.2 gives the PK parameters for meropenem and piperacillin. These data are compared with published data from healthy volunteers [127, 128]. Both drugs show a larger V_d in the studied patients compared to the healthy volunteers. CL of meropenem is 50% lower than healthy volunteers but CL is only slightly lower for piperacillin. The estimated percentage of antibiotic cleared through the surgical drains whilst not clinically significant; was still notable (3.8% and 8.2% for both meropenem and piperacillin, respectively).

Linear regression analyses of the percentage of antibiotic cleared through the surgical drains and the volume of surgical drains fluid, are shown in Figure 4.3. Correlations were observed for meropenem (r^2=0.89; P=0.05) and piperacillin (r^2=0.63; P=0.20). Note that this analysis was performed with only 4 of the 5 subjects for both meropenem and piperacillin with the remaining patients having inadequate drain fluid volumes for assay and surgical drains AUC calculation.

Figure 4.2: Time concentration profile of meropenem and piperacillin in critically ill patients with indwelling surgical drains. The brown lines represent plasma concentrations, the dark green lines are surgical drains fluid concentrations and the dashed green line is the EUCAST MIC breakpoint for *P. aeruginosa* (16mg/L)



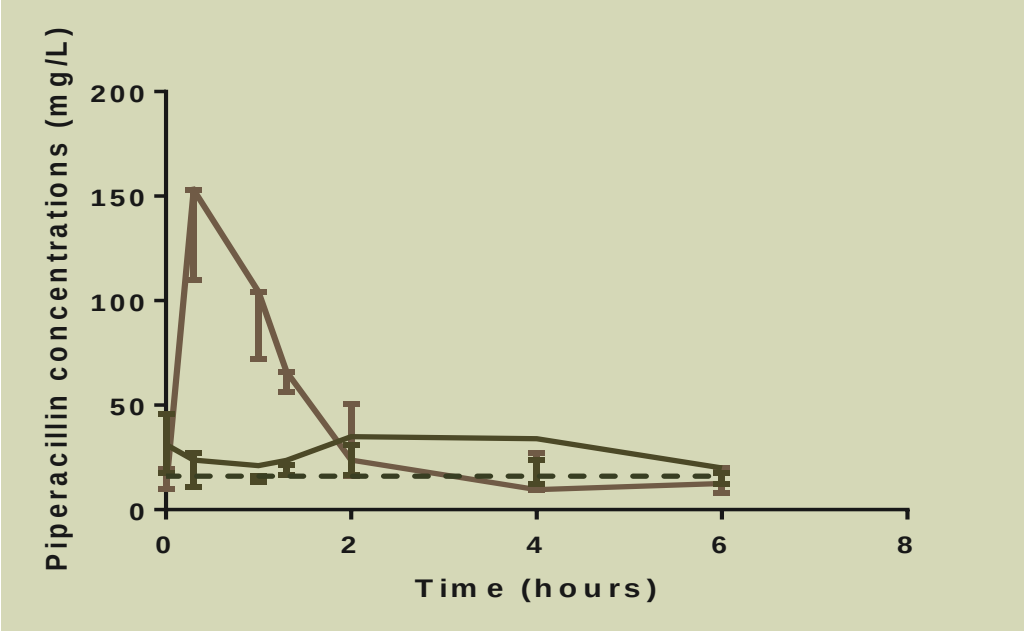
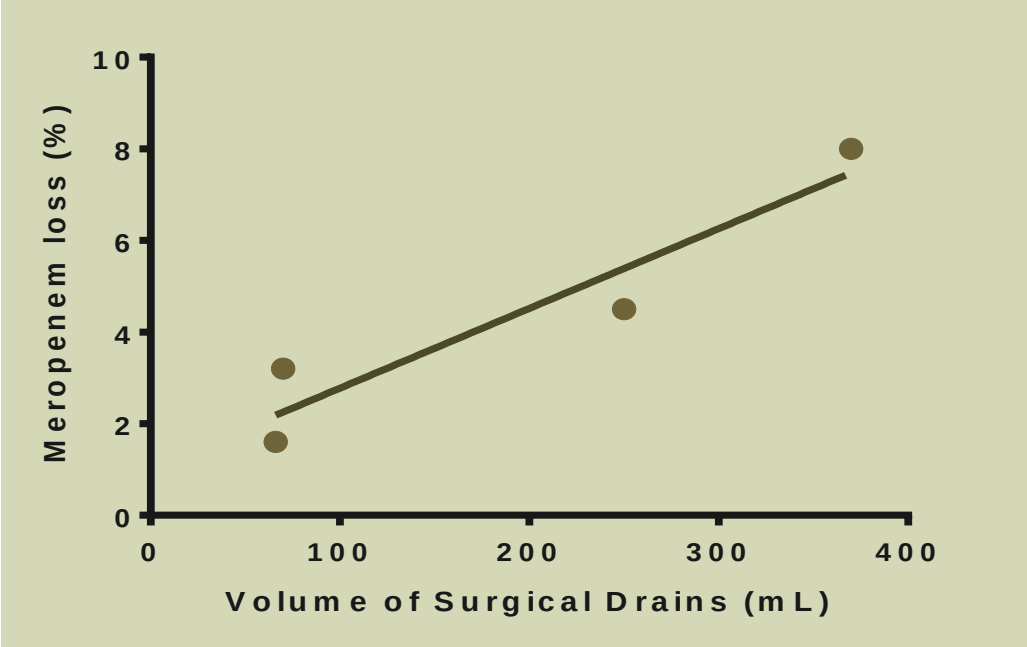


Figure 4.3: Linear regression analysis showing relationship between meropenem drain clearance and output volume of surgical drains (r^2=0.89; *P*=0.05) and piperacillin drain clearance and output of surgical drains (r^2=0.63; *P*=0.20).



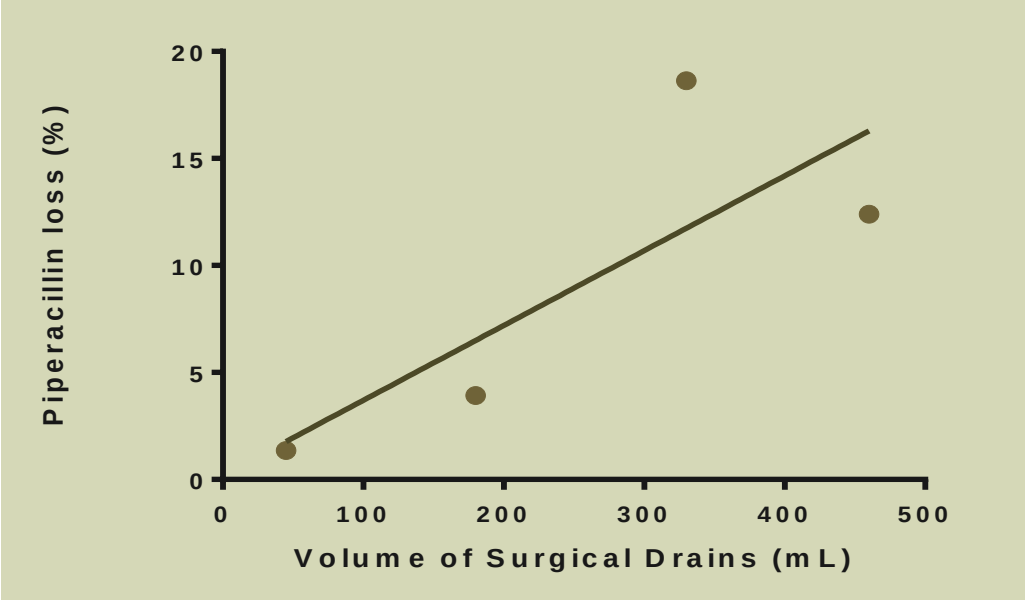


Table 4.2: Pharmacokinetic parameters of meropenem and piperacilin in critically ill patients with indwelling surgical drains compared with healthy volunteers

Pharmacokinetic parameter	***Patients with surgical drains***		***Healthy volunteers [127,***	
	Meropenem	Piperacillin (Unbound)	Meropenem	Piperacillin
Half-life (h)	3.18 (3.05-4.66)	1.85 (1.34-2.19)	0.98	0.98
Clearance (L/h)	5.7 (5.1-10.5)	13.1 (12.9-13.2)	11.3	12.1
Volume of distribution (L/kg)	0.41 (0.35-0.56)	0.63 (0.38-1.28)	0.17	0.21
AUC (plasma)(mg.h/L)	128.7 (95.3-176.7)	344.3 (341.1-348.4)	77.5	253.0
AUC (drain fluid)(mg.h/L)	19.6 (13.5-26.0)	NA	NA	NA
$AUC_{drain} : AUC_{plasma}$	0.2 (0.1-0.2)	NA	NA	NA
Antibiotic clearance via drain (%)	3.8 (2.8-5.4)	NA	NA	

Discussion

The present study describes the PK of meropenem and piperacillin in critically ill patients with indwelling surgical drains. The AUC in plasma for both drugs was found to be larger than that reported in healthy volunteers [127, 128]. For the comparative AUC in drain fluid, a lower AUC was shown than in plasma (78% and 75% lower for meropenem and piperacillin, respectively). Rapid clearance through the surgical drains could explain this difference. Similar trends have been observed in other studies [120-122]. The antibiotic concentrations in the surgical drains fluid exceed the MIC of most common pathogens of intra-abdominal infections, although these concentrations may not be sufficient for higher MIC organisms such as *P. aeruginosa* [129].

The increased V_d of both meropenem and piperacillin suggests extensive distribution but also the potential for lower than expected concentrations, thereby increasing the risk of therapeutic failure [130]. Indeed, these data are in keeping with the pathophysiological changes encountered in the critically ill, which in the context of the majority of patients studied here who had intra-abdominal pathology, the presence of a capillary leak syndrome, will lead to a larger V_d and longer half-life [131].

Interestingly, the increased V_d was present with an increased AUC which is an unusual finding. We would consider that the use of a non-compartmental PK analysis as opposed to a more mechanistic compartmental approach may be the reason for this. If a two compartment models were used, it may have better characterised the effect of the peripheral compartment. Nevertheless, when comparing the meropenem patients with non-critically ill patients that underwent elective abdominal surgery without surgical drainage, the AUC in the patients in this study were 34% lower than the comparators [132].

PK studies of both meropenem and piperacillin/TZP, in patients with complicated intra-abdominal infections have also shown increased V_d and support the current findings [120-122]. The majority of patients in our study had intra-abdominal infections and the pathophysiological changes in these critically ill patients has been recently reviewed [131]. Fluid expansion due to an increase in fluid volume within the peritoneal cavity is the main factor that leads to wider drug distribution. Fluid shifts from a capillary leak syndrome often

require fluid administration to maintain intravascular volume. At a tissue level, fluid accumulation from the capillary leak syndrome can result in increased V_d of the antibiotic.

An increase in V_d suggests that a higher initial dose is required to rapidly achieve therapeutic concentrations [130]. However, the magnitude of such a dose adjustment is yet to be defined although dose-adjustment using beta-lactam therapeutic drug monitoring could be considered useful in these settings [7].

The lower total CL shown for meropenem is likely due to a decreased glomerular filtration rate (GFR) which could be expected with these older patients. This is despite the GFR measured using the Cockcroft-Gault Equation, being within normal range. However, this equation may not be appropriate for use in ICU patients and so the reliability of this result could be questioned. Thus, it remains unknown why the meropenem cohort had a lower CL than that described in the comparator healthy volunteers.

Neither antibiotic displayed high drain fluid concentrations. Compared to plasma concentrations, the AUC ratio of drain fluid concentrations was 0.2 (0.1-0.2) and 0.2 (0.2-0.3) for meropenem and piperacillin, respectively. In fact, the plasma concentrations for piperacillin were also rarely above the MIC breakpoint for *P. aeruginosa,* which may support altered dosing approaches if this pathogen is suspected (Figure 4.2). As such, the present data shows a correlation between antibiotic clearance through the drain and the volume of surgical drains output. However, they also suggests that only in the presence of very high fluid output drains (>1000 mL/day) would supplemental doses of antibiotic would be necessary.

Although this study is limited by the small patient numbers which could not provide definitive dosing guidance, the pharmacokinetic variability described emphasises the effect of changes in V_d and CL owing to pathophysiological changes. Dosing for the individual patient tailored to the altered physiology is strongly recommended.

Conclusion

This study has shown that patients with indwelling surgical drains receiving either meropenem or piperacillin/TZP have a greatly increased V_d compared with healthy volunteers. This increased V_d is likely to be due to the inflammatory pathology at the site of the drain as well as other pathophysiological changes commonly seen in critically ill patients. Therefore, this data appears to support the need for larger initial antibiotic doses

in these patients, although the magnitude of such increased doses is yet to be defined. We would also propose that other ongoing higher antibiotic dosing in these patients would only be required in the presence of high output drains (>1000 mL/day). Clearly more data is required to determine how to optimize dosing in critically ill patients with indwelling surgical drains to prevent under-exposure of antibiotics and potential therapeutic failure or the development of bacterial resistance.

Acknowledgement

We would like to acknowledge Suzanne Parker, Gregory A Medley and Lu Jin from Burns, Trauma and Critical Care Centre, School of Medicine, The University of Queensland for their technical assistance with the HPLC assays.

Funding: This research has received funding from Royal Brisbane and Women's Hospital Research Foundation. Dr Roberts is funded, in part by a National Health and Medical Research Council of Australia Research Fellowship (NHMRC APP1048652).

Competing Interest: None declared.

Ethical Approval: This study was approved by the Ethics Committee at Royal Brisbane and Women's Hospital (HREC/09/QRBW/372) and The University of Queensland.

4.4 Conclusion on pharmacokinetics of beta-Lactam antibiotics in intra-abdominal disease in critically ill patients

This chapter has described the available data from PK studies of beta-lactam antimicrobials in intra-abdominal disease. Together, these studies demonstrate highly variable antimicrobial concentrations at the infection site as well as variable drug clearance. Studies including IAI patients consistently show extensive drug distribution suggesting that an initial higher dose may be required when a less susceptible pathogen is suspected in causing IAI. Conversely, in abdominal surgery patients, prolonged drug clearance was evident and less frequent dosing may be necessary. Although achieving a high peritoneal fluid: plasma ratio is preferred for antimicrobials in patients with intra-abdominal disease; this data is not readily available for all antimicrobials included in the review. There is also data available of suggesting some level of impaired antimicrobial penetration as well as possible drug clearance through surgical drains. The PK study on two commonly prescribed antimicrobials, meropenem and piperacillin, in critically ill patients with indwelling surgical drains in this chapter has shown marked PK alterations, which could be due to the inflammatory pathology in these patients. This PK alteration could lead to therapeutic failure from low antimicrobial concentrations and dosing modifications are recommended, particularly if less susceptible pathogens are suspected. Additionally, a linear correlation was shown between antimicrobial clearance and the volume of surgical drain fluid produced. Thus, additional dosing may also be considered when high volumes of surgical drains fluid are produced. In conclusion, inappropriateness of standard dosing of both meropenem and piperacillin, in critically ill patients with indwelling surgical drains, is a possibility that is likely to require non-standard dosing to ensure therapeutic concentrations are achieved.

5. Chapter 5: The Potential Use of Ampicillin/Sulbactam in Treating Infections in Critically Ill Patients

5.1 Synopsis

The aim of this chapter is to describe the potential use of ampicillin/sulbactam in treating infections in critically ill patients. In this chapter, this antibiotic was reviewed from several aspects, such as pharmacology, microbiology, pharmacodynamic, pharmacokinetic and dosing modalities, which led to a published manuscript of this review. An additional chapter of the potential use of ampicillin/sulbactam as combination therapy against MDR *Acinetobacter baumannii* (MDR-Ab) was also included in this review. We found that PK studies are required to better define the necessary doses of ampicillin/sulbactam in critically ill patients. This chapter also includes the study of PK of ampicillin/sulbactam in critically ill patients at risk of MDR-*A*b infections. The chromatographic methods used in analysing ampicillin/sulbactam in PK samples will be described, followed by findings of the PK study. Additionally, the appropriateness of standard doses of ampicillin/sulbactam in achieving PK/PD targets in critically ill patients at risk of MDR-Ab infections will also be evaluated.

5.2 Published manuscript entitled, "Ampicillin/Sulbactam : Its Potential Use in Treating Infections in Critically Ill Patients"

The manuscript entitled, "Ampicillin/Sulbactam : Its Potential Use in Treating Infections in Critically Ill Patients has been accepted for publication by International Journal of Antimicrobial Agents (2013; 42(5):384-9).

All data collection, data interpretation and drafting of the paper were undertaken by the PhD candidate, Syamhanin Adnan, assisted by Dr Shanthi Ratnam and Dr Suresh Kumar. Prof Jason Roberts assisted with the data collection and data interpretation. Prof David Paterson and Prof Jeffrey Lipman oversaw all aspects of this chapter.

The manuscript is presented as published; except figures and tables have been inserted into the text at slightly different positions. Also, the numbering of pages, figures and tables has been adjusted to fit the overall style of the Thesis. The references are found alongside the other reference of the Thesis, in the section `Bibliography'.

"Ampicillin/Sulbactam : Its Potential Use in Treating Infections in Critically Ill Patients"

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Keywords : Ampicillin/sulbactam, multi-drug resistant, critically ill, pharmacokinetics, pharmacodynamics

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Abstract

The purpose of this paper was to review the potential utility of ampicillin/sulbactam as a therapy for serious infections in critically ill patients. Data for this review were identified by searches of PubMed and of the reference lists of the included articles. We found that ampicillin/sulbactam appears to have a number of characteristics that support its use in the treatment of serious infections in critically ill patients. Ampicillin/sulbactam demonstrates extensive penetration into many infection sites, supporting its use in a wide range of infection types. Microbiologically, sulbactam has strong intrinsic antibiotic activity against multidrug-resistant (MDR) bacteria, including *Acinetobacter baumannii,* which supports its use for the treatment of infections mediated by this pathogen. Of some concern, there have been reports showing a decline in susceptibility of some bacteria to ampicillin/sulbactam. As such, use of lower doses (4/2 g/day), particularly for MDR *A. baumannii*, has been linked with a 30% reduced success rate in critically ill patients. The therapeutic challenges for ensuring achievement of optimal dosing of ampicillin/sulbactam do not however, result partly from bacterial susceptibility but also from the pharmacokinetic alterations common to β-lactam agents in critical illness. These pharmacokinetic changes are likely to reduce the ability of standard dosing to achieve the concentrations observed in non-critically ill patients. Optimisation of therapy may be more likely with the use of higher doses, administration by 4-h infusion or by combination therapy, particularly for the treatment of infections caused by MDR pathogens.

Introduction

Ampicillin/sulbactam is a β-lactam/β-lactamase inhibitor combination, licensed for parenteral use, which was developed to overcome resistance to ampicillin. It is approved by the US Food and Drug Administration (FDA) for skin and skin-structure infections, intra-abdominal infections (IAIs) and gynaecological infections. It can also be used for other infections caused by ampicillin-susceptible bacteria [133]. However, in recent years there has been an increasing amount of surveillance data reporting decreased susceptibility to ampicillin/sulbactam in *Escherichia coli* [134]. Since *E. coli* is one of the most common pathogens causing IAIs, ampicillin/sulbactam is probably not a good option in treating IAIs in patients at risk of having ampicillin/sulbactam-resistant strains of *E. coli* [135].

A few clinical outcome studies have been undertaken that demonstrate the utility of ampicillin/sulbactam for treating resistant pathogens such as multidrug-resistant (MDR)

Acinetobacter baumannii [136, 137]. Optimised dosing strategies are of paramount importance in an era of limited antibiotic options and a diminishing antibiotic development pipeline [138]. Antibiotic therapy is defined to be inappropriate if the empirical drug selection, dose and method of administration are not effective against the causative pathogen [139]. The presence of MDR pathogens will increase the likelihood of inappropriate antibiotic therapy both in terms of drug choice and dose. In addition, bacteria such as MDR *A. baumannii* are particularly problematic given their capacity to survive environmentally on inanimate objects for extended periods of time [140, 141].

Management of infections in critically ill patients becomes more complicated given their acute pathology as well as the presence of pre-existing co-morbidities. The complicated physiological changes that occur in the critically ill can present a significant challenge to clinicians for procuring effective antibiotic doses because of dramatic changes in PK(PK)s [142]. The additional wide variability of PKs is likely to cause different antibiotic concentrations to those observed in non-critically ill patients. Thus, the use of standard dosing could yield different clinical effects in critically ill patients. Since drug concentrations are a direct result of the dosage administered, these variations can be overcome by appropriate modification of the dosing regimen[26]. Dose optimisation of antibiotics has become a key factor for improving the probability of a favourable outcome in critically ill patients.

The objective of this paper was to review the potential utility of ampicillin/sulbactam as a therapy for infections in critically ill patients.

Search Strategy

Data for this review were identified by searches of PubMed (1987 to July 2013) as well as references from relevant articles. Studies including information relating to bacterial susceptibility, PKs, pharmacodynamics, and efficacy or failure of ampicillin/sulbactam in the treatment of critically ill patients were included. Forty papers were found to be relevant, with twelve papers excluded because they discussed oral sultamicillin, contained only animal model data, were case studies or were not written in English.

Antibiotic characteristics of ampicillin/sulbactam

Pharmacology

Ampicillin/sulbactam a combination of a β-lactam/β-lactamase inhibitor with an extended spectrum of antibiotic activity. Ampicillin works by binding to penicillin-binding proteins (PBPs) and inhibiting bacterial cell wall synthesis, causing disruption of the bacterial cell wall and ultimately resulting in bacterial cell death. However, resistant pathogens may produce β-lactamase enzymes that can hydrolyse ampicillin. This activity is inhibited by the presence of sulbactam through reversible formation of an acyl-enzyme intermediate [143]. Sulbactam is also capable of bindings to the PBP of *Bacteroides fragilis* and *Acinetobacter* spp. even when it is administered alone [144]. In vitro studies have reported clinically relevant activity of sulbactam against *Acinetobacter* spp., making it distinctive from other β-lactamase inhibitors (e.g. tazobactam and clavulanic acid) [145].

Microbiology

Ampicillin/sulbactam exhibits a broader spectrum of antibiotic activity than ampicillin alone as a result of a synergistic effect with sulbactam. Inhibition of β-lactamases by sulbactam enables ampicillin to remain active in the presence of β-lactamases and effective against Gram-positive bacteria that commonly cause respiratory tract infections and skin and soft-tissue infections, e.g. meticillin/oxacillin-susceptible *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Streptococcus viridans* [146-148]. However, ampicillin/sulbactam is not effective against meticillin-resistant *S. aureus* (MRSA) and coagulase-negative staphylococci, even though it may appear to be active in vitro [149]. Furthermore, it does not add any clinical advantage over other antibiotics for the treatment of Groups A, B, C and G streptococci as these particular pathogens do not produce any β-lactamases [149]. Ampicillin/sulbactam may be useful for treating β-lactamase-producing enterococcal isolates, although high doses or altered administration techniques such as continuous infusion would be required [150].

Gram-negative bacteria that are susceptible to ampicillin/sulbactam include the β-lactamase-producing and non-β-lactamase-producing Gram-negative bacteria such as *Haemophilus influenzae*, *Moraxella catarrhalis* and *Neisseria gonorrhoeae.* However, it has limited activity against *Pseudomonas aeruginosa* and Enterobacteriaceae that produce extended spectrum β-lactamases (ESBLs) [151-153]. In a recent surveillance

study of Gram-negative bacteria in IAIs, a declining susceptibility of *E. coli* towards ampicillin/sulbactam was demonstrated [134]. Similar findings were seen in other surveillance data on *E. coli* bloodstream infections [154]. Exposure to penicillins, including ampicillin/sulbactam, is considered the only significant independent risk factor associated with the emergence of ampicillin/sulbactam-resistant *E. coli* [155]. It follows that ampicillin/sulbactam may be problematic for those patients at risk of infections mediated by ESBL-producing organisms [135]. Furthermore, suboptimal outcomes in a study of ventilator-associated pneumonia (VAP) in trauma patients suggest that ampicillin/sulbactam is associated with decreased susceptibly for common pathogens associated with VAP, although *A. baumannii* was not among the common pathogens in this study [156]. A list of studies comparing the effectiveness of ampicillin/sulbactam with its comparators were discussed in detail in a review article by Lode [146].

Despite limited activity against most Gram-negative pathogens, in vivo and in vitro studies conducted during the development of sulbactam demonstrated a strong affinity for a PBP of *A. baumannii* [143]. Recent evidence suggests that low inhibitory concentrations of ampicillin/sulbactam for PBP3 may contribute to the effectiveness of this combination against *A. baumannii* [157]. Nevertheless, increasing rates of bacterial resistance of *A. baumannii* towards ampicillin/sulbactam have been documented in several recent surveillance reports, which are of concern [146, 158].

Similar to other β-lactam/β-lactamase inhibitors combinations, ampicillin/sulbactam also displays an inoculum effect, with an increasing bacterial load associated with significant increases in the minimum inhibitory concentration (MIC) of the pathogen [159].

Pharmacodynamics

Ampicillin is a time-dependent antibiotic with its bacterial killing largely dependent on the time the free concentration is maintained above the MIC during a dosing interval ($fT>_{MIC}$). The duration of exposure will therefore determine the rate and extent of bacterial killing. In vivo and in vitro studies have shown that the penicillin group of antibiotics require 50–60% $fT>_{MIC}$ for maximum bactericidal activity [160]. This suggests that prolonged ampicillin concentrations will be more likely to result in therapeutic success. However, when ampicillin is administered as co-formulation for sulbactam, bacterial re-growth has been reported when sulbactam levels fall below ‘critical’ concentrations [161]. Therefore, like

other β-lactamase inhibitors, sulbactam is probably best described as an AUC-dependent drug where bacterial killing is associated with the area under the concentration—time curve (AUC) [162]. To date, there are no studies measuring the importance of antibiotic exposure with development of resistance to ampicillin/sulbactam but, like other antibiotics, underdosing may lead to resistance [163].

Pharmacokinetics

Ampicillin and sulbactam have similar PK profiles that appear unaffected by co-administration of each other. The PK observed in healthy volunteers contrasts that observed in various other patient populations is described in Table 5.1 [164], where the variability of key PK parameters is clearly shown.

Table 5.1 : Pharmacokinetic parameters of ampicillin/sulbactam in various populations

Reference	*Study population*	*Samples*	*Antibiotic*	C_{max} *(mg/L)*	$AUC_{0-\infty}$ *(mg·h/L)*	$t_{1/2}$ *(h)*	*Vd*		CL_{total} *(L/h)*	CL_{renal} *(L/h)*
							L	**L/kg**		
Foulds et al., 1985 [164]	Healthy volunteers	*n* = 40	Ampicillin	120	N/A	1.1	–	0.2	17.6 ± 3.5	14.2 ± 3.7
			Sulbactam	60	N/A	1.0	–	0.2	15.7 ± 3.5	15.7 ± 3.4
Blum et al., 1989 [165]	Renal disease	*n* = 4;	Ampicillin	N/A	379.7 ± 59.6	3.3 ± 1.9	–	0.3 ± 0.1	89.7 ± 16.5	39.4 ± 23.6
		CL_{Cr} = 21.8 ± 9.9 mL/min	Sulbactam	N/A	262.2 ± 77.3	3.7 ± 2.1	–	0.2 ± 0.1	69.3 ± 26.2	34.2 ± 22.3
	Haemodialysis	*n* = 4; CL_{Cr} = 2.7 ± 3.4 mL/min	Ampicillin	N/A	1654.8 ± 1170.2	17.4 ± 8.0	–	0.4 ± 0.1	31 ± 21	0.4 ± 0.6
			Sulbactam	N/A	432.2 ± 206.3	13.4 ± 7.4	–	0.6 ± 0.2	45.3 ± 19.5	0.5 ± 0.6
Yokoyama et al., 2012 [166]	Post-surgical patients	*n* = 40; cardiovascular surgery	Ampicillin	N/A	N/A	1.3 ± 0.9	13.2 ± 3.1	0.2	8.5 ± 3.3	N/A
			Sulbactam	N/A	N/A	1.3 ± 0.6	14.6 ± 3.2	0.2	8.8 ± 3.3	N/A
Meyers et al., 1991 [167]	Healthy elderly	*n* = 8; 65–85 years old	Ampicillin	112.4 ± 34.3	182 ± 57.8	1.3 ± 0.3	26.3 ± 8.8	0.3	198 ± 55.6	71.5 ± 28.3
			Sulbactam	59 ± 20	110.4 ± 32.7	1.6 ± 0.3	23.5 ± 7.7	0.3	162.7 ± 46.2	66 ± 24.5
Nahata et al., 1999 [168]	Paediatric patients	*n* = 10; 1–6 years old	Ampicillin	200 ± 118	179 ± 79.2	0.7 ± 0.1	–	0.3 ± 0.2	5.1 ± 2.4	N/A
			Sulbactam	102 ± 64	90.5 ± 42.8	0.7 ± 0.1	–	0.3 ± 0.2	5.1 ± 2.4	N/A
Rohde et al., 1997 [169]	ICU patients with CVVHD	*n* = 4	Sulbactam	N/A	N/A	6.0 ± 0.8	19.0 ± 5.1	0.2	45.0 ± 7.9	5.0 ± 5.2

Distribution

Ampicillin and sulbactam are hydrophilic antibiotics and have a volume of distribution (V_d) consistent with the volume of extracellular body water, and both have a similar V_d (0.2 L/kg) in healthy volunteers. Patients on haemodialysis, elderly patients as well as paediatric patients show a slightly increased V_d (Table 5.1) [167-169]. Pathophysiological changes associated with critical illness are also likely to increase the V_d of hydrophilic drugs and, as such, larger doses may be required to rapidly achieve target concentrations in these circumstances [24, 170].

Both ampicillin and sulbactam exhibit high concentrations in cerebrospinal fluid (CSF), exceeding the MIC of important bacterial pathogens, particularly in the presence of inflamed meninges [171-173]. Both concentrations have shown a strong correlation; however, these concentrations decline rapidly 7 h after dosing [172]. Conventional sulbactam dosing (administered as 2/1 g of ampicillin/sulbactam every 6 h) has been suggested to be appropriate for achieving adequate penetration across the blood–brain barrier, depending on the degree of meningeal inflammation and the susceptibility of the organism [172, 174]. Similarly, favourable penetration resulting in therapeutic exposure has been shown for other sites including costal cartilage, middle ear fluid, peritoneal fluid, intestinal mucosa, prostatic and appendicular tissue, sputum and peritonsillar abscess pus [165, 175]. For infections involving the lower respiratory system, particularly VAP, achieving effective epithelial lining fluid concentrations of antibiotics can be challenging for some therapies [176, 177], although ampicillin/sulbactam appears to penetrate well [178, 179]. Adequate concentrations in abdominal tissues against common pathogens, i.e. *S. aureus*, *E. coli* and *B. fragilis*, have also been reported for ampicillin/sulbactam [180]. Both agents have moderate protein binding (38% for sulbactam and 28% for ampicillin).

Metabolism and elimination

The half-life of ampicillin is ca. 1 h, whether alone or in combination with sulbactam, with elimination primarily by the urinary system (75% excreted unchanged in the urine) [164]. Tubular secretion is considered a significant process for elimination as well, given that the half-life of ampicillin is prolonged with co-administration with probenecid [143]. Only a small amount is excreted through the biliary system; in the

presence of normal liver function, it was estimated to be ca. 0.2% of the sulbactam dose and 2.8% of the ampicillin dose [143]. Based on these data, the common dosing frequency for ampicillin/sulbactam is every 6–8 h. PK data have shown prolonged clearance in the elderly [167], renal disease patients [165] and critically ill patients on renal replacement therapy [169]. In contrast, reduced clearance was shown in paediatric [168] and post-operative patients [166] (refer to Table 4.1). Thus, adjustment in dosing frequency may be required in these patient subtypes.

Dosing modalities

Given the potential for variations in function of the eliminating organs, dose adjustment will be required at times to optimise antibiotic exposure in critically ill patients. Data from healthy volunteers have been used to procure the current approved ampicillin/sulbactam dosing regimens whereby 6-hourly dosing is suggested for severe infections [143]. Further to this, the manufacturer has recommended that the daily sulbactam dose does not exceed 4 g/day. More recently, a PK/pharmacodynamic (PK/PD) modelling study of sulbactam using PK data from healthy volunteers has advocated a regimen of 3 g of sulbactam every 8 h as a 4-h infusion as necessary to achieve optimal antibiotic exposures for less susceptible pathogens [181]. However, this study may not be confidently extrapolated to critically ill patients considering the PK variations common to these patients as well as the frequently higher MICs of bacterial pathogens. At this time, the relevant PK/PD studies to define the necessary doses for use in critically ill patients remain elusive.

The PK parameters of principal importance for optimal dosing are V_d and clearance, as described in Fig. 2.2. Both of these parameters can be greatly affected by critical illness-driven physiological changes, as shown in Fig. 2.1. Of caution to routinely increasing ampicillin/sulbactam doses, high doses of this combination product may occasionally cause elevated liver enzymes, anaemia, thrombocytopenia and leukopenia, and most of the toxicity data regarding sulbactam is related to its use in combination with ampicillin. Attainment of high CSF concentrations of β-lactams may also result in neurological adverse effects, including seizures [182].

Scenarios requiring altered dosage

Changes in renal function

The renal system provides the major elimination pathways for ampicillin and sulbactam and therefore changes in renal function will in turn greatly affect its clearance. A strong relationship between ampicillin and sulbactam clearance and creatinine clearance was shown in a PK study involving patients prescribed ampicillin/sulbactam for surgical prophylaxis [166]. This high renal clearance means that a prolonged half-life can be expected in renal failure patients to the extent that only once-daily dosing is required for those undergoing maintenance haemodialysis [165]. However, there is a paucity of data for dosing in continuous renal replacement therapies [183].

Another important phenomenon common to critically ill patients is that of enhanced renal elimination of drugs, a phenomenon known as augmented renal clearance (ARC) [142, 184]. Enhanced renal elimination of solutes will also increase clearance of renally cleared antibiotics, reducing their concentration and jeopardising their therapeutic effect [26]. Although very little data exist describing the effect of ARC on ampicillin/sulbactam, a recent study has demonstrated a significant correlation between ARC and subtherapeutic unbound plasma trough concentrations of various other β-lactam antibiotics in critically ill patients [185]. The dynamic nature of the renal function of critically ill patients could result in frequent fluctuations in dosing requirements for ampicillin/sulbactam and this will require repeated clinical evaluation for potential dose adjustment.

Changes in distribution

Given its hydrophilic nature, the fluid shifts that commonly occur in critically ill patients are likely to lead to an increase in the V_d of drugs such as ampicillin/sulbactam. Common causes of fluid shifts include sepsis, fluid resuscitation, hepatic failure, burns and hypoalbuminaemia. This increased V_d can lead to low drug concentrations and potential therapeutic failure [26]. Little data is presently available for ampicillin/sulbactam to quantify these potential changes in critically ill patients.

Monotherapy of ampicillin/sulbactam for treatment of multi-drug resistant *Acinetobacter baumanni*

The intrinsic activity of sulbactam against *A. baumannii* means that unlike most infections for which it is used to treat, sulbactam is the active agent in the ampicillin/sulbactam co-formulation [186]. Of the available studies undertaken to determine the optimum dose of ampicillin/sulbactam for treating this resistant pathogen, most are clinical studies that trial one fixed dose versus another fixed dose and are not necessarily based on data from PK studies. This should be considered problematic as there is little data supporting the doses that are chosen and, potentially, none of them may be optimal.

For those studies involving susceptible *A. baumannii* in non-critically ill patients, doses of 2–4 g/day of sulbactam had been shown to have a favourable response rate (ca. 90%) [187-190]. However, when the same dose was used in studies involving critically ill patients with more resistant strains of *A. baumannii*, the success rate was lower, with only 68% of patients improving on therapy [136]. Higher doses (up to 12 g/day) have also been studied in similar settings and have shown similar success rates to those achieved with colistin therapy [191]. Another study comparing ampicillin/sulbactam with imipenem/cilastatin in the treatment of VAP showed similar efficacy in both groups although the doses used were not reported [192].

A review article [193] has listed the studies reporting ampicillin/sulbactam treatment of resistant *A. baumannii* and described clinical improvement rates of 46–75% with doses of between 6 g/day and 9 g/day. For this reason, papers by Koulenti and Rello [194] and Urban et al. [195] suggest that doses of ≥6 g/day are required for treatment for infections by MDR *A. baumannii*.

The available data do not adequately define the appropriate dose of ampicillin/sulbactam for treatment of resistant pathogens in critically ill patients because the PKs of these compounds have not been well defined.

Combination therapy with ampicillin/sulbactam for treatment of multi-drug resistant *Acinetobacter baumanni*

Combination therapies are supported mostly by in vitro and in vivo studies. These studies can define potential additive, synergistic or even antagonistic effects, although the significance of these combinations should always be evaluated clinically [196].

In vitro synergism towards MDR *A. baumannii* of ampicillin/sulbactam and meropenem, imipenem, fosfomycin, rifampicin and colistin has been shown [197-204]. Triple combination therapy of meropenem, sulbactam and colistin has consistently shown very high levels of synergy [197, 198]. Other combination therapies, without ampicillin/sulbactam, have also shown high levels of synergy against MDR *A. baumannii*, including colistin with a carbapenem [197]. Other combination therapies do have effects that are as strong as the above combinations, i.e. imipenem with tigecycline, amikacin or ciprofloxacin [198, 199].

In vivo murine studies have shown good results with rifampicin-based combination therapy [201, 202, 204]. Higher clinical success rates with combination therapy for MDR *A. baumannii* infections were shown in a recent study by Santimaleeworagun et al. [205]. Most patients received either sulbactam co-formulated with cefoperazone or fosfomycin as combination therapy, with success rates of 60.3% and 81.0% for monotherapy and combination therapy groups, respectively (P = 0.04). In multivariate analysis, renal impairment [Odd Ratio (OR) = 8.9, 95% confidence interval (CI) 1.2–39.5], bloodstream infection (OR = 6.4, 95% CI 1.2–33.7) and an inappropriate antimicrobial regimen (OR = 0.0, 95% CI 0.0–0.1) were independent predictors of treatment failure.

Conclusion

Serious bacterial infections that are caused by poorly susceptible organisms are a great challenge in the management of critically ill patients. Indeed, in most countries the treatment options for these patients are very limited. Ampicillin/sulbactam may be a useful agent for treating some of these MDR infections, in particular MDR *A. baumannii*, because of its good tissue penetration, good safety profile and availability in most countries. However, despite its safety and efficacy, ampicillin/sulbactam

suffers from several drawbacks; surveillance data have shown declining susceptibility of ampicillin/sulbactam for several common pathogens encountered among critically ill patients. There are many factors that could contribute to this scenario, including potential antibiotic under dosing. Limited PK/PD data in critically ill patients makes it difficult to predict its optimum doses that should be used to increase the likelihood of therapeutic success. This should be considered an important part of the optimal use of ampicillin/sulbactam, as clinical studies have shown inferior outcomes when lower doses are compared with higher doses.

Funding: JAR is funded in part by a National Health and Medical Research Council of Australia Research Fellowship (NHMRCAPP1048652)

Competing Interests: None declared.

Ethical approval: Not required.

5.3 Submitted manuscript `Simultaneous determination of Ampicillin and Sulbactam in human plasma and urine by UHPLC-MS/MS'

The manuscript entitled, `Simultaneous determination of Ampicillin and Sulbactam in human plasma and urine by UHPLC-MS/MS` is to describe the materials and method used for the sample analyses of ampicillin/sulbactam used throughout this Thesis. Ultra high performance liquid chromatography analysis was developed to determine ampicillin/sulbactam concentrations in plasma and urine samples.

All data collection, data interpretation and drafting of the paper were undertaken by Dr Steven Wallis, assisted by the PhD candidate, Syamhanin Adnan, Jenny Lisette Ordóñez Meija and Suzanne Parker. Prof Jason Roberts, Prof David Paterson and Prof Jeffrey Lipman oversaw all aspects of this chapter.

The manuscript is presented as submitted; except figures and tables have been inserted into the text at slightly different positions. Also, the numbering of pages, figures and tables has been adjusted to fit the overall style of the Thesis. The references are found alongside the other reference of the Thesis, in the section `Bibliography'.

"Simultaneous determination of Ampicillin and Sulbactam in human plasma and urine by ultra high performance liquid chromatography-tandem mass spectrometry"

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Keywords: validation, ultra high performance liquid chromatography, triple quadrupole, electrospray, critically ill

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Abstract

A rapid, sensitive, and simple method for simultaneous analysis of ampicillin and sulbactam in human plasma and urine was developed and validated. Plasma samples were treated with acetonitrile and dichloromethane to remove proteins and lipid soluble components, whilst urine was diluted. Separations were by ultra high performance liquid chromatography on a C18 column with a formic acid – acetonitrile gradient. Ampicillin and the internal standard (cefotaxime) were detected in positive mode and sulbactam in negative mode by tandem mass spectrometry. The calibration range for ampicillin was 1 to 200 µg/mL in plasma and 25 to 5000 µg/mL in urine, whilst for sulbactam is was 0.5 to 100 µg/mL and 125 to 2500 µg/mL, respectively. The method has been used in a pharmacokinetic study of co-formulated ampicillin and sulbactam in critically ill patients.

Introduction

Ampicillin is a beta-lactam antibiotic that binds to penicillin-binding proteins (PBPs) and inhibits bacterial cell wall synthesis, causing disruption of the bacterial cell wall and subsequently, bacterial cell death. However, resistant pathogens may produce β-lactamase enzymes that can hydrolyse ampicillin and reduce its efficacy.

Sulbactam has weak antibacterial activity, but is an effective beta-lactamase inhibitor. When co-administered with ampicillin, sulbactam extends the spectrum of activity to ampicillin-resistant gram negative and gram-positive bacteria [206].

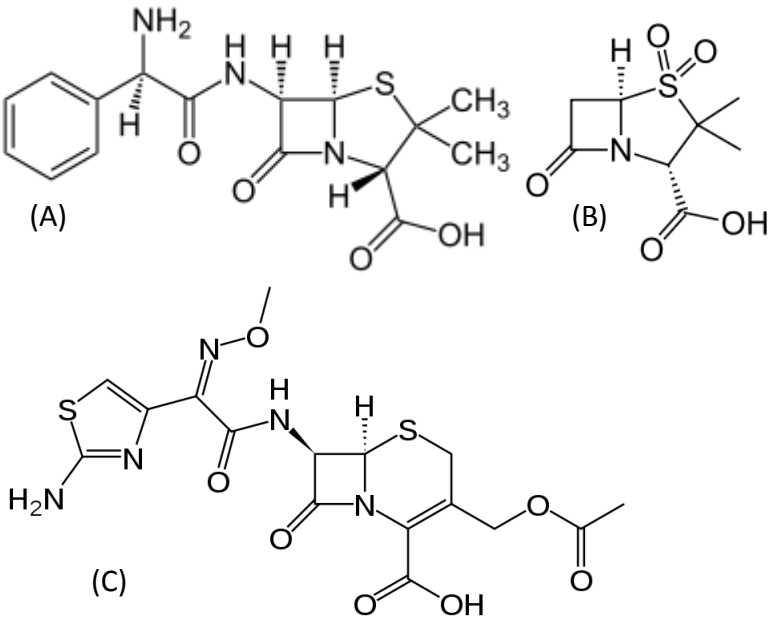
Ampicillin/sulbactam is β–lactam/β-lactamase inhibitor antibiotic that is co-formulated in a 1:2 ratio to maximize effectiveness. It is used for skin and skin-structure infections, intra-abdominal infections, gynaecological infections and other infections caused by ampicillin-susceptible bacteria.

Ampicillin/sulbactam has been of recent interest as one of the limited options available for the treatment of multi-drug resistant *A. baumannii* (MDR-Ab) [207]. However, the dose used has not been based on pharmacokinetic (PK) studies and to perform such investigation, it is necessary to measure both drug concentrations in plasma and urine, preferably simultaneously [207].

The analysis of ampicillin in plasma has been achieved by high performance liquid chromatography (HPLC) with [208, 209] and without [210, 211] derivatisation. Ampicillin has also been measured in urine [212]. In recent years, published methods for analysis of multiple antibiotics in plasma by ultra high performance liquid chromatography-mass spectrometry (UHPLC)/mass spectrometry (UHPLC-MS/MS) and high performance liquid chromatography-ultra-violet (HPLC-UV) have included ampicillin, although without analysis of sulbactam [213-218]. Sulbactam has been measured in plasma alone and with antibiotics other than ampicillin [219-222].

Methods for simultaneous analysis of ampicillin and sulbactam have been presented for quantification in bovine milk by UHPLC-MS/MS [223] and in pharmaceutical formulations by HPLC-UV [224, 225]. The authors could find only one method for simultaneous analysis of ampicillin and sulbactam in human plasma and/or urine [226]; Cazorla-Reyes' method, which was recently published and measures ampicillin and sulbactam with 19 other analytes in plasma and urine. The aim of this work was to produce a reliable, quick and sensitive method for simultaneous measurement of ampicillin and sulbactam (structures in Figure 4.1) in plasma and urine suitable for use in a clinical PK study.

Figure 5.1: Structure of two analytes and IS. (A) Ampicillin; (B) Sulbactam; (C) Cefotaxime



Materials and methods

Chemicals and Reagents

Ampicillin was sourced from Aspen (St Leonards, Australia), sulbactam from Sigma Chemical Co (St Louis, USA) and cefotaxime from Hospira (Melbourne, Australia). Acetonitrile was gradient grade (Merck, Darmstadt, Germany), while dichloromethane (Merck, Darmstadt, Germany) and formic acid (Ajax, Taren Point, Australia) were analytical grade. Distilled, deionised water was sourced from a Permutit system (Hartford, UK). Blank plasma was sourced from the blood bank and urine from volunteers.

Chromatography

UHPLC analysis was performed on a Shimadzu Nexera system equipped with dual pumps, autosampler with a sample compartment set to 5°C, and column oven set to 40°C. The column was a Shimadzu Shim-pack XR-ODS III (2.0 x 75 mm, 1.6 µm) with a Phenomenex C18 SecurityGuard ULTRA pre-column. The mobile phase was a gradient of solution A (0.1% formic acid in water) and solution B (0.1% formic acid in acetonitrile) as per Table 5.2. The mobile phase was delivered at 0.3 mL/min and generated a backpressure of approximately 5700 psi. A post-column valve directed mobile phase eluent to either waste or a Shimadzu 8040 triple quadrupole mass spectrometer. A volume of 0.2 µL was injected and the run time was 4.5 min.

Table 5.2: Time program for gradient elution

Time from injection (min)	***Composition of mobile phase***
0.0 to 0.5	7.5% B
0.5 to 1.3	7.5% B to 95% B
1.3 to 3.0	95% B
3.0 to 3.4	95% B to 7.5% B
3.4 to 4.5	7.5% B

Mass Spectrometry

The Shimadzu 8040 was fitted with an electrospray ionisation source operated with a nebulising gas (N_2) flow of 3 L/min, drying gas (N_2) flow of 15 L/min, heat block temperature of 400°C and a desolvation line temperature of 250°C. Ampicillin and the internal standard (cefotaxime) were measured by positive mode multiple reaction monitoring (MRM), whilst sulbactam was measured in negative mode. The mass spectrometry settings are presented in Table 5.3. A 50 ms dwell time was used. The collision gas was argon. A reference ion was monitored for peak purity in addition to the target ion used for quantitation.

Table 5.3: Mass Spectrometry Settings

Analyte	***Ampicillin***		***Cefotaxime***		***Sulbactam***	
Ion type	Target	Reference	Target	Reference	Target	Reference
Parent Ion	350	350	456.2	456.2	232.3	232.3
Daughter Ion	106.1	192	125	156	187.9	139.95
Q1 Pre Bias (V)	-30	-24	-30	-30	23	23
CE (V)	-21	-17	-45	-21	10	13
Q3 Pre Bias (V)	-20	-20	-24	-17	18	26

Solutions

Aqueous calibration standard stocks with combined ampicillin/sulbactam concentrations (in µg/mL) of 200/100, 100/50 and 50/25 were prepared and stored at -80°C. On the day of assay these stocks were serially diluted ten-fold with water to ampicillin/sulbactam concentrations of 20/10, 10/5, 5/2.5, 2/1 and 1/0.5 µg/mL. A separate set of ampicillin/sulbactam stocks, were prepared at 3000/1500, 600/300 and 60/30 µg/mL in water for quality control (QC) preparation. These stocks were diluted with blank plasma or blank diluted urine to quality control (QC) ampicillin/sulbactam concentrations of 150/75, 30/15 and 3/1.5 µg/mL, and aliquots stored at -80°C.

Sample Preparation

Samples were analysed in batches comprising standards, a blank and double blank, duplicate QCs and clinical samples. For plasma analysis, 100 µL of water (or calibration solution for standards), 100 µL of plasma (blank plasma for standards and blanks) and 100 µL of internal standard (100 µg/mL cefotaxime in water, or merely water for the double blank) were combined in a microfuge tube. Acetonitrile (400 µL) was added to precipitate proteins, followed by vortex mixing and centrifugation (5 min at 12000 rpm). Supernatant (500 µL) was transferred to a clean microfuge tube and 500 µL of dichloromethane added to partition the acetonitrile and lipid-soluble components, followed by vortex mixing and centrifugation (5 min at 12000 rpm). A 50 µL aliquot of the aqueous supernatant was transferred to an autosampler vial for analysis.

For urine analysis, 40 µL of urine sample was initially diluted with 960 µL of water. Then 100 µL of calibration standard, QC, diluted urine sample or blank was combined with 100 µL of internal standard (100 µg/mL cefotaxime in water, or merely water for the double blank). Following vortex mixing and centrifugation to settle any particulate matter, a 50 µL aliquot was transferred to an autosampler vial for analysis.

Validation

The method performance was validated separately for plasma and urine in accordance with the US Food Drug and Administration (FDA) guidance for industry on bioanalysis [125]. Linearity was assessed over 3 calibration curves, with the acceptance criterion for individual standards being within ±15% of nominal. Lower limit of quantification (LLOQ) was assessed by analysis of 5 replicates of the lowest calibrator with precision and accuracy criteria of being within ±20%. Matrix effects were assessed by quantifying ampicillin and Sulbactam at spiked at high, low and blank concentrations in 5 separate batches of matrix, with the criteria of precision and accuracy within ±15% and a blank signal of <20% of the LLOQ peak area. Precision and accuracy of the assay were assessed both intra-batch and inter-batch with 5 replicates at each QC level with criteria of ±15%. Stability was validated in terms of aqueous stock storage at -80°C, long term sample storage at -80°C, and

three freeze-thaw cycles. Recovery was assessed for the plasma method by comparing ampicillin, sulbactam and cefotaxime areas spiked pre-extraction with those obtained when spiked post-extraction. Precision was calculated as the percentage relative standard deviation (% RSD, being SD/mean as a percentage) and accuracy was calculated as the mean/nominal concentrations as a percentage.

Application

The method was used to measure concentrations of ampicillin and sulbactam in plasma and urine from critically ill patients at the Intensive Care Unit, Hospital Sungai Buloh, Malaysia. The study was approved by the ethics committee and informed consent was obtained from the patient or his/her substitute decision maker.

Results and Discussion

Mass Spectrometry

For all three analytes the molecular ion was formed in abundance and was used as parent ion, albeit the sulbactam parent was in negative mode whereas ampicillin and cefotaxime were formed in positive mode. Positive ion multiple reaction monitoring (MRM) of sulbactam was significantly poorer than negative ion. Two daughter ions were monitored for each parent; one ion was monitored as a reference ion with relative intensity for reference/target of 40%, 100% and 40% for ampicillin, sulbactam and cefotaxime, respectively. Ions were selected manually from precursor and product ion scans, and software-directed auto-optimisation routines conducted to confirm the absence of more preferable MRMs and to optimise voltages.

Chromatography

Chromatography for samples in plasma and urine are shown in Fig 5.2 and 5.3, respectively. Various gradient conditions were trialled to achieve the chromatography. Sufficient retention of the analytes was achieved so that the diverter valve could be used to send the poorly retained fraction of the injection to waste before directing the analyte-containing eluent into the MS for measurement. The pressure generated in the gradient (5700 psi) was modest for a UHPLC application and flow rate could have been increased, however the run time was suitable for our purposes and so was not pushed further for shorter analyses.

Figure 5.2: Chromatograms of ampicillin and sulbactam extracted from plasma. Depicted are a drug-free urine blank (A); a LLOQ calibration standard at ampicillin/sulbactam concentration of 0.5/1 µg/mL; an incurred sample containing ampicillin/sulbactam at 130/63 µg/mL

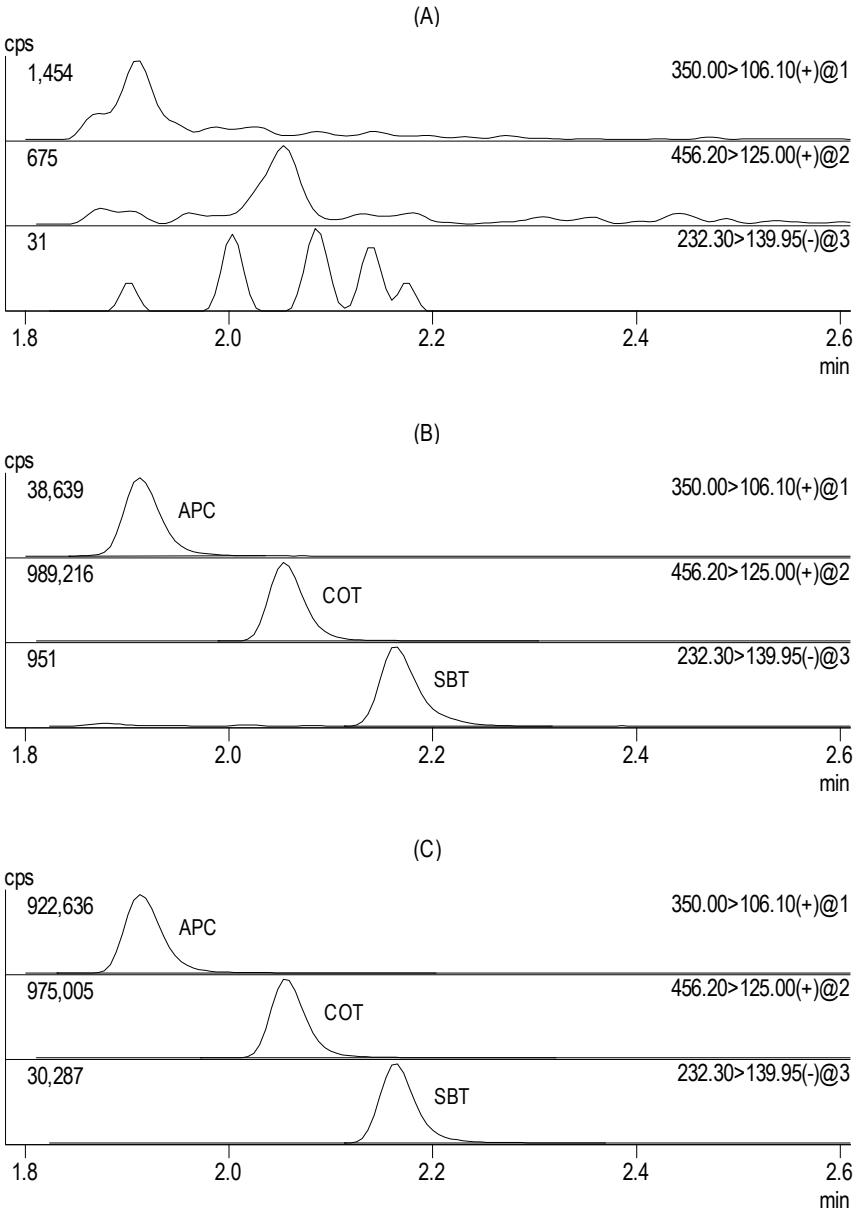
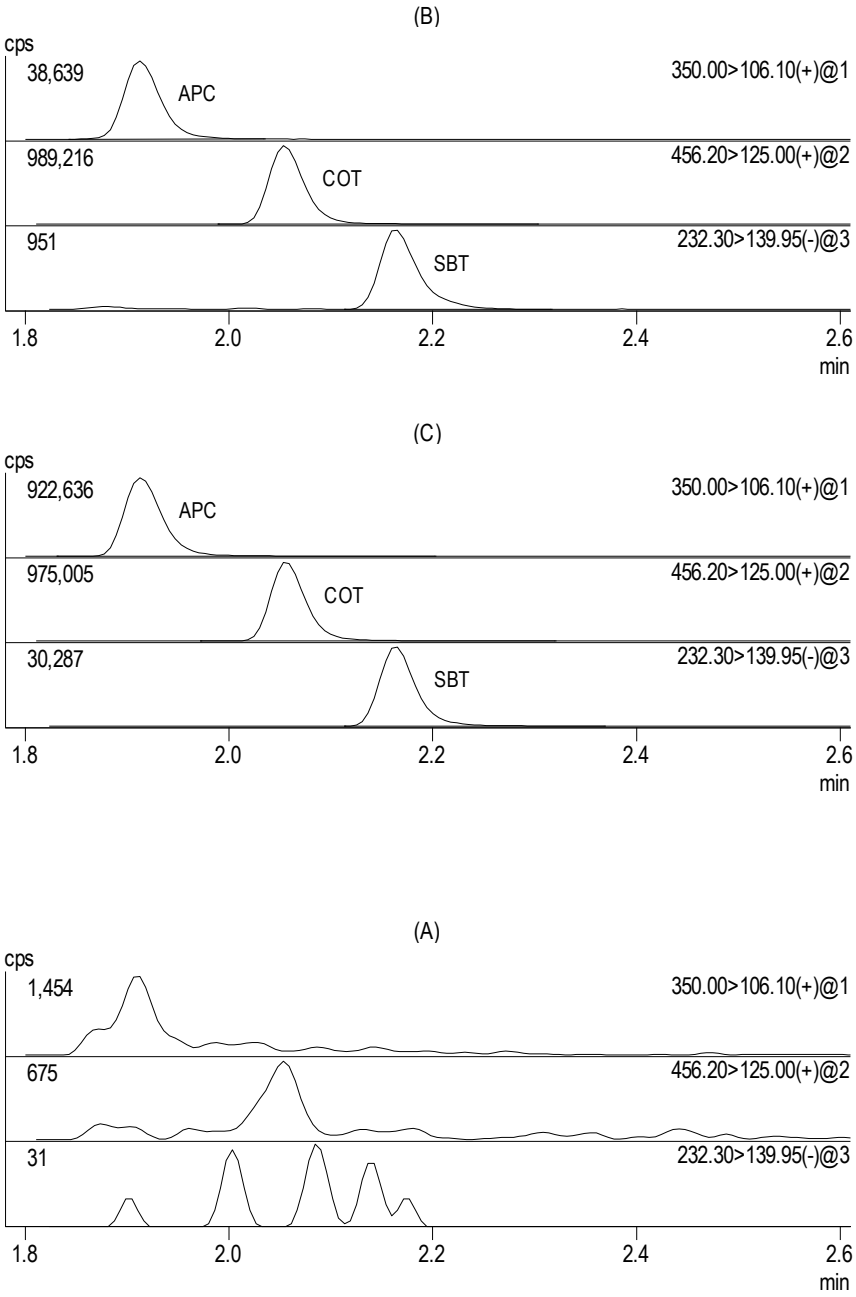


Figure 5.3: Chromatograms of ampicillin and sulbactam from urine samples. Depicted are a drug free urine blank (A), a calibration standard at ampicillin/sulbactam concentration of 0.5/1 µg/mL (B); an incurred urine sample at ampicillin/sulbactam concentration of 668/438 µg/mL



Sample Preparation

Protein precipitation is a quick, easy and cheap method of plasma sample preparation. However, crashing out the proteins still leaves a significant proportion of plasma components in the supernatant, including phospholipids. The dichloromethane wash, whereby an equal volume of dichloromethane is added to supernatant, partitions the acetonitrile and lipid soluble components away from the aqueous phase to produce a cleaner and more concentrated extract for injection.

Urine samples were diluted 25-fold to bring concentrations to the range that matches the plasma assay. Hence, the QCs at 150/75, 30/15 and 3/1.5 µg/mL represent sample concentrations of 3750/1875, 750/375 and 75/37.5 µg/mL and the calibration range covers undiluted urine concentrations of 25/12.5 to 5000/2500 µg/mL.

Validation

A straight line linear regression with a 1/concentration^2 weighting provided an adequate calibration equation with no indication of a quadratic relationship within the concentration range. The mean slope and intercept of the 3 calibration curves, mean regression coefficient (r^2) and the maximum % deviation (inaccuracy) of the calibrators of all 3 calibration curves are presented in Table 5.4.

Table 5.4: Calibration curve linearity validation results

	Plasma			***Urine***		
	Equation	r^2	Maximum % deviation	Equation	r^2	Maximum % Deviation
Ampicillin	y = 0.0363x - 0.0004	0.9971	±7.0%	y = 0.0278x +0.0010	0.9979	±10.6%
Sulbactam	y = 0.0034x +0.0001	0.9925	±13.3%	y = 0.0035x +0.0001	0.9952	±10.6%

The precision and accuracy of the plasma and urine assays at the LLOQ, as well as within and between batches at the QC levels, are presented in Tables 5.5 and 5.6 for Ampicillin and sulbactam. The acceptance criteria were met in all cases.

Table 5.5 : LLOQ, inter-and intra-batch precision and accuracy for ampicillin in plasma and urine

	Plasma			***Urine***		
	Mean (SD) (µg/mL)	Precision (%)	Accuracy (%)	Mean (SD) (µg/mL)	Precision (%)	Accuracy (%)
LLOQ	0.98 (0.02)	2.4	97.8	0.95 (0.04)	4.4	94.6
Intra-	3.12 (0.21)	6.6	104.0	3.05 (0.12)	3.8	101.6
batch	30.8 (1.4)	4.6	102.6	30.5 (1.3)	4.3	101.7
	149 (8)	5.0	99.1	154 (5)	3.0	102.8
Inter-	2.97 (0.07)	2.5	98.8	2.95 (0.06)	2.0	98.3
batch	28.8 (1.2)	4.0	95.8	29.9 (1.0)	3.3	99.8
	144 (6)	3.9	95.8	150 (8)	5.2	99.9

Table 5.6 : LLOQ, inter-and intra-batch precision and accuracy for sulbactam in plasma and urine

	Plasma			***Urine***		
	Mean (SD) (µg/mL)	Precision (%)	Accuracy (%)	Mean (SD) (µg/mL)	Precision (%)	Accuracy (%)
LLOQ	0.524 (0.054)	10.3	104.7	0.536 (0.054)	10.1	107.1
Intra-	1.49 (0.06)	4.3	99.2	1.45 (0.04)	3.1	96.5
batch	15.1 (0.4)	2.8	100.4	14.8 (0.2)	1.2	98.5
	80.3 (4.5)	5.6	107.0	74.5(1.1)	1.5	99.4
Inter-	1.45 (0.05)	3.5	96.6	1.47 (0.04)	2.6	97.8
batch	15.7 (0.8)	5.3	104.3	14.8 (0.2)	1.2	99.0
	74.9 (4.6)	6.2	99.8	76.1 (2.1)	2.8	101.4

The matrix testing indicated that the precision and accuracy of the assays were acceptable across multiple batches of matrix, as depicted in Table 5.7.

Table 5.7 : Matrix testing for ampicillin and sulbactam in plasma and urine

	Plasma			***Urine***		
	Mean (SD) (µg/mL)	Precision (%)	Accuracy (%)	Mean (SD) (µg/mL)	Precision (%)	Accuracy (%)
Low Ampicillin	0.97 (0.02)	1.8	97.2	10.08 (0.40)	3.9	100.8
High Ampicillin	98.4 (2.6)	2.6	98.4	48.8 (1.0)	2.0	97.6
Low sulbactam	-[a]	-[a]	-[a]	4.39 (0.35)	7.9	87.8
High sulbactam	49.5 (0.9)	1.9	99.0	23.8 (0.6)	2.7	95.0

[a] Not quantified at this concentration.

Validation revealed adequate recoveries for ampicillin (59.5%), sulbactam (70.1%) and cefotaxime (60.0%) from plasma. The performance of the method was not adversely affected by the less than complete recovery as demonstrated by there being sufficient signal at the LLOQ and acceptable precision and accuracy validation.

The specificity testing of the methods showed negligible change in peak area for Ampicillin or sulbactam in the presence of cefepime, ceftazidime, piperacillin, tazobactam, amoxicillin, ciprofloxacin, fluconazole, meropenem, ceftriaxone, doripenem and vancomycin.

Ampicillin and sulbactam met stability criteria under the storage conditions tested. Aqueous stocks stored at -80°C for 6 weeks demonstrated a %deviation of -7.5% for ampicillin and +6.1% for sulbactam. Plasma and urine QCs stored at -80°C for 6 weeks, and that underwent 3 freeze-thaw cycles prior to assays met stability criteria of ±10% in the majority of cases, except for sulbactam in urine at the lowest QC level (within ±12%) (Tables 5.8 and 5.9).

Table 5.8 : Stability validation results for ampicillin in plasma and urine

	Plasma			***Urine***		
	Mean (SD) (µg/mL)	Precision (%)	Accuracy (%)	Mean (SD) (µg/mL)	Precision (%)	Accuracy (%)
3x	2.86 (0.02)	0.7	95.4	3.16(0.23)	7.1	105.3
FT	28.1 (0.8)	2.7	93.6	30.0 (0.6)	1.9	100.0
	140 (4)	2.5	93.6	150 (13)	8.7	99.8
LTS	2.86 (0.16)	5.5	95.2	2.89 (0.03)	1.1	96.4
	28.0 (0.6)	2.0	93.3	29.9 (1.6)	5.3	99.7
	146 (3)	2.1	97.6	151 (6)	4.0	100.7

3x FT: Three freeze-thaw cycles; LTS: long term storage for 6 weeks at -80°C

Table 5.9 : Stability validation results for sulbactam in plasma and urine

	Plasma			***Urine***		
	Mean (SD) (µg/mL)	Precision (%)	Accuracy (%)	Mean (SD) (µg/mL)	Precision (%)	Accuracy (%)
3x	1.47 (0.05)	3.1	98.0	1.32 (0.06)	4.4	88.2
FT[a]	15.6 (0.8)	4.8	104.0	15.4 (0.6)	3.9	102.7
	74.8 (0.9)	1.1	99.7	75.4 (3.8)	5.0	100.6
LTS[b]	1.46 (0.05)	3.1	97.3	1.49 (0.17)	11.5	99.1
	14.7 (1.0)	7.1	98.0	14.2 (0.5)	3.5	94.9
	66.9 (2.0)	3.0	89.2	-[c]	-[c]	-[c]

[a] Three freeze-thaw cycles; [b] long term storage for 6 weeks at -80°C; [c] Not quantified at this concentration.

Application

This method has been successfully applied to a 10-patient clinical trial with 70 plasma and 9 urine samples. A representative plasma concentration – time profile is presented in Fig 4; the samples were collected from a 54.3 kg, 33 year old male on receiving his seventh dose of 2 g ampicillin / 1 g sulbactam (four hourly intravenous administration). The concentration in urine collected over the 6-hour sampling period was 668 µg/mL ampicillin and 438 µg/mL sulbactam.

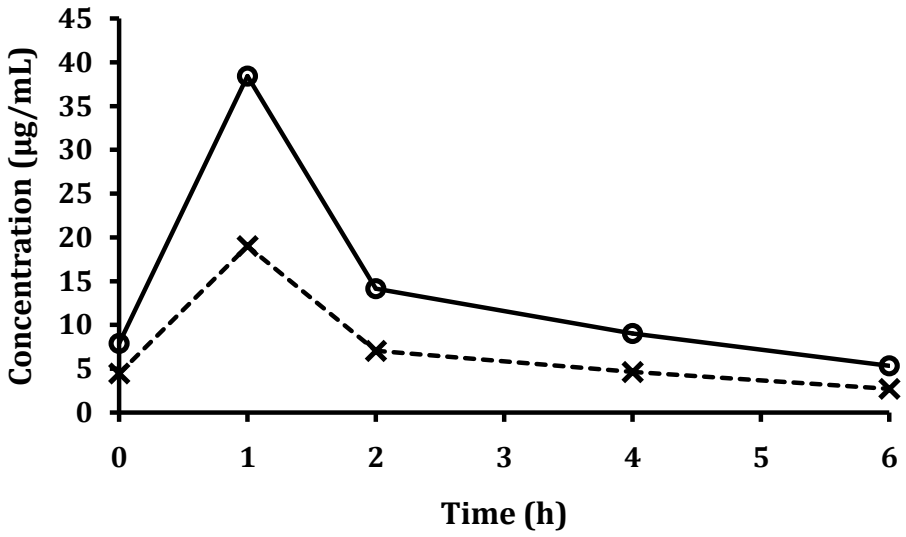


Figure 5.4 : Plasma concentration-time plot for ampicillin (circles, solid line) and sulbactam (crosses, dotted line) in a patient.

Conclusion

The method presented here is the only method yet published for exclusive, optimised measurement of ampicillin and sulbactam in plasma and urine. The assay performance is accurate and precise, with sufficient sensitivity, quick and easy sample preparation, and robust instrumental analysis. It has been demonstrated to be suitable for PK study applications and could be used where therapeutically-relevant concentrations require measurement.

Acknowledgements

Jason Roberts is funded by a Career Development Fellowship from the National Health and Medical Research Council of Australia (APP1048652).

5.4 Submitted manuscript `Pharmacokinetics of Ampicillin/Sulbactam in Critically Ill Patients at Risk of Multi-Drug Resistant *Acinetobacter baumannii* Infections'

The manuscript entitled, `Pharmacokinetics of Ampicillin/Sulbactam in Critically Ill Patients at Risk of Multi-Drug Resistant *Acinetobacter baumannii* Infections' is to describe the PK study of ampicillin/sulbactam in critically ill patients at risk of MDR *A. baumannii* infections..

All data collection, data interpretation and drafting of the paper were undertaken by the PhD candidate, Syamhanin Adnan, assisted by Dr Shanthi Ratnam and Dr Suresh Kumar. Dr Steven Wallis assisted with bio-analysis of the PK samples. Prof Jason Roberts assisted with the data analysis and data interpretation. Prof David Paterson and Prof Jeffrey Lipman oversaw all aspects of this paper.

The manuscript is presented as submitted; except figures and tables have been inserted into the text at slightly different positions. Also, the numbering of pages, figures and tables has been adjusted to fit the overall style of the Thesis. The references are found alongside the other reference of the Thesis, in the section `Bibliography'.

"Pharmacokinetics of Ampicillin/Sulbactam in Critically Ill Patients at Risk of Multi-drug Resistant Acinetobacter baumannii Infections"

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Keywords: pharmacokinetic, Ampicillin/sulbactam, critically ill, multi-drug resistant Acinetobacter baumannii infections

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Abstract

Ampicillin/sulbactam is one of the few antibiotic options available for treatment of multi-drug resistant *Acinetobacter baumannii*. The aim of this study is to describe the pharmacokinetics of ampicillin/sulbactam in critically ill patients at risk of multi-drug resistant *A. baumannii* infections. Serial plasma samples were collected over at least one dosing interval. Drug concentrations were measured using validated chromatographic method. Nine patients, (8 male, 1 female) were recruited in this study with median age of 33 years. The median (inter quartile range) pharmacokinetic parameter estimates for ampicillin were: area under concentration-time curve for dosing interval (AUC): 195.7 (78.1-490.5) mg.h/L, clearance (CL): 10.2 (5.5-25.6) L/h, volume of distribution (Vd): 0.6 (0.4-0.7) L/kg. For sulbactam, the pharmacokinetic parameter estimates were: AUC: 108.1 (43.8-430.0) mg.h/L, CL: 9.2 (2.4-22.8) L/h, Vd: 0.5 (0.3-0.6) L/kg. The median estimate for drug clearance was also found to be 72% lower for ampicillin and 54% lower for sulbactam compared to data from healthy volunteers. Both ampicillin and sulbactam clearance was correlated with creatinine clearance (r^2=0.9; P=0.001 and r^2=0.8; P=0.0003 respectively). Two thirds of patients did not have sulbactam concentrations exceeding target concentrations (4mg/L) throughout the dosing interval. Higher initial doses for those patients with a high Vd and adjustment of subsequent doses in accordance with renal function should be considered to optimize dosing in these challenging patients.

Introduction

Ampicillin/sulbactam is β–lactam/β-lactamase inhibitor antibiotic that was co-formulated to maximize its effectiveness. It is used for skin and skin-structure infections, intra-abdominal infections, gynaecological infections and other infections caused by ampicillin-susceptible bacteria [133]. Sulbactam has been shown to have intrinsic activity against *Acinetobacter baumannii* and it is one of the limited options available for the treatment of multi-drug resistant *A. baumannii* (MDR-Ab) [144]. In Malaysian ICU, MDR-Ab has been reported to be the most common isolate associated with ventilator associated pneumonia [227]. For MDR-Ab, susceptibility is mostly limited to only sulbactam-combination antibiotics and colistin [141]. Of concern, a recent review had also highlighted the decreasing trend in susceptibility of sulbactam-combination antibiotics and in some countries, which has also been observed in Malaysia [228]. In some countries, colistin may be the only option available for MDR-Ab [229]. More recently, a retrospective study was conducted to compare monotherapy of colistin and combination therapy of colistin/sulbactam with promising results for the combination of colistin/sulbactam [230]. Furthermore, another retrospective study also emphasises the utility of sulbactam in this context and compared colistin and ampicillin/sulbactam for MDR-Ab and showed similar clinical responses between therapies [231]. Both of these studies have used high doses of sulbactam; between 3g – 4g per day. However, the dose used has not been based on pharmacokinetic (PK) studies and an enhanced understanding of ampicillin/sulbactam PK may help procure better dosing for these patients. The target exposure for antibiotics is guided by the minimum inhibitory concentration (MIC) of the target bacterial pathogen. Ampicillin is a time-dependent antibiotic ($fT_{>MIC}$) whereas the pharmacodynamic of sulbactam is not as well characterised but has been considered to be dependent on the ratio of the area under curve (AUC) to MIC, i.e. AUC/MIC [160, 161]. Emerging data suggests that $fT_{>MIC}$ may also be important for sulbactam [181, 232] . According to the Clinical and Laboratory Standards Institute (CLSI), the equivalent MIC breakpoints for ampicillin/sulbactam for susceptible, intermediate and resistant *A. baumannii* are 8/4 mg/L, 16/8 mg/L and 32/16 mg/L, respectively. Since sulbactam is a bacteriostatic agent, it is recommended for use as high doses; which of course, can increase the risk of toxicity. Empiric therapy with high doses of ampicillin/sulbactam, is common in

countries where there is a high risk of MDR-Ab. To date, we are unaware of any studies describing the PKs of ampicillin/sulbactam in critically ill patients. A recent review of the dose recommendations from other patient groups has proposed that doses between 4g – 12g/day may be required for critically ill patients [233]. The aim of this study is to describe the PKs of ampicillin/sulbactam in critically ill patients at risk of MDR-Ab infections.

Materials and methods

This was a prospective open labelled PK study conducted at Intensive Care Unit, Hospital Sungai Buloh. Critically ill patients who met the following criteria were eligible for inclusion: a) written informed consent had been obtained from the patient or his/her substitute decision maker; b) clinical indication for ampicillin/sulbactam therapy; c) 18 years old or above and d) an intra-arterial catheter in situ (for the purposes of blood sampling). Patients were excluded from the study if one or more of the following criteria were met: a) renal replacement therapy and b) history of allergy to study antibiotics and expected antibiotic cessation within 24 h of identification of expected sampling.

Antibiotic administration and sample collection

All samples were collected over a single dosing interval. Standard doses, in accordance to local practice (2g/1gm intravenous (i.v) every 4 h) were administered. Blood samples were drawn at five time points, this was at 0 (pre-dose), 1.0 (end of infusion), 2, 3 and 4 hours post dose. All samples were immediately placed in polypropylene tubes on ice and were centrifuged at 3000 rpm for 10 minutes within 4 hours of collection. The plasma and supernatant were removed; aliquots of the plasma were placed into labelled polypropylene screw-cap cryo-vials and stored at -80°C until assay. All samples were sent frozen on dry ice from Hospital Sungai Buloh, Malaysia to Burns, Trauma and Critical Care Research Centre, The University of Queensland, Australia where they were stored at -80°C until assay.

Data collection

Data were collected while the patient was in the ICU, from the patient medical record obtained through computerized system. Data collected included the following: a) relevant medical history, b) demographic data, c) antibiotic therapy details, d) SOFA (Sequential Organ Failure Assessment) score [51] and f) blood biochemistry. Creatinine clearance was calculated using the Cockcroft-Gault equation [234].

Assay

Ultra high performance liquid chromatography analysis with reverse phase chromatography was performed on a Shimadzu Nexera system. Ampicillin and sulbactam in plasma were measured using a Shimadzu 8040 triple Quadruple mass spectrometer. Ampicillin was measured by positive mode MRM, whilst sulbactam was measured in negative mode.

All samples were assayed alongside calibration standards and quality controls prepared by spiking drug into matching drug-free biological matrix. Assays were validated and conducted using criteria from the US Food and Drug Administration guidance on bioanalysis [125].

The precision and accuracy of the methods were validated to be within 7% in plasma at low, medium and high concentrations of the calibration range. Range of linearity is 1-200 µg/mL for ampicillin and 0.5-100 µg/mL for sulbactam.

PK Analysis

A non-compartmental PK analysis was performed to describe the disposition of ampicillin and sulbactam. The C_{max} was the observed maximum concentration at the end of infusion and trough concentration (C_{min}) was the observed minimum concentration prior to drug administration. The area under the concentration-time curve (AUC) from 0 to 4 hours was calculated using the trapezoidal rule. The AUC extrapolated to infinity ($AUC_{0-\infty}$) was calculated using AUC and the elimination rate constant (k_{el}). The kel was calculated as the negative slope of the non-weighted squares curve fit of the final 2 sampling points during the elimination phase. Clearance (CL) was calculated as dose/$AUC_{0-\infty}$. The volume of distribution (V_d) was

calculated as CL/ k_{el}. Half-life was calculated at 0.693/k_{el}. All data are presented as median (inter quartile range (IQR)).

Statistical Analysis

Statistical analysis was performed using Graphpad Prism version 5.0 (GraphPad Software Inc., La Jolla, CA). Linear regression describing the relationship between drug clearance and creatinine clearance as well as volume of distribution with body weight was performed. P-values <0.05 were considered significant.

Results

Nine patients, (8 male, 1 female) were recruited for this study. Demographic and clinical data are presented in Table 5.10. A wide range of age and renal function was observed in this cohort with median age of 33 (IQR : 23-62) years and median creatinine clearance of 119 (IQR : 76-150) mL/min. The median body weight was 61 (IQR : 54-67) kg and Sequential Organ Failure Assessment (SOFA) score was 3.5 (3.0-5.0). One third of the patients in this study were above 60 years old, with creatinine clearance less than 100 mL/min. Moreover, 55% (n=5) of the studied patients also displayed an elevated creatinine clearance (>130mL/min). PK parameter estimates for both ampicillin and sulbactam contrasted against published data from healthy volunteers [173] are shown in Table 5.11. Compared to healthy volunteers' data, both study drugs had a prolonged half-life and a reduced CL which was 72% and 54% lower for both ampicillin and sulbactam, respectively. The PK parameter estimates for both drugs, as shown in Table 4.3, were widely variable. The V_d was found to be 50% larger for ampicillin and 60% larger for sulbactam. Figure 5.5 displays the concentration-time profile for ampicillin and sulbactam in plasma compared to relevant MIC values. The percentage of patients that did not achieve 100% $fT_{>MIC}$ for sulbactam at 4 mg/L, 8mg/L and 16 mg/L on the first sampling occasion were 66%, 78% and 78%, respectively. Linear regression analyses of antibiotic clearance and creatinine clearance are shown in Figure 5.6. Significant correlations with creatinine clearance were observed for both ampicillin (r^2=0.9; P=0.001) and sulbactam (r^2=0.8; P=0.0003). For V_d, correlations with body weight, were not statistically significant for either ampicillin (r^2=0.2, P=0.15) or sulbactam (r^2=0.2,P=0.2).

Table 5.10 : Demographic and clinical data of included patients

Parameter	***Median (IQR)***
Age (years)	33 (23-62)
Weight (kg)	61 (54-67)
SOFA	3 (3-5)
Creatinine clearance (mL/min)	119 (76-150)
Serum creatinine concentration (µmol/L)	52 (51-65)

Table 5.11 : Pharmacokinetic parameter estimates of ampicillin and sulbactam in critically ill patients at risk of multi-drug resistant *Acinetobacter baumannii* infections compared to published healthy volunteer data. Data presented either as median (Inter Quartile Range) or mean (Standard Deviation).

Pharmacokinetic parameter	***ICU patients at risk of Acinetobacter baumannii infections***		***Healthy volunteers*** [164]	
	Ampicillin	Sulbactam	Ampicillin	Sulbactam
Half-life (h)	2.1 (0.9-4.0)	2.2 (0.9-5.2)	1.1	1.0
Clearance (L/h)	10.2 (5.5-25.6)	9.3 (2.4-22.8)	17.6 ± 3.5	14.2 ± 3.7
Volume of distribution (L/kg)	0.6 (0.4-0.7)	0.5 (0.3-0.6)	0.2	0.2
Area under curve 0-4 h (mg.h/L)	121.0 (73-204.7)	67.4 (39.1-111.5)	NA	NA
Maximum concentration (mg/L)	58.0 (42.7-103.6)	35.4 (22.4-53.8)	120	60
Minimum concentration (mg/L)	8.0 (3.4-51.7)	3.7 (1.7-27.2)	NA	NA

Figure 5.5 : Ampicillin (top panel) and sulbactam (low panel) concentration-time profile. The clinical and Laboratory Standards Institute susceptibility breakpoints for ampicillin/sulbactam against *A. baumannii* are shown as dotted lines. For sulbactam, minimum inhibitory concentrations 4mg/L is susceptible, 8mg/L is intermediate and 16mg/L is the resistant breakpoint.

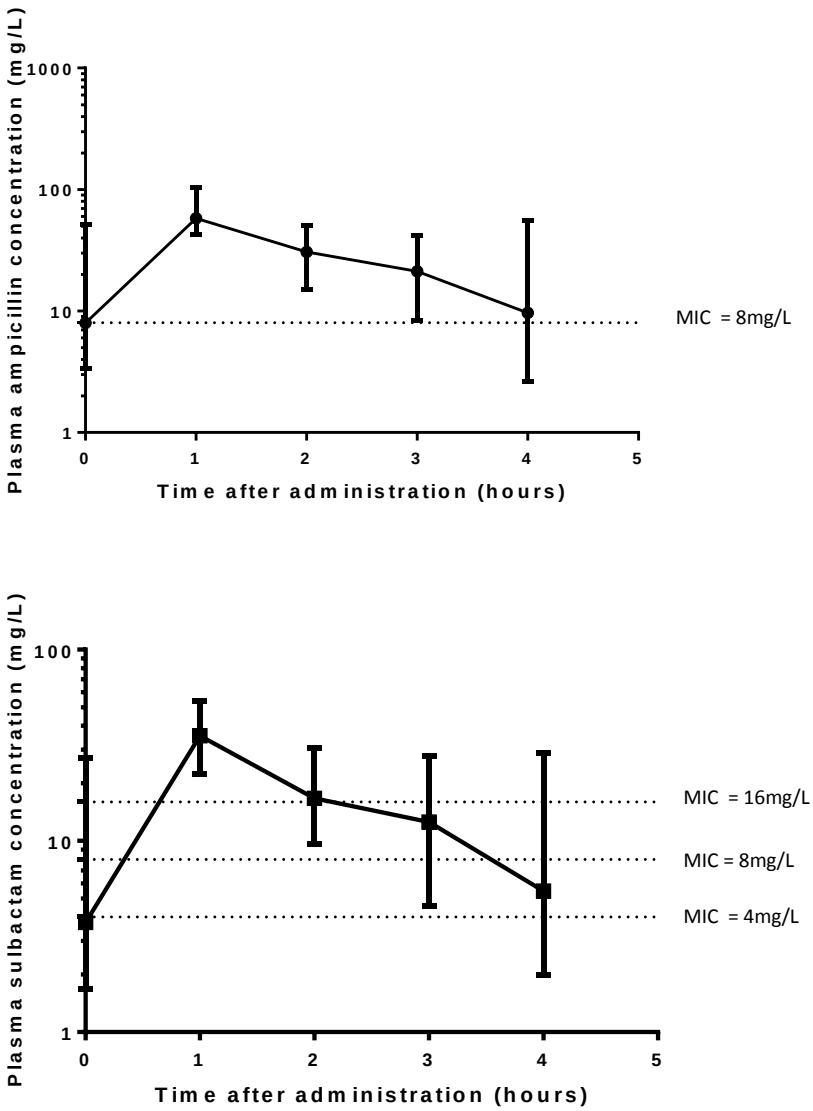


Figure 5.6a : The relationship between creatinine clearance and ampicillin clearance. The solid line is the line of linear regression (r^2=0.9, P=0.001)

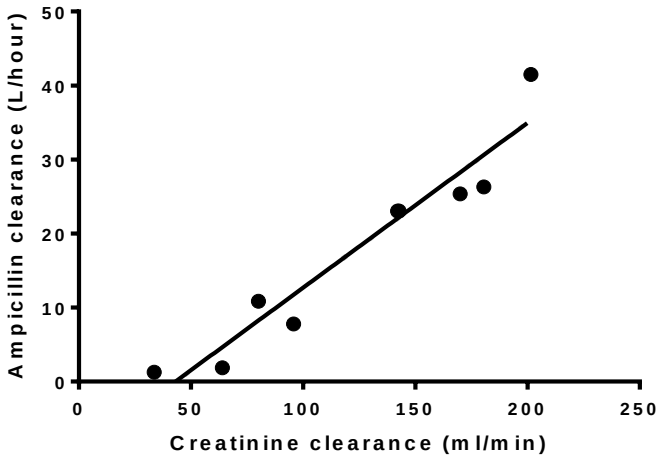
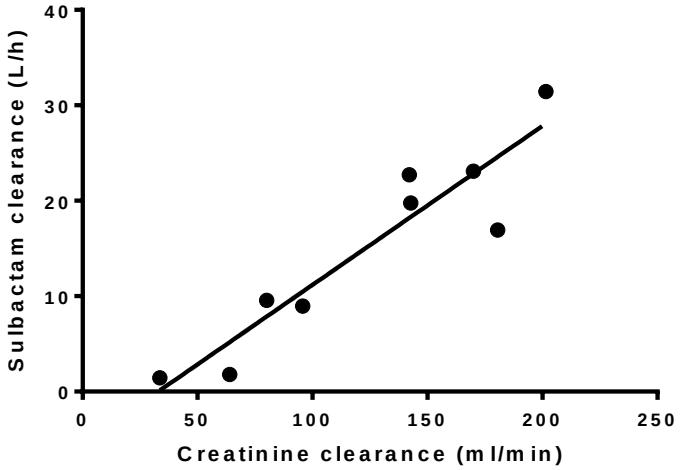


Figure 5.6(b) : The relationship between creatinine clearance and sulbactam clearance. The solid line is the line of linear regression (r^2=0.8, P=0.0003)



Discussion

To the best of our knowledge, this is the first PK study of ampicillin/sulbactam in critically ill patients. We have has shown significant differences in PK parameters of ampicillin/sulbactam between critically ill patients, at risk of *A. baumannii* infections, compared with healthy volunteers'. The renal function of this studied cohort shows marked variability although this is not unexpected; given that severity of illness may differ significantly among critically ill patients.

The reduced drug clearance shown in this study was due to the fact that some of the studied patients manifested elevated serum creatinine concentrations indicative of acute kidney injury. We observed a significant correlation between drug clearance and creatinine clearance (P=0.001) as has been previously shown for many renally cleared antibiotics. These findings suggest that that there is a risk of excessive drug concentrations with decreased renal function and vice versa; and inadequate drug concentrations with increased renal function. This was clearly seen in this study; high doses of ampicillin/sulbactam (2g/1g every 4 hours) resulted in concentrations greater than 50 mg/L of ampicillin for those subjects with decreased creatinine clearance, and greater than 20 mg/L for sulbactam. Similarly, for those subjects with an elevated creatinine clearance (>130mL/min), trough concentrations were found to be low, less than 10 mg/L to ampicillin and less than 5 mg/L for sulbactam. This data support dose adjustment of ampicillin/sulbactam according to the renal function to avoid potential toxicity.

Elevated renal clearance, also known as augmented renal clearance (ARC) has considerable consequences for altered antimicrobial concentrations and often occurs in those who do not have renal impairment and have received adequate fluid resuscitation during their intensive care unit (ICU) admission [185]. ARC is more prominent in young traumatized and postoperative patients and those with low illness severity scores [45]. However, since the creatinine clearance in this study was estimated using the Cockcroft-Gault equation [234], which has been reported to have significant bias and imprecision compared to a measured urinary creatinine clearance, this equation may not have been able to identify ARC patients [43] and thus should be considered a limitation of this study.

The increased V_d of both study drugs implies extensive distribution out of the blood stream and the potential for lower than expected concentrations; at least in the initial phase of therapy. Indeed, inadequate trough concentrations were observed in some of the studied subjects, which did not exceed the susceptibility breakpoint for *A. baumanni,* thereby increasing the risk of therapeutic failure. It was also observed that there is no significant correlation of V_d with body weight, a possible explanation for this is because of the molecular characteristics of ampicillin and sulbactam. Ampicillin and sulbactam are hydrophilic antibiotics which are typically distributed into plasma and the interstitial fluid of tissues with poor penetration into adipose tissues [143]. Therefore, changes in V_d for these antibiotics are not dramatically affected by variations in body weight but are greatly affected by fluid shifts in extravascular compartments [235, 236]. Fluid expansion is a common phenomenon seen in critically ill patients and can lead to inadequate drug concentrations, as clearly shown in this study [142]. Therefore, this study would support giving higher initial doses for those patients with a high V_d and adjustment of subsequent doses in accordance to renal function [237].

There are some limitations of this study we wish to declare, firstly the small sample size may mean that not all the likely PK variability in the critically ill population has been captured in this cohort. The measurement of renal function using the Cockcroft-Gault equation may not be accurate for patients with unstable serum creatinine concentrations. Finally, the generally low body weight of the included patients may mean that some PK observations may not be indicative of the PKs of higher weight and obese patients.

Conclusion

The wide variability in PKs seen between subjects in this study and its significant differences from those observed in healthy volunteer data, illustrate the potential problems with current dose recommendations. This study has shown a wide range of drug exposures including inadequate ampicillin and sulbactam exposures which increases the likelihood of sub-optimal patent outcomes. Dose modifications based on estimates of renal function are particularly important in critically ill patients.

5.5 Conclusion on ampicillin/sulbactam: its potential use in treating infections in critically ill patients

This chapter has described the characteristics of ampicillin/sulbactam and its potential use in treating infections in critically ill patients. From this chapter, ampicillin/sulbactam is shown to have extensive tissue penetration making it a suitable antibiotic choice for many infection sites. It also has intrinsic activity against *A. baumannii;* an increasingly important pathogen in intensive care settings. Nevertheless, decreasing susceptibility of pathogens to ampicillin/sulbactam limits its use. Dose recommendations are also variable and not evidence-based, ranging from 4g/day – 12g/day. For those available PK studies; none were conducted in critically ill patients and therefore, PK studies to define the necessary doses of ampicillin/sulbactam in these patients are an important next step to ensure better use this valuable drug combination in this patient population. Our subsequent PK study has shown that there are significant differences in the PK of ampicillin/sulbactam between critically ill patients with healthy volunteers with wide variability of PK parameter estimates common to the critically ill. In our study, almost two thirds of patients did not have sulbactam concentrations exceeding target concentrations throughout the dosing interval. We also observed a significant correlation between drug clearance with creatinine clearance. We therefore propose that higher initial doses of ampicillin/sulbactam should be used for those patients likely to have increased volumes of distribution, whilst dosage adjustment according to renal function is also strongly suggested.

6. Summary and direction for future research

The importance of antimicrobial treatment of critically ill patients with severe infections is undeniable. The main objective of this Thesis is to improve the use of antimicrobials in common clinical scenarios encountered by critically ill patients, particularly for hospitals that commonly have patients admitted with trauma and infectious diseases. Chapter 1 describes the challenges associated with three different clinical scenarios.

Critically ill patients are a special patient population with marked changes in their physiology and this is the main reason why standard antimicrobial doses are unable to produce consistent therapeutic responses. These physiological changes discussed in detail in Chapter 2. Four principle physiological changes that affect antimicrobial PK are fluid shifts, organ dysfunction, changes in protein binding and circulatory failure. Sepsis, AKI, hepatic failure, fluid resuscitation and burns are among factors that could lead to fluid shifts in critically ill patients. All these changes can affect the two PK parameters that define dosing requirements, Vd and Cl. Changes in Vd could cause changes in PK particularly during the initial phase of treatment with changes in Cl having implications for the later phase of treatment. These changes may affect the likelihood of achieving adequate drug concentrations and subsequently, require changes in drug dosing.

Elevation of renal clearance, also known as ARC, is one of the physiological changes that may occur in critically ill patients and this was described in Chapter 3. This Chapter of the Thesis looks into the incidence of elevated renal clearance in Malaysian critically ill patients. The study included in this Thesis has shown that ARC is common in ICU patients. It was observed that nearly half of the recruited patients had ARC, with most patients being male, admitted post trauma and having undergone emergency surgery. Convenient bedside methods to identify these patients are required. Significant imprecision was demonstrated when comparing estimated Cockcroft-Gault creatinine clearance and a measured urinary creatinine clearance, with a larger bias in ARC patients. It follows that those patients at risk of ARC require more careful evaluation of their kidney function; with accurate determination only possible at this stage using a measured creatinine clearance. The

present study did not describe the relationship between ARC and sub-therapeutic drug concentrations and whether this correlates with a negative patient outcome. It is proposed that this aspect be further examined in future studies.

For this reason, PK studies conducted in critically ill patients are required to describe drug behaviour and therefore, to provide guidance for more accurate dosing that improves the likelihood of positive clinical outcomes for critically ill patients.

IAI is one of the common infections among critically ill patients, especially in trauma centre hospitals. Chapter 4 of this Thesis addresses this topic, which includes a published manuscript that reviews all PK studies of beta-lactam antibiotics used in intra-abdominal disease. In this review, higher Vd values were shown for most beta-lactam antibiotics in IAI patients, twice as high for both meropenem and piperacillin and 20% higher for ceftazidime. Higher Cl were observed for post abdominal surgery patients, four times greater for imipenem-cilastin, three times higher for cefepime and two times greater for doripenem. A higher initial dose would be required for less susceptible pathogens in the management of IAI. For beta-lactams, more frequent dosing would be indicated for post-operative patients. The possibility of drug clearance through surgical drain in IAI patients was also highlighted in this review, which has led to a published PK study, conducted in this patient group.

Surgical drains are a fairly common procedure for critically ill patients, especially for patients with intra-abdominal pathologies, like IAI. This invasive procedure, together with the intra-abdominal disease, as discussed above, can cause marked changes in the patient's physiological condition and therefore, will lead to changes to antimicrobial PK. A study conducted in this Thesis in these patients has shown that the standard doses of the studied drugs (1 gm IV 8 hourly for meropenem and 4.5 gm IV every 6 hourly for piperacillin/TZP) have extensive drug distribution and larger doses are recommended to avoid low drug concentrations. This study has also found that the amount of the drug cleared through the surgical drains correlates with the volume of output of inflammatory fluid through the surgical drains. Therefore, in the presence of a larger volume of drain fluids, exceeding 1000 mL per day, supplemental antimicrobial doses should be considered. However, this study could

not specify the magnitude of the supplemental dose to be given and this is suggested as an important area of future research.

Chapter 5 of this Thesis also looked into the potential use of ampicillin/sulbactam in critically ill patients, particularly for those at risk of infection by the resistant pathogen, MDR-Ab. This chapter includes a published review of ampicillin/sulbactam; specifically on its use in critically ill patients. Various doses have been studied and standard doses, 2-4g/day of sulbactam, used in non-critically ill patients, have shown lower success rates when used in critically ill patients. Higher doses, up to 12g/day of sulbactam, have been shown to have similar outcomes compared to colistin. The available PK studies for this particular drug support a dosing regimen of 3g of sulbactam given as a 4 hour infusion, 8 hourly in order to achieve optimal exposure for less susceptible pathogens. However, this PK study was not conducted in critically ill patients and the absence of PK studies on ampicillin/sulbactam involving critically ill patients, together with the challenges in managing resistant pathogens does not permit this particular antimicrobial to be used optimally. Therefore, a PK study was carried out in this Thesis, on the standard doses used locally in Malaysian intensive care, 3g ampicillin/sulbactam IV 4 hourly. PK analysis has shown marked PK variability, which is significantly different to that reported in healthy volunteers. A significant correlation was found between drug clearance and creatinine clearance in this study supporting dosing that is adjusted to the patient's renal function. Higher drug concentrations were observed in patients with decreased renal function and vice versa; low concentrations were seen in patients with elevated renal function. Therefore, it is suggested that renally impaired patients should be given lower doses than are currently used. Patients with ARC require higher than maximum doses. For those patients that had received excessive fluid resuscitation, larger doses may be needed during the initial phase of the treatment. However, due to several drawbacks in this study, more specific dosing guidelines are not able to be produced. This dosing guideline could probably be made available if a study with larger sample size is carried out and a population PK analysis is performed which would be the natural next step for research in this area.

7. Reference

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