THE ASSOCIATION OF ANTIBIOTIC PHARMACODYNAMIC INDICES WITH SURVIVAL IN HUMAN SEPTIC SHOCK

BY

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

ACCP/SCCM: American College of Chest Physicians/Society of Critical Care Medicine

APACHE II: Acute Physiology and Chronic Health Evaluation

AST: Antimicrobial Susceptibility Testing

AUC: Area Under the serum Concentration-time curve at steady-state over 24 h unless otherwise stated. It is equivalent to a single dose AUC0–α

BAP: Blood Agar Plate

CA-MRSA: Community Acquired – Methicillin Resistant S. aurous

CAP: Community-Acquired Pneumonia

CDC: the Centers for Disease Control and Prevention

CI: Confidence Interval

CL: total clearance

CLSI: Clinical and Laboratory Standard Institute

C_{max} or C_{peak} concentration: the highest concentration reached or estimated

C_{min}: trough concentration or the lowest concentration reached.

Css: Steady state concentration

CO: Cardiac Output

COPD: Chronic Obstructive Pulmonary Disease

CrCl: Creatinine clearance

DBW: Dosing Body Weight

DM: Diabetes Mellitus

DRC: Dynamic Response Concentration

ED: Emergency Department

ESBL: Extended Spectrum β-Lactamase

Fu: Fraction of drug excreted in urine

GFR: Glomerular Filtration Rate

HSC: Health Sciences Centre, Winnipeg, Manitoba

HTM: *Haemophilus* Test Medium

IAI: Intra-abdominal infection

ICD-9-CM: International Classification of Diseases, 9th Revision, Clinical Modification codes for principal discharge diagnosis

IQR: interquartile range

ICU: Intensive Care Unit

ke: elimination constants rate

LOS: Length of Stay

MBC: Minimal Bactericidal Concentration

MIC: Minimal Inhibitory Concentration

MODS: Multiple Organ Dysfunction Syndrome

MAP: Mean Arterial Pressure

MH: Mueller-Hinton Agar

NHDS: National Hospital Discharge Survey database

OR: Odds Ratios

PAMPs: Pathogen-Associated Molecular Patterns

PBP: Penicillin-Binding Proteins

PK/PD: Pharmacokinetics /Pharmacodynamic

PROGRESS: Promoting Global Research Excellence in Severe Sepsis database

PRRs: pattern Recognition Receptors

P value: $P \leq .05$ was considered statistically significant

SBP: Systolic Blood Pressures

$S_{cr::}$ Serum creatinine

SIRS: Systemic Inflammatory Response Syndrome.

SS: Severe Sepsis

SSTI: Skin and Soft Tissue Infection (other than wound)

T: Time

TBW: Total Body Weight

$T_{1/2}$: elimination half-time of the drug

t’ = infusion time

UTI: Urinary tract infection

VAP: ventilator-associated pneumonia

Vd: Volume of drug distribution

VRE: Vancomycin- Resistant Enterococci

VRSA: Vancomycin Resistant S. Aureus

WBC: White Blood Cells count

ABSTRACT

Septic shock and sepsis associated multiple organ failure are a major cause of morbidity and mortality in intensive care units (ICUs) globally. Approximately 50% of all patients with septic shock can be expected to die during their hospitalization unfortunately; the incidence of sepsis and septic shock continues to increase, driven by a rising frequency of underlying comorbidities. Despite the fact that major improvements have been made in therapy of serious infections, the treatment of patients with septic shock remains one of the major challenges to ICU clinicians.

In the decades since the introduction of the first modern antibiotic, penicillin, pathogens have continuously evolved under selective antimicrobial pressure. This has resulted in a lack of significant improvement in clinical effectiveness in the antimicrobial therapy of septic shock despite the ever more broad-spectrum and potent drugs have been used. Available evidence suggests that source control and an early administration of appropriate antimicrobial therapy remain the most important and powerful clinical interventions for mitigation of sepsis-associated mortality. However, it has not been clear whether manipulation of antimicrobial therapy beyond early administration can achieve incremental reductions in sepsis-associated mortality. Despite the lack of supportive clinical data, animal studies suggest that the key to significant improvement in the outcome of septic shock may lie with improvements in delivery of existing antimicrobials (dosing optimization). Thus, understanding the pharmacokinetic / pharmacodynamic (PK/PD) indices may be the cornerstone in the path to improvement of sepsis outcomes.

In this research program, 342 bacteremic patients with septic shock were determined to have been treated with a β-lactam as the only effective antimicrobial (β-lactam monotherapy). Of these, males accounted for 59.1% of the total cases. The average (± standard deviation) age of patients was 63.87 (± 15.6) years. Average Acute Physiology and Chronic Health Evaluation II (APACHE II) was 26.97 (± 8.8). Gram-negative organisms were isolated in 190 (representing 55.56 %) cases while 152 Gram-positive organisms were isolated in 152 (representing 44.44 % of the total).

Objective: To show that key pharmacokinetic indices for a wide variety of β–lactams are associated with outcome in human septic shock caused by a range of bacterial pathogens.

Methods: β-lactam pharmacodynamic (PD) indices including time above MIC and four times above MIC (*f*T>MIC, *f*T>4X MIC); and peak drug concentration and area under the drug concentration curve divided by MIC (Cpeak/MIC, AUC/MIC) over the 1st 24 hours of therapy were developed using the antibiotic dosing schedule, estimated creatinine clearance, estimated volume of distribution and assayed pathogen MIC for the utilized β-lactam.

Measurements and Main Results: Logistic regression modeling was used to examine survival to hospital discharge as a function of β-lactam PD indices as a continuous variable. The same effect was stratified over the entire group and specific subgroups. The average age and APACHE II score of patients was 63.9 (± 15.6 SD) years and 27.0 (± 8.8 SD) respectively. Males accounted for 59.1% of the total. The median time to appropriate drug administration was 3.67 (1.0-7.5 IQR) hrs following documentation of hypotension. Gram-negative organisms accounted for 55.6% of the total pathogens.

Logistic regression analysis demonstrated that 1st 24 hour time above MIC (OR 1.175 95% CI 1.073-1.288 per 10% increment, p=.0005) and 1st 24 hour time above 4X MIC (OR 1.127 95% CI 1.057-1.203 p=.0003) were both strongly associated with the improved survival of septic shock. Neither the 1st 24 hour Cpeak/MIC (p=.0762) or 1st 24 hour AUC/MIC (p=.0872) achieved significance in relation to outcome in logistic regression analysis. Logistic regression of subgroups suggested that time related PD indices were associated with survival most strongly for Gram-negatives including enterobacteriaceae among the organism groups. No specific organism grouping (e.g. *Staphylococcus aureus*, *Escherichia coli*, etc.) achieved significance. The effect was most pronounced for cephalosporins with a similar trend for carbapenems but not for penicillins. In a multivariate regression model with age, time to appropriate antibiotic and APACHE II score, the relationship between time above the MIC (OR 1.126 95% CI 1.048-1.295, p=.0067) and time above four time the MIC (OR 1.130 95% CI 1.044-1.226, p=.0027) and hospital survival remained significant.

DEDICATION

I dedicate this work to my little family, my lovely wife Tahani, and my beautiful girls Elaf and Taif and my handsome son Eyad. Thank you all for your continuous support and patience throughout my study.

I also dedicate this work to my family back in Saudi Arabia, my parents and my sisters Batoul, Zohour, and Bodour, and my brothers Khalid , Adel, and the twins Mazen and Yasser ,this would not have been possible without their unending encouragement and support and keeping me in touch. I owe my deepest gratitude to my parents, whom have been a constant encouragement and source of inspiration and strength not only during my study in Canada, but actually in everything throughout my life, since they raised me, taught me, loved me, and made of me a strong man able to face the challenges.

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1 Introduction

The intervention of modern antimicrobial therapy following the discovery of penicillin during the 1940's yielded remarkable improvements in case fatality of serious infections including septic shock. Since then, pathogens have continuously evolved under selective antimicrobial pressure resulting in a lack of significant further improvement in clinical effectiveness in the antimicrobial therapy of septic shock despite ever more broad-spectrum and potent drugs. In addition, although substantial effort and money has been expended on the development of novel non-antimicrobial therapies of sepsis in the last 30 years but clinical progress in this regard has been limited. The key to significant improvement in the outcome of septic shock may lie, in great part, with improvements in delivery of current antimicrobials.

1.1 Sepsis

1.1.1 Overview

Sepsis-associated multiple organ failure and septic shock due to infection in critically ill patients are a major cause of morbidity and mortality in intensive care units (ICUs) of the developed world (Friedman, Silva et al. 1998; Jimenez-jimenez, Perez-paredes et al. 2003). Two decades ago, in 1990, the estimated number of sepsis cases was about 450,000 per year in the United States, with >100,000 deaths. A more recent study; estimated that more than 750,000 patients diagnosed with sepsis in American hospitals were accompanied by about 210,000 deaths annually (comparable Canadian data does not exist) (Angus, Linde-Zwirble et al. 2001; Martin, Brunkhorst et al. 2009). Consequently, the incidence of sepsis far exceeds the incidence of colon cancer, breast cancer, AIDS or first myocardial infarctions (Angus, Linde-Zwirble et al. 2001; Alberti, Brun-Buisson et

al. 2002; Balk 2004). The mortality rate in severe sepsis cases has been reported as high as 75%, in some cases, such as pseudomonal septic shock. Accordingly, approximately up to 50% of all patients with severe sepsis are expected to die during their hospitalization (Roberts and Lipman 2006; Roberts, Hons et al. 2007).

Although the incidence rate of sepsis is expected to rise in the following decades. Sepsis is already the tenth most common cause of death in the US (Miniño, Heron et al. 2006). Moreover, increased mortality can be anticipated with the expansion of aggressive invasive surgical procedures and the rapid development of antibiotic resistance in both community and hospital acquired infections (Angus, Linde-Zwirble et al. 2001; Roberts and Lipman 2006)

1.1.2 Historical Review

Ancient Greeks used the term "sepsis" to apply to any type of decomposition of a living organism such as animal, vegetable or any other organic matter. More than 2700 years ago in the poems of Homer, the term ''sepsis'' was used medically. Hippocrates, a Greek physician known as the father of medicine, used herbs to cure human infection and sepsis at that time (Baron, Baron et al. 2006; Funk, Parrillo et al. 2009).

Marcus Terentius Varro (around 100 BC), was one of the first to speak about the notion of contagion. He suggested, ''small creatures invisible to the naked eye, fill the atmosphere around us, and breathed through the nose cause dangerous diseases.'' In 1546, Hieronymus Fracastorius illustrated clearly what has become known as the ''germ theory'', which suggested that three different forms of contagion may result in infection.

These included 1) direct contact 2) indirect contact through the infected articles and /or 3) transmission through air (airborne infection).(Thurston 2000; Funk, Parrillo et al. 2009).

In 1841, Ignaz Semmelweiss (1818–1865), an obstetrician in Vienna, noticed a high mortality rate from puerperal sepsis (childbed fever) among those women who delivered at hospital. Surprisingly, he noted that childbed fever was rare for those women who delivered at home. The hospital mortality rate ranged from 2% for women delivered by midwives, while it reached up to 16% for those women delivered by medical students. Noting that medical students routinely performed autopsies (without subsequent hand-washing) whereas midwives did not, Semmelweiss introduced an infection control measure (hand washing policy) in his maternity ward before and between patient's examinations. As a result, the rates of puerperal sepsis among patients managed by medical students dropped dramatically to less than 3% in a short period of time. Ironically, Semmelweiss died of sepsis in 1865 from a wound infection acquired during one of his operations (Jay 1999; Thurston 2000; De Costa 2002). Semmelweiss was the first to demonstrate the connection between aseptic technique and reduced infection rate.

Later, Joseph Lister (1827–1912), a surgeon, observed that infection of open wounds can lead to sepsis and death at a later time. He used carbolic acid as an antisepsis therapy for wound dressings and was clearly able to reduce wound infections, thus preventing sepsis and death in his hospital. In 1877, under an unprecedented aseptic technique, he was able to perform an open patellar repair operation successfully with no postoperative infection. His approach resulted in a remarkable reduction of postoperative infectious complications (Thurston 2000; De Costa 2002; Baron, Baron et al. 2006; Funk, Parrillo et al. 2009).

At about the same time, Louis Pasteur (1822–1895) was working on his germ theory of disease. Pasteur clearly identified and connected hemolytic streptococci as the causative agent of puerperal sepsis. Robert Koch (1843–1910), also conducted experiments on the germ theory of septic illness. Koch was able to define the criteria required to relate a microbial agent as the cause of disease: (1) the agent must be isolated from the diseased animal or from a patient's body fluid or tissue; (2) the organism must be grown in culture media; (3) infecting a healthy animal with the cultured organism must produce the disease (animal model); and finally, (4) the same organism must be recovered from the newly infected animal.(Baron, Baron et al. 2006; Funk, Parrillo et al. 2009). Firm acceptance of the germ theory as a cause of infection and sepsis by the medical community was achieved and subsequently, substantial efforts have been directed towards finding a way to eradicate these pathogenic microorganisms during infection.

Central to this effort was the discovery of penicillin by Sir Alexander Fleming (1881–1955) that paved the road for the development of the antimicrobial chemotherapy industry. In 1941, Ernest Chain and Howard Florey were able to produce penicillin in large amounts. As a result, this drug was came to be used widely in the hospital setting and in the battle field as well during the Second World War. It has been suggested that penicillin played an important role in saving thousands of soldier's lives from septic death. In 1945, Fleming, Chain, and Florey were awarded the Nobel Prize for their efforts. The same year, the first aminoglycosides antibiotic, streptomycin was developed.(Rolinson 1998; Khardori 2006; Funk, Parrillo et al. 2009).

1.1.3 Definitions

Standardized definition of sepsis and its related conditions are highly desirable in order to facilitate clinical care and research. Consensus definitions were developed in 1991 at a consensus conference sponsored by the American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine (SCCM) (ACCP 1992) and revised in 2001.(Levy, Fink et al. 2003). The sepsis-associated definitions recommended by the consensus panel were as follows;

Infection

Defined as a pathological process in which an inflammatory response is evoked in reaction to the presence of microorganisms. It is caused by the invasion of normally sterile host tissue, fluids or body cavity by pathogenic micro-organisms (Levy, Fink et al. 2003).

Systemic Inflammatory Response Syndrome (SIRS)

A condition considered to be present when patients present with a combination two or more of the following clinical findings in response to an appropriate clinical insult. These are; increased temperature (body temperature higher than 38°C) or hypothermia (lower than 36°C), tachycardia (heart rate higher than 90/min), or tachypnea (hyperventilation evidenced by respiratory rate higher than 20/min or PaCO2 lower than 32 mmHg), leukocytosis (white blood cell count higher than 12,000 cells/ µl) or leukopenia (WBC lower than 4,000/ µl) (Bone 1996; Angus, Linde-Zwirble et al. 2001).

Sepsis

A serious clinical syndrome defined by the presence of infection causing systemic inflammatory response (SIRS) to that infection (Bone 1996; Levy, Fink et al. 2003).

Severe sepsis (sepsis with organ dysfunction)

Sepsis causing acute organ dysfunction such as acute renal or liver failure.(Angus, Linde-Zwirble et al. 2001).

Septic Shock

A state of acute circulatory failure that is associated with persistent arterial hypotension despite adequate fluid resuscitation, in conjunction with perfusion abnormalities. Standard abnormalities in an adult include Mean Arterial Pressure (MAP) MAP<60 mm Hg, systolic blood pressures <90 mm Hg or a drop in systolic blood pressure of >40 mm Hg from baseline. Septic shock is the form of severe sepsis associated with failure of the cardiovascular system.

The consensus definitions of sepsis not only help the clinicians to be able to differentiate between infectious and noninfectious causes of SIRS. but also set a standard frame work for both epidemiologists and therapists of sepsis (Danai and Martin 2005).

1.1.4 Epidemiology

Sepsis as a clinical syndrome represents a main cause of morbidity and mortality in intensive care units (ICUs) globally. In the last two decades, the availability of computerized patient information databases on a large scale has substantially improved the ability to study the epidemiology of sepsis (Martin, Mannino et al. 2003; Danai and Martin 2005). In 1990, in the USA, the Centers for Disease Control and Prevention

(CDC) reported that there were about 450,000 cases of septicemia annually in the American hospitals, with >100,000 deaths (Angus, Linde-Zwirble et al. 2001). This report warned the public that the incidence of septicemia was increasing. However, the CDC study counted cases of septicemia (i.e. with positive blood culture), not severe sepsis, which often occurs in patients without positive blood cultures.

By using the National Hospital Discharge Survey (NHDS) database, and data from the Clinical Modification of the International Classification of Diseases, 9th Revision (ICD-9) codes, epidemiologists attempted to identify cases of sepsis and propose a national estimate of the incidence of severe sepsis with more detailed information on ICU utilization and the cost of the healthcare related to this critical condition. Over the 22-year period, between 1979 and 2000, Martin et al estimated that the number of cases of sepsis that increased from 164,000 cases (82.7 per 100,000 population) to nearly 660,000 cases (240.4 per 100,000 population) with an annual increase in the incidence of sepsis of 8.7% (Martin, Mannino et al. 2003).

Studies conducted in the US, UK, and in Australia and New Zealand have consistently estimated the incidence of severe sepsis at a rate between 50-80 annually /100,000 populations. Similar data on Canadian incidence has never been reported. Finfer et al. reported severe sepsis at the rate of 77 cases per 100,000 people in Australia and New Zealand (Finfer, Bellomo et al. 2004). Padkin et al. found 51 patients with severe sepsis per 100,000 population in the United Kingdom (Padkin, Goldfrad et al. 2003). Further studies found severe sepsis to occur at the rate of 81 cases per 100,000 people in the United States in 2000 (Danai and Martin 2005). These cases of severe sepsis represented from 10% to 15% of total ICU admissions. Moreover, nearly 25% of sepsis

cases and about 50-75% of severe sepsis cases progress to septic shock. Septic shock cases represent between 5 to 8% of all ICU admissions and accounts for the bulk of the mortality rate of sepsis (Brun-Buisson C 1995; Annane, Aegerter et al. 2003; Finfer, Bellomo et al. 2004; Danai and Martin 2005).

The incidence of sepsis and severe sepsis, while high, is also increasing according to most studies. Sundararajan et al found the American incidence of severe sepsis increased from 65 patients per 100,000 in the year 1999 to 76 per 100,000 in the year 2002. (Sundararajan, MacIsaac et al. 2005). The incidence of sepsis is also increasing at an annual rate of 9%. Consequently, 934,000 and 1,110,000 cases by the years 2010 and 2020 have been estimated for the Unites States (Angus, Linde-Zwirble et al. 2001; Martin, Mannino et al. 2003; Sundararajan, MacIsaac et al. 2005).

Reasons for this increase may include A) increasing advanced age population with increased predisposition to illness; B) high prevalence of patients with chronic organ failure (liver disease, renal failure, cardiomyopathy, and pulmonary obstructive diseases) and diseases such as diabetes, malignancy, AIDS, etc.; C) excessive use of invasive procedures for diagnostic and therapeutic purposes (indwelling catheters and devices); and D) extensive use of immune suppressor drugs for treating a wide range of critical illness (Brun-Buisson C 1995; Friedman, Silva et al. 1998; Angus, Linde-Zwirble et al. 2001; Martin, Mannino et al. 2003; Danai and Martin 2005).

Comorbidities are commonly found in most sepsis patients. Epidemiologic studies have shown that, more than 50% of the severe septic patients have at least one chronic comorbid medical condition. Diabetes, obstructive pulmonary disease, congestive heart

failure, liver and kidney disease, hypertension, and cancer, each can be found in 10-20% of sepsis patients. Notably, >90% of septic shock patients have one or more of these major comorbidities conditions (Angus, Linde-Zwirble et al. 2001; Martin, Mannino et al. 2003; Danai and Martin 2005).

According to Angus et al, and others, the overall hospital mortality rate for severe sepsis is ranges from 20% to 30%. For patients without underlying comorbidity, mortality increased with age from 10% in younger children to more than 38% in elderly over 85 years. For patients with underlying comorbidities, mortality was much higher and changed dramatically throughout different adulthood stages (Angus, Linde-Zwirble et al. 2001; Martin, Mannino et al. 2003; Danai and Martin 2005). The average age of patients who develop sepsis has been consistently reported between 55 and 65 years of age.

The risk of death is directly related to the severity of the septic illness. Mortality for sepsis patients is <15%, severe sepsis is 25-50% and mortality exceeds 50% for septic shock patients. The variation in death rates is primarily explained by the intensity of acute illness. A strong correlation has also been shown to exist between the risk of death and the number of organ failure or associated comorbidity (Alberti, Brun-Buisson et al. 2005; Danai and Martin 2005). Consequently, sepsis as a syndrome ranks as the leading cause of death for patients in noncoronary intensive care units (ICUs) and the 10th leading cause of death in the United States. Furthermore, sepsis adversely impacted the quality of life for survivors (Martin, Mannino et al. 2003; Miniño, Heron et al. 2006).

Regarding the site of infections associated with sepsis syndromes, the greatest percentage (25-50%) are due to respiratory tract infections (Brun-Buisson C 1995;

Rangel-Frausto, Pittet et al. 1995; Alberti, Brun-Buisson et al. 2002; Martin, Mannino et al. 2003; Sundararajan, MacIsaac et al. 2005; Kumar, Roberts et al. 2006). Intra-abdominal infections account for a disproportionate fraction of cases of severe sepsis (10-32% compare to their contribution to ICU infections (5-7%) (Rangel-Frausto, Pittet et al. 1995; Vincent, Bihari et al. 1995). A recent study has shown that, approximately 29% of cases of septic shock were due to intra-abdominal infections (Kumar, Roberts et al. 2006). On the other hand, the urinary tract infection (UTI) accounts only about 8-11% of cases of severe sepsis and septic shock, but about 16-31% of the total ICU infections. (Rangel-Frausto, Pittet et al. 1995; Vincent, Bihari et al. 1995; Alberti, Brun-Buisson et al. 2002; Annane, Aegerter et al. 2003; Guidet, Aegerter et al. 2005).

Throughout the last five decades, there has been a gradual change in the distribution of the pathogens that are been isolated from septic patients. The introduction of antibiotics in the mid of the last century has a major impact on this change. In the early 1950's Gram-positive organisms (*Staphylococcus aureus* and *streptococci*) were sensitive to penicillin. Two decades later, in the 1960's and 70's, the selective pressure of penicillin use against Gram-positives drove the development of penicillin-resistant *S. aureus* and helped Gram-negative organisms to become the dominant nosocomial pathogens. During the 1980's, a new era of antibiotic resistance evolved with Gram-positive organisms becoming able to resist a broader spectrum of antibiotics (Methicillin-resistant *S. aureus*, coagulase-negative *staphylococci*, penicillin-resistant *Streptococcus pneumoniae* and vancomycin-resistant *Enterococcus*). In the 1990's, Gram-positive cocci were isolated in 40-50% of single isolates in sepsis and septic shock patients (Brun-Buisson C 1995; Rangel-Frausto, Pittet et al. 1995; Michael J. Richards 1999; Alberti,

Brun-Buisson et al. 2002; Kumar, Roberts et al. 2006). The most recent studies have shown an increase in the incidence of yeast and other fungi to about 5-10% of total blood cultures per year; representing 5% of total sepsis infections and up to 8.2% of septic shock isolates (Michael J. Richards 1999; Alberti, Brun-Buisson et al. 2002; Martin, Mannino et al. 2003). The emergence of increasingly resistant pathogens as a cause of sepsis is escalating and is another major concern that complicates the existing challenges of treating septic shock patients. These "super bugs" include pan-resistant *Pseudomonas aeruginosa* and highly resistant extended spectrum β-lactamase (ESBL) Gram-negatives, vancomycin-resistant *enterococci* (VRE) (Murray 2000), and methicillin-resistant *S. aureus* (MRSA) including community strains (i.e. CA-MRSA). In addition, cases of vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) are growing (Murray 2000; CDC 2002; Giamarellou 2005).

The impact of sepsis and septic shock are of substantial financial consequence. The associated cost of sepsis syndrome is a major economic burden on the health care systems globally. According to epidemiologic studies, the average hospital's costs for treating severe sepsis patients ranging between $22,000 and $60,000 per episode in the US resulting in a total estimates near $17 billion annually (Angus, Linde-Zwirble et al. 2001; Danai and Martin 2005).

Despite the fact that huge improvements have been made in the medical field, treatment of patients with septic shock remains one of the major challenges to the ICU clinicians. The evidence available to date suggests that source control of the pathogen and early administration of appropriate antimicrobial therapy remain the most important and powerful clinical intervention (Roberts, Hons et al. 2007). Although a significant amount

of research has recently been directed toward treatment modalities that modulate cellular mechanisms such as human recombinant activated protein C (Gordonr. Bernard 2010) and the use of steroids (Hotchkiss and Karl 2003), the impact of such therapies remain significantly inferior to the benefit with early and appropriate antibiotic therapy. There is an urgent need for extensive research to improve treatment outcomes for this population of patients (Annane, Aegerter et al. 2003; Hotchkiss and Karl 2003; Baron, Baron et al. 2006; Roberts and Lipman 2006)

1.2 Antimicrobial Therapy

1.2.1 Overview

Since 1928, when Alexander Fleming first noted the inhibition of *staphylococcus* from a mold belonging to the genus *Penicillium* fungus, the isolation and development of novel antimicrobial agents has been an intense area of research (Craig 2004). Fleming did not extend his work to clinical studies because he was not able to purify enough penicillin for the experiments. So, while the discovery was made in 1928, the use of penicillin as a therapeutic agent to treat infections did not happen until the following decades. In August 1940, the successful use of penicillin to cure infections in animal experiments was reported (Kong, Schneper et al. 2009). A year later, penicillin was taken up for clinical trial. By 1946, a year after the Nobel Prize for Medicine was awarded to Fleming, Florey, and Chain, penicillin was finally available in the open market (Rolinson 1998).

With the discovery and development of penicillin for clinical use, there was a great deal of stimulus in the research for new antibacterial agents. This led to the discovery of many other antibiotics including streptomycin, chloramphenicol, tetracycline, erythromycin, vancomycin and kanamycin. However, to this day, penicillins and related

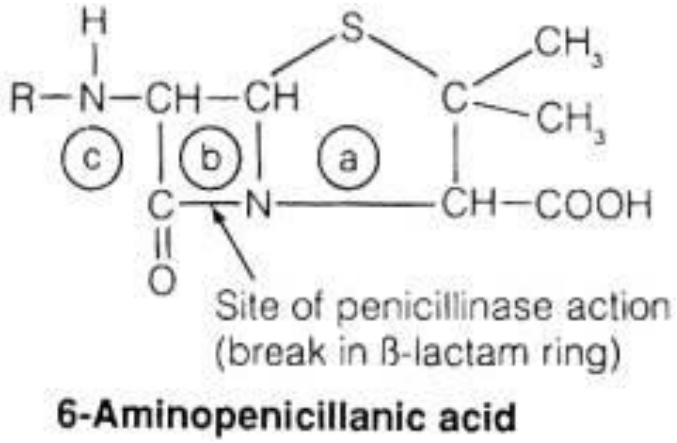
β–lactams remain the most widely used antibiotics in humans. During 1940s and 1950s, the β–lactam group comprised of two compounds, namely penicillin G and penicillin V (Rolinson 1998).

1.2.2 Types of β-Lactam Antibiotics

1.2.2.1 Penicillin

Penicillin is a 6-aminopenicillanic acid consisting of a thiazolidine ring, an attached β-lactam ring, and a side chain (Figure 1). Manipulations of the side chain have altered β-lactamase susceptibility, antibacterial spectrum, and pharmacokinetic properties. Other groups of antibacterial agents that contain the β-lactam ring include cephalosporins, carbapenems, and monobactams (Khardori 2006; Kong, Schneper et al. 2009). Penicillins and other closely related compounds are bactericidal against most susceptible bacteria (except *Enterococcus)* and have an excellent safety profile. The initial introduction of aqueous penicillin G for treatment of streptococcal and staphylococcal infections in 1941 to 1944 was followed by the emergence of penicillinase-producing (a form of β-lactamase) *S. aureus* (Khardori 2006). This finding prompted the development of penicillinase-resistant (i.e. semi synthetic) penicillins (methicillin, cloxacillin, oxacillin, and nafcillin), in which an acyl side chain prevents disruption of the β-lactam ring by penicillinase.

Figure 1: Chemical Structure of Penicillin.



a) 6-aminopenicillanic acid consisting of a thiazolidine ring, b)an attached β-lactam ring, and c) a side chain. (Google image)

With the discovery of 6-aminopenicillanic acid **(**6-APA), many types of penicillin with varying side chains were designed, and semi synthetic β-lactam compounds were developed. That side chains can alter the susceptibility of the inactivating enzymes (β-lactamases). Methicillin, was the first semi synthetic penicillin introduced to resist hydrolysis by *S. aureus* penicillinase (Kong, Schneper et al. 2009). The first semi-synthetic penicillin was followed by a variety of penicillin derivatives including; oxacillin, cloxacillin, dicloxacillin and flucloxacillin, in addition to carboxypenicillins (carbenicillin and ticarcillin). The development of the semi-synthetic penicillins was quickly followed by the development of the cephalosporins and other β-lactam type antibiotics (Rolinson 1998; Shahid, Sobia et al. 2009).

The aminopenicillins (ampicillin and amoxicillin) were developed to cover the need for antibiotics with resistance to the β-lactamases of common Gram-negative bacteria. Aminopenicillins initially were effective against *E. coli*, *Proteus mirabilis, Shigella, Salmonella*, *Hemophilus*, and *Neisseria species* (Rolinson 1998). Carboxypenicillins (carbenicillin and ticarcillin), and ureidopenicillins (mezlocillin, azlocillin, and piperacillin) offer additional activity against enterobacteriaceae, such as *Klebsiella* and *Enterobacter*, and *Pseudomonas aeruginosa*. Many Gram-negative bacteria, including enterobacteriaceae and *Hemophilus influenzae*, are now resistant to many types of penicillin because of β-lactamase production.

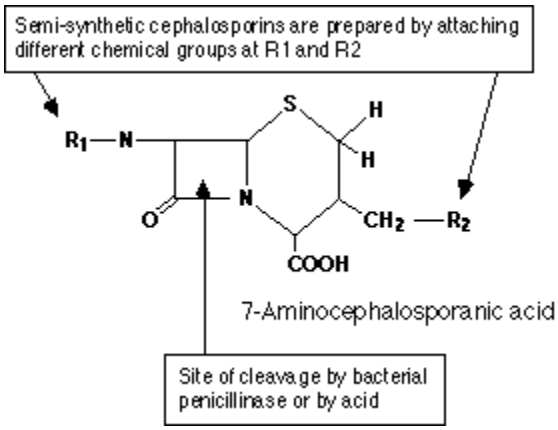
β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam inhibit β-lactamase of resistant bacteria. They are combined with penicillins to generate increased breadth and potency of antimicrobial activity. These drugs are available as combinations of amoxicillin-clavulanate, ampicillin-sulbactam (in the USA only), ticarcillin-

clavulanate, and piperacillin-tazobactam. The current penicillin combination group of drugs is ineffective against Gram-negative bacteria that produce other types of β-lactamase, including ESBL.

1.2.2.2 Cephalosporins

Though the generation of semi-synthetic compounds presented a great opportunity, natural sources continued to be explored. Abraham and Newton isolated cephalosporin C from a strain of *Cephalosporium acremonium* (Kong, Schneper et al. 2009). This compound generated an entirely new family of β-lactam antibiotics because, instead of 6-APA, it possesses a nucleus of 7-aminocephalosporanic acid (7-ACA) (Figure 2). Several generations of cephalosporins with potent broad-spectrum activity have been synthesized using 7-ACA as the precursor (Khardori 2006). These cephalosporins were introduced for clinical use in the 1960s. They are bactericidal and have favorable pharmacokinetic profiles and exhibit low rates of drug-associated toxicity. In general, first generation cephalosporins (cefazolin, cephalexin) are most active against aerobic, Gram-positive cocci, including methicillin-susceptible *S. aureus*. Second-generation cephalosporins are more active against few Gram-negative organisms, such as *Klebsiella spp, E. coli*, and *Proteus spp*. Cefoxitin and cefotetan are active against anaerobic bacteria as well. Third-generation cephalosporins are the most active against Gram-negative aerobic organisms. From this group, ceftazidime and cefoperazone are active against *P. aeruginosa*. Cefepime, classified as a fourth-generation cephalosporin, has an extended spectrum of activity against both Gram-positive and Gram-negative organisms, including *P. aeruginosa* (Page 2004). The newest fifth generation cephalosporins such as ceftobiprole and ceftaroline (currently available in the USA) have activity for MRSA.

Figure 2: Chemical Structure of Cephalosporins.



This compound possesses a nucleus of 7-aminocephalosporanic acid (7-ACA) instead of 6-APA in penicillin. Several generations of cephalosporins with potent broad-spectrum activity have been synthesized using 7-ACA as the precursor represented by different "R" sides. (Google image)

1.2.2.3 Carbapenem

Many naturally occurring compounds that exhibited antibiotic activity have been isolated from certain bacteria. Some of these include carbapenems from *Streptomyces spp*, and monobactam from *Pseudomonas acidophila, Chromobacterium violaceum* and *Agrobacterium radiobacter* (Kong, Schneper et al. 2009). Carbapenems and monobactams contain different β-lactam nuclei than 6-APA and 7-ACA. These compounds led to the development of new β-lactams with potent activity (Khardori 2006). Carbapenems are a class of β-lactam antibiotics with a broad spectrum of activity against aerobic and anaerobic Gram-positive and Gram-negative organisms. Imipenem in combination with cilastatin (to inhibit imipenem degradation by the renal brush border peptidase enzyme) became available in 1985; meropenem followed a decade later in 1996. The spectrum of activity of these carbapenems includes *Streptococcus*, methicillin-susceptible S. *aureus*, *Neisseria spp, Haemophilus spp*, anaerobes, and aerobic Gram-negative pathogens, including *P. aeruginosa. Stenotrophomonas maltophilia* is typically resistant to carbapenems. Imipenem is more active against Gram-positive cocci *in vitro*. Meropenem exhibits a better *in vitro* activity against Gram-negative bacilli. A third carbapenems, ertapenem, has become available recently; however, it does not have activity against *P. aeruginosa* or *Enterococcus species* (Khardori 2006; Shahid, Sobia et al. 2009). Doripenem has also been recently introduced and possesses activity similar to meropenem.

1.2.2.4 Monobactams

Aztreonam is a synthetic compound with a β-lactam ring and is the only member of the monobactam group. The antibacterial spectrum of aztreonam is limited to aerobic

Gram-negative bacilli because of the lack of its affinity for the penicillin binding proteins in Gram-positive bacteria. However, it is not nephrotoxic and has low anaphylactic cross sensitivity with penicillins. Therefore, aztreonam, in susceptible bacteria, is a useful non-nephrotoxic alternative to the aminoglycosides and may be used with caution in patients with significant penicillin hypersensitivity reactions (Khardori 2006; Shahid, Sobia et al. 2009).

1.2.3 Mechanism of Action

The β-lactams inhibit enzymes (transpeptidase, carboxypeptidase, and endopeptidase) located beneath the cell wall that are termed the ‘‘penicillin-binding proteins.’’ This binding/inhibition prevents the development of normal peptidoglycan structure of the cell wall, because these enzymes are involved in creating the cross-linkage between the peptide chains. All β-lactam antimicrobial agents including the carbapenems exhibit bactericidal activity by binding to penicillin-binding proteins (PBPs). The binding of the β-lactam molecule to the PBPs prevents bacteria from completing transpeptidation (cross-linking) of peptidoglycan strands, thus preventing the synthesis of an intact bacterial cell wall. Subsequent activation of the endogenous autolysis of bacteria by β-lactams initiates cell lysis and death thereafter (Khardori 2006; Zhanel, Wiebe et al. 2007; Shahid, Sobia et al. 2009). The bactericidal activity of β-lactams is completely dependent on inhibition of transpeptidation activity associated with bacterial replication. Without bacterial growth transpeptidation activity is absent and the cidal activity of β-lactams is lost. There are various PBPs which contribute to differences in activity among β-lactams and resistance (e.g. PBP 1 in *E. coli* and PBP 2a in MRSA).

1.2.4 Minimum Inhibitory Concentration (MIC)/ Minimal Bacteriocidal Concentration (MBC)

The susceptibility of an organism to a specific antibiotic is determined by performing a minimal inhibitory concentration (MIC) assay. The minimal concentration of an antibiotic required to completely inhibit the visible growth of an organism in either broth (i.e. no turbidity in broth) or agar medium (no bacterial colonies) is called the MIC. Subculture of the broth dilutions from an MIC assay to agar media is used to determine the minimal bactericidal concentration (MBC); since the MIC concentration only inhibits bacterial growth but does necessarily eliminate the organism, subculture of organisms from an MIC well into antibiotic free medium will result in re-invigorated organism growth. The concentration required to kill the organism is often significantly higher than the concentration needed to inhibit growth. Therefore the MBC is the concentration of antibiotic where no growth of colonies following sub-inoculation onto agar or into broth occurs (the MBC is typically several concentrations higher than the MIC). Based on the potential ability to achieve effective blood concentrations to an antibiotic in the body, organisms are classified as susceptible, intermediate susceptible, or resistant to the antibiotic. An agent's ability to kill an organism is dependent upon the pharmacodynamic (PD) properties of the drug in addition to the sensitivity (i.e. MIC) of the pathogen. These properties, taken together, are used in dose optimization and to design the dosing regimens that can be effective against a defined group of organisms (Zhanel, Wiebe et al. 2007; Eagye, Kuti et al. 2009).

1.2.5 Pharmacokinetics (PK) Considerations

Pharmacokinetics describes the absorption, distribution, metabolism, and elimination characteristics of a drug in the human body (i.e. what the body does to the drug). To

achieve bacterial eradication for β-lactams, antimicrobial dosing regimens must provide sufficiently high free antibiotic concentrations (above the MIC of the pathogen) at the infection site for a sufficient period of time. The possibility of bacterial eradication can be best achieved through an awareness of pharmacokinetics.

Although the MIC is an important measure of antimicrobial activity, other factors like patient's clinical situation, characteristic of the antimicrobial agent and pathogen-related issues influence the outcome of antimicrobial therapy. Currently, there is an increase in antibiotic resistance in pathogens resulting in treatment failures. Particularly among critically ill patients, this indicates that there is an urgent need to re-evaluate dosing strategies with available antimicrobials to maximize its effectiveness and in order to limit or minimize the spread of microbial resistance. The goal of antibacterial therapy is generally to maximize *in vivo* drug concentrations or the duration of exposure, depending on the class of antibiotic.

The efficacy of bacterial clearance from the host is best assessed by merging drug pharmacokinetic and pharmacodynamic (PK/PD) data with pathogen's MIC data. Different classes of antibiotics have different patterns of bactericidal activity based on PK/PD characteristics, and these patterns can actively determine treatment outcomes. By integrating the MIC data with PK/PD characteristics of an antimicrobial, optimal dosing regimens can be determined and the bacteriologic and clinical outcomes can be optimized. This approach might significantly improve the antimicrobial efficacy in one hand and may limit or minimize development of further antimicrobial resistance in pathogens on the other hand, as it was suggested by Andes and Necolau (Andes, Anon et al. 2004; Nicolau 2008).

1.2.5.1 Pharmacokinetic Changes Observed in Critically Ill Patients.

There are substantial pathophysiological changes that occur in critically ill patients that may alter the pharmacokinetics of antimicrobial drugs resulting in sub-therapeutic concentrations at the infection site. These changes must be considered when seeking optimal dosing regimens.

Volume of Distribution (Vd):

The volume of distribution (Vd) of a drug is mathematically derived by dividing the amount of the given drug in the body by the plasma concentration (Livornese LL 2001). Patients with sepsis and septic shock often exhibit marked increases in extracellular water. As a consequence, during severe sepsis and septic shock, increased Vd can reduce serum (and infection site) drug concentrations. Bacterial and fungal pathogens possess unique microbial components called pathogen-associated molecular patterns (PAMPs); examples include lipopolysaccharide (also known as endotoxin) in Gram-negative bacteria and peptidoglycan in Gram-positive bacteria (Adib-Conquy and Cavaillon 2007). These molecules (toxins) can stimulate host innate-immune responses by binding to cell receptors known as 'pattern recognition receptors'(PRPs), which include Toll-like receptors and several other types of cytoplasmic receptors (e.g. NOD1 and NOD2) (Akira, Uematsu et al. 2006). Receptor binding results in the activation of intracellular signaling cascades that lead to an enhanced production of inflammatory cytokines, such as tumor necrosis factor, TNFα, interleukin 1β and gamma-interferon, IFNɤ. These result in upregulation of adhesion molecule expression, stimulation of humoral and cell-mediated immune responses involved in inflammatory responses, and activation of vascular endothelial cells (Gerlach and Toussaint 2010). Once the vascular endothelium

is stimulated vasoconstriction or vasodilatation may result. In addition, endothelial damage and maldistribution of blood flow and increased capillary permeability may occur. The end result of this diffuse inflammatory stimulation is the generation of SIRS with associated fluid shifts from the intravascular compartment to the interstitial space "third-spacing" due to vascular leakage. This process normally results in marked tissue edema and increased Vd. Resuscitation-related increased fluid administration further results in a significantly increased Vd of hydrophilic (water-soluble) drugs. The Vd of β-lactams (which are highly water soluble) may decrease significantly which decreases their plasma drug concentration. The Vd of hydrophilic drugs may also be increased somewhat in critically ill patients when they are subjected to mechanical ventilation, hypoalbuminaemia (by increased capillary leakage), extracorporeal circuits (e.g., plasma exchange, cardiopulmonary bypass), post-surgical drains, or in patients with significant burn injuries. However, lipophilic drugs typically already have a large Vd because of their distribution into adipose tissue so the clinical impact of such increases is relatively modest compared to hydrophilic drugs like β-lactams (Roberts, Hons et al. 2007; Roberts and Lipman 2009).

Antibiotic Half-Life ($T_{1/2}$)

The rate of drug plasma clearance expressed as the time taken for elimination of half of the concentration of an antibiotic from the body is known as elimination half life ($T_{1/2}$). The $T_{1/2}$ of an antibiotic is directly dependant on the volume of distribution (Vd) and plasma clearance (CL) of the compound from the body and is represented by the equation (Livornese LL 2001):

$$T_{1/2} = 0.693 * Vd \ / CL$$

This relationship is such that an increase in Vd leads to increased $T_{1/2}$ and an increase in plasma clearance (CL) leads to reduced $T_{1/2.}$ $T_{1/2}$ is also dependant on the disease progression in critically ill patients and the interventions required by the clinicians. Critically ill patients develop hypotension at the initial phase which is due to redistribution of intravascular volume into the extra vascular space and loss of venous tone. The standard clinical intervention is to administer intravenous fluids and, if that fails to improve the hemodynamic profile, then intravenous administration of vasopressores/inotropes (e.g. nor epinephrine or dopamine) is used to support cardiac output/perfusion and blood pressure. The intervention of fluid resuscitation may have some influence on drug distribution space or Vd; interventions such as pressor or inotropic may increase cardiac output and thus enhance renal and or hepatic drug clearance of antibiotic.

Early in sepsis, before the development of organ dysfunction, there will be a significant increase of renal perfusion leading to enhanced creatinine clearance (augmented renal clearance) (Udy AA 2010). This leads to elimination of hydrophilic compounds at an increased rate. The measurement of creatinine clearance rate, a marker of glomerular filtration, in these patients thus plays a significant role in determining the dosing frequency of hydrophilic antibiotics. In addition, some reports suggest that there will be an increase in the clearance of the antibiotics when critically ill patients are ventilated (Roberts and Lipman 2009). Further, an increase in intra-thoracic pressure resulting from the use of mechanical ventilation results in homeostatic mechanisms that increase intra- and extra-vascular fluids and, therefore, Vd (Power, Forbes et al. 1998). All of these factors may play a role in reducing the effectiveness of antibiotics by

reducing their overall $T_{1/2}$ and tissue concentrations early in sepsis and thus time above some inhibitory concentration.

Later in sepsis and septic shock, the situation may change. The endogenous inflammatory response leads to myocardial depression and vascular failure with resultant impairment of micro vascular circulation, decreased cardiac output and decreased organ perfusion. Sepsis-associated with multi-organ dysfunction syndrome which may include hepatic and/or renal dysfunction lead to decreased clearance of the antibiotics from the body and prolonged $T_{1/2}$. This results in the presence of the antibiotic for a longer time in the body with an increased risk of drug related toxicity due to unnecessarily high concentrations of the antibiotic as well as their metabolites (Livornese LL 2001; Trotman RL 2005). In addition, high levels of ventilatory support and intravascular volume depletion in late sepsis can also result in decreased cardiac output and organ perfusion resulting in marked impairments in CL.

In situations where patients develop significant organ dysfunction and diffuse edema, alterations in antimicrobial therapy may be necessary in order to prevent further damage of these organs and to maximize antimicrobial efficacy while limiting toxicity. This may include altered drug choice or dosing regimen of β-lactams in response to changes of organ function and third spacing (Livornese LL 2001; Roberts and Lipman 2006; Roberts and Lipman 2009).

Protein Binding

Along with the above mentioned factors, another important PK parameter which affects PD is the protein concentration in the plasma of the infected patients. Only free

(unbound) antibiotic fraction is important for microbiologic activity; decreased protein binding will induce higher antibiotic levels (Drusano 2004). Knowing that, different classes of compounds have different binding affinities to proteins in the plasma. Protein binding influences the volume of distribution (Vd) and clearance (CL) of the compound from the body. In general, drugs that are highly protein bound or water-soluble are found mainly in the vascular space and have a small Vd (smaller than the estimate of the total body water volume); drugs that are highly lipid soluble have a large Vd, as they are able to penetrate body tissues more easily. For these drugs, a Vd that exceeds the total volume of body water can be calculated mathematically. Moreover, Vd is important in calculating the plasma half-life $T_{1/2}$ of a drug (Power, Forbes et al. 1998; Livornese LL 2001). A remarkable example of this pharmacokinetic alteration in β-lactams exists for one of the third generation cephalosporin, ceftriaxone, which is 95% bound to albumin in normal patients; the small free fraction may results in a relatively high Vd, decreased drug clearance, a high $T_{1/2}$ (5.8-8.7 hours) and adequacy of once daily dosing. In contrast, most β-lactams have shorter elimination half-lives (JD. 1998), and an increased Vd and the need for multiple daily drug doses (Roberts, Hons et al. 2007).

Tissue Penetration

Achieving the concentration of the antibiotic at the target site, usually tissues, is an important element for favorable clinical outcome (Ryan 1993). Consideration of tissue concentrations is particularly important in critically ill patients as the circulation and penetration of the antibiotics may be highly impaired (McKenzie 2011). This is often due to dilution of antibiotic concentration because of the increase in interstitial (Extra-cellular) fluid and increased Vd. Micro dialysis is an *in vivo* sampling technique that can

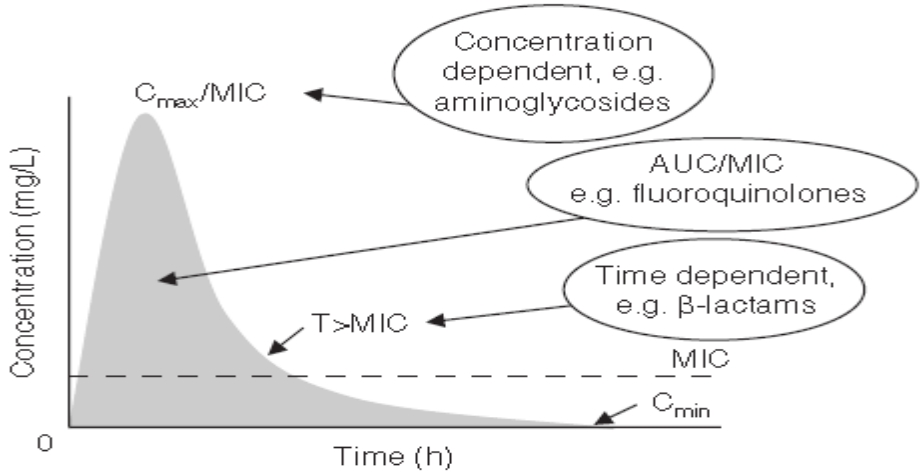
be used to determine the tissue concentrations of the antibiotic in critically ill patients (Roberts, Roberts et al. 2011). The current evidence suggests that the antibiotic penetration into patient's tissues with septic shock is highly impaired with five to ten times lower concentrations than in healthy volunteers. In other patients with sepsis but without shock, a less significant effect on tissue concentrations had been reported (Roberts and Lipman 2009). For that reason, in patients with sepsis and septic shock, high end dosing of antibiotics should be exploited to maximize tissue penetration. Dosing of the antibiotic should be individualized based on the anticipated PK alternations and health condition of the critically ill septic patient. Ideally, adjustment of antibiotic dosing depending on the idealized pharmacokinetic parameters might optimally facilitate clinical outcomes (Mouton, Theuretzbacher et al. 2008; Roberts and Lipman 2009).

1.2.6 Pharmacodynamic (PD) Considerations

The science of pharmacodynamic is defined as the study of the action or effects of drugs on living organisms (i.e. what the drug does in the body). Different classes of antibiotics have different patterns of bactericidal activity based on pharmacokinetic and pharmacodynamic characteristics, and these patterns can actively determine treatment outcomes. By integrating the MIC data with PK/PD characteristics of an antimicrobial, optimal dosing regimens can be determined and the bacteriologic and clinical outcomes can be optimized. This approach might significantly improve the antimicrobial efficacy and may limit or minimize development of further antimicrobial resistance in pathogens as suggested by Andes and Necolau (Andes, Anon et al. 2004; Nicolau 2008).

The primary determinants of the efficacy of a drug can be evaluated by specific PD indices (Figure 3) such as the percentage of time that the free plasma concentrations

Figure 3: Pharmacodynamic (PD) indices of different class of antibiotics



Correlation of Pharmacokinetic PK and Pharmacodynamic PD parameters of antibacterials as expressed in serum as a concentration vs. time curve. AUC = Area Under the serum Concentration-time curve; C*max* = Peak serum drug concentration; C*min* = Minimum serum drug concentration; MIC = Minimum Inhibitory Concentration; T>MIC = time for which the free serum concentration of a drug remains above the MIC of the organism for a dosing intervals (Roberts and Lipman 2006).

remain above the MIC (% *fT*>MIC), and the 24-h AUC over MIC ratio (AUC/MIC) (Kitzes-Cohen, Farin et al. 2002). The kill kinetics of β-lactams is time-dependent but concentration-independent. This means that the duration of time that the free serum concentration of the drug exceeds the MIC for the causative pathogen is the most important predictor of bacterial killing. This also suggests that the key pharmacodynamic parameter that drives clinical efficacy of β-lactams will be the percentage of time that the serum concentration of the free, unbound fraction of the drug is maintained above the MIC (% *f*T > MIC) of the pathogen for the most of the time (Andes, Anon et al. 2004; Mouton, Dudley et al. 2005; Nicolau 2008). Although mathematically linked to the percentage of *f*T>MIC, peak drug concentration (C_{peak} or C_{max} / MIC) and the area under the concentration time curve to MIC ratio are less closely associated with bacterial killing/drug efficacy (Power, Forbes et al. 1998; Jacobs 2001). Dosing regimens that maximize bacterial killing using this approach have been suggested to maximize the rate of response to the treatment and minimize the development of antibiotic resistance and have been recommended for critically ill patients (Roberts, Hons et al. 2007; Roberts and Lipman 2009)..

Although not applicable to β-lactams, the maximum serum concentration above the MIC (C_{max}/MIC) for aminoglycosides and AUC/MIC for fluoroquinolones are the key parameters for predicting successful eradication of infectious pathogens and clinical efficacy.

Pharmacodynamic of β –lactam

As noted, pharmacodynamic deals with the effects of the drug on the body which include therapeutic effects (Craig 2003). The key pharmacodynamic elements for

effective antibacterial therapy takes into account MIC and duration of bactericidal effects (Taccone FS 2010), including post antibiotic effects (PAE) (JD. 1998), killing rate of the compound, and rate of development of resistant mutants (Drusano 2004).

The post-antibiotic effect is another pharmacodynamic property of antimicrobials and describes the ability of an antibiotic to induce bactericidal effect even after its levels have fallen below the MIC in the serum (Andes, Anon et al. 2004; Drusano 2004). Some antibiotics, particularly those that inhibit the protein or nucleic acid synthesis in bacteria, have the ability to induce post-antibiotic effect against Gram- negative and Gram-positive bacteria *in vivo* (Andes, Anon et al. 2004). Although some β-lactam antibiotics exhibit short PAEs against *S. aureus*, most do not exhibit any against Gram-negative bacteria and *streptococci* (Craig 1995; JD. 1998). Limited post antibiotic effects have been seen *in vitro* and *in vivo*, with carbapenems against strains of *P. aeruginosa* (Hollander 1994). Gram-negative bacilli generally exhibit no persistent effects with most β-lactams. This explains, in part, why β-lactams that show time-dependant killing of microorganisms have a very short PAE with little contribution of late enhanced antimicrobial effect (Andes, Anon et al. 2004; Geli 2009). Antimicrobials that are able to inhibit protein and nucleic acid synthesis are generally found to have a substantial PAE compared to β-lactams antibiotics (Power, Forbes et al. 1998). Generally, antibiotics that exhibit prolonged PAEs have to be administered less frequently. Thus, PAE has a significant impact on the dosing optimal regimen of some antibiotics (McKenzie 2011). Since most drugs with prolonged PAE exhibit concentration-dependent microbial killing, use of less frequent but higher doses (as with aminoglycosides once-daily dosing) is possible while still maintaining effective bacterial killing. Understanding of PAE of an antibiotic can

help optimize dosing and ultimately improve the outcome of critically ill septic patients (Andes, Anon et al. 2004).

Optimal dosing strategies for β-lactams

For compounds like β-lactams that exhibit time-dependant efficacy, suppression and/or killing of the pathogenic bacteria occurs once the concentration of the compounds attain a critical serum threshold and the suppression/killing efficacy decreases as the serum concentration falls below the threshold for increasing period of time (McKenzie 2011). The most commonly applied threshold is that which defines bacterial growth suppression (i.e. the MIC) although the threshold that defines bactericidal activity is usually higher (i.e. the MBC which is usually several times -four to five times- higher than MIC). Regardless, the critical issue is the duration of time that bacteriostatic/bactericidal concentrations are achieved in the body rather than the absolute concentrations achieved.

The free drug concentration is the actual antibiotic concentration that is available and relevant for exerting the therapeutic effect. However, in clinical practice, the free concentration of β-lactam antibiotics is rarely maintained above the MIC for the entire dosing interval due to relatively short $T_{½}$ of most β-lactams (Mouton, Dudley et al. 2005; Crandon, Bulik et al. 2010). Because of the increased body water, most critically ill patients have a markedly increased Vd for β-lactams and this is often expressed as a lower trough concentrations in patient's plasma; this can be problematic for these time-dependent antibiotics such as β-lactams (Roberts, Hons et al. 2007). Free time of a compound above MIC can be optimized by a variety of methods including 1) dosing

more frequently (for fixed daily dose) 2) continuous infusion or 3) using different formulations that result in sustained release of the compound in the body (Roberts, Paratz et al. 2008). In addition, use of a repository dosage form, or co-administration of a drug that inhibits the inactivation or elimination of the antimicrobial (e.g., probenecid with some β-lactams) can also increase % *f*T>MIC (J. E. Leggett 1989). It is of critical importance that for any antibiotic for which time above MIC drives antimicrobial efficacy, achievement of concentrations of the compound much higher than the MIC will not provide any additional reduction in growth of the pathogen (Jaruratanasirikul, Sriwiriyajan et al. 2005; Jason A. Robertsa 2008).

In *in vivo* animal experiments with a neutropenic mouse infection model, the survival rate of the animals after challenge with *S. pneumoniae* was maximal when serum concentrations of amoxicillin or amoxicillin-clavulanate exceeded the MIC of the pathogen for about 40% of the dosing interval (Erlendsdottir 2001). In other animal infection models respiratory tract infection and tissue infection with *S. pneumoniae,* bactericidal activity was observed when penicillin exceeded the MICs for 65% and 35 % of the dosing interval respectively (Erlendsdottir 2001). A bacteriostatic effect was observed in animal infection models for *Enterobacteriaceae* and *S. pneumoniae* when the cephalosporins compound was maintained above MICs for 35 to 40 % of dosing interval. In related study, maximum bactericidal effect for cephalosporins was observed when β-lactam concentrations were maintained above MIC for 60% to 70 % of the dosing interval for both strains (Craig 1995; Craig 1998). Crandon and co-workers found that free drug concentrations of cefepime in patients infected with *P. aeruginosa* exceeding the MIC of the organism for more than 60% of the dosing interval (i.e.% *fT* >MIC >

60%) was associated with improved clinical outcome (Crandon, Bulik et al. 2010). With staphylococcal infections, excellent efficacy achieved when the concentration of β-lactams was maintained above MIC for at least 40 % of the time of the dosing interval (B. Vogelman 1988). Notably, for the carbapenems, a shorter percentage of time above the MIC is appeared to be required for optimum efficacy (20% to 30%) than for other β-lactams (Mouton JW 2000) .

In treating immuno-competent individuals, bacteriostatic antibiotics are thought to be acceptable for positive clinical and microbiological outcomes, because host defense will play an important role in eradicating infection (McKinnon, Paladino et al. 2008). For β-lactams, significant bacterial reduction and clinical response is achieved even when concentrations are maintained above the MIC for approximately 40% to 50% of the dosing interval (consistent with a bacteriostatic effect) regardless of infecting pathogen and level of resistance (Fantin 2006).

The bacteriostatic effect after 24 hours of β-lactams therapy against multiple bacterial pathogens has been studied in the neutropenic animal model (Craig 2004). The optimal time of the free drug above MIC of the bacterial pathogen was higher with cephalosporins (35% to 53%) than for penicillins (29% to 34%), which were higher again than carbapenems (20% to 26%). Differences in killing were attributed to differences in the rates of killing by each class of β-lactam separately, which were fastest with the carbapenems and slowest with the cephalosporins. In these studies, total (not free) drug concentrations were used in calculating the PK/PD parameters (Craig 2003).

Standard Bolus or Intermittent Administration

Pharmacodynamic analysis has been used in the development of optimal antibiotic administration regimens that maximize antibacterial effects (Jaruratanasirikul, Sriwiriyajan et al. 2005). The standard approach – based on manufacturers' instructions - for β-lactams usually involves administration of β-lactams as multiple, intermittent boluses or injections throughout the day with the frequency varying between different drugs of the class depending on PK indices. Some β-lactam antibiotics can be dosed just once a day (primarily ceftriaxone) though most require multiple daily doses except in the context of renal failure. With this mode of administration, the high peak concentrations of β-lactams do not enhance the bactericidal activity of these agents unlike what is seen with aminoglycosides and fluoroquinolones. In addition, the development of antibiotic resistance can be accelerated with this mode of administration; when the concentration of the drug falls below the MIC, the pathogen is able to re-grow at that sub-therapeutic level of drug within the dosing intervals since, there is no significant PAE for β-lactam antibiotics (except for carbapenems). This can engender selection of intrinsically resistant clones.

As pathogens become more resistant, antibiotic treatment for these pathogens will be extremely challenging. This increases the need for newer strategy and/or newer antimicrobial agents (Jacobs 2001). One way that been proposed to prevent emerging resistance is via administration of higher β-lactam doses to increase efficacy of therapy. However, this strategy is not ideally cost effective nor does it optimize the exposure to β-lactam antibiotics (Roberts, Hons et al. 2007; Nicolau 2008; Roberts and Lipman 2009). Optimizing the therapeutic drug dosing strategy according to PK/PD principles for

maximal efficacy against emerging resistant microbial pathogen needs to be considered in designing effective antimicrobial regimens and in selecting suitable empirical therapy (Jacobs 2001).

A number of pathophysiological changes occur in critically ill patients that alter the PK indices of the drug resulting in suboptimal clinical and microbiological response. These must be considered in designing optimal β-lactams dosing regimens in the critically ill. Taccone *et al* (2010), recently published a study answering the question of whether the first dose of β-lactams (piperacillin-tazobactam, ceftazidime, cefepime, and meropenem) would result in adequate (i.e. sufficient to kill the microbial pathogen) serum and tissue concentrations in critically ill patients. In this study, only meropenem resulted in adequate therapeutic level of four times the MIC, following the initial bolus dose for *P. aeruginosa*. On the other hand, standard first doses bolus regimens for piperacillin-tazobactam, ceftazidime and cefepime were insufficient to generate therapeutic serum concentrations greater than four times the MIC of *P. aeruginosa* in critically ill patients. Initial adequate serum concentration levels were achieved only for very susceptible microbial pathogens (i.e. MICs of 1 μg/mL or less for cefepime, and MICs of 2 μg/mL or less for ceftazidime and piperacillin-tazobactam). This was of significant concern since the organisms isolated from critically ill patients in the ICUs often carry higher MICs than similar community pathogens.(Taccone FS 2010).

Extended or continuous infusion

An alternate dosing approach involving continuous or extended intermittent infusions of drug provides a better strategy for administering β-lactams. This approach, based on basic PK/PD principles, maintains the therapeutic serum drug level above the MIC for

most or all of the dosing interval while undesired high peak (C_{peak} or C_{max}) and sub optimal MIC- trough concentrations (C_{min}) - found with intermittent dosing strategy are eliminated (Buijk, Gyssens et al. 2002; Roberts, Hons et al. 2007). Notably, following the development of penicillin early in 1940s, continuous infusion of the drug was the standard dosing approach. However, intermittent dosing regimen was adopted (without supportive clinical studies) for ease of administration before creation the mechanical infusion pumps (Nicolau 2003; Nicolau 2008).

Continuous infusion maximize β-lactam antimicrobial and clinical efficacy by maintaining adequate serum and target tissue antibiotics in steady state concentrations above the MIC, throughout the therapeutic course of treatment (Roberts, Hons et al. 2007; Roberts, Paratz et al. 2008). This approach can potentially have a significant impact in the treatment of immunocompromised patients, critically ill patients and/or when a high MIC pathogen is involved (Daenen S 1988). Another potential advantage of continuous infusions is that it reduces the amount of drug required and the cost of the antibiotic treatment (Lodise, Lomaestro et al. 2007). Continuous infusion of ceftazidime was found to be more efficacious than standard intermittent bolus dosing strategy, in an *in vitro* pharmacokinetic model, (Power, Forbes et al. 1998), and *in vivo* human model, when high sustained plasma concentrations were required for killing of *P. aeruginosa* infection in critically ill patients (Burgess 1999; Nicolau 2003).

A study conducted by Lodise et al. (2007), convincingly changed the clinical practice at Albany Medical Center Hospital (Albany, New York). The study attempted to evaluate the clinical and microbiological outcomes of extended infusion (EI) of piperacillin-tazobactam therapy for critically ill patients infected with *P. aeruginosa*. The

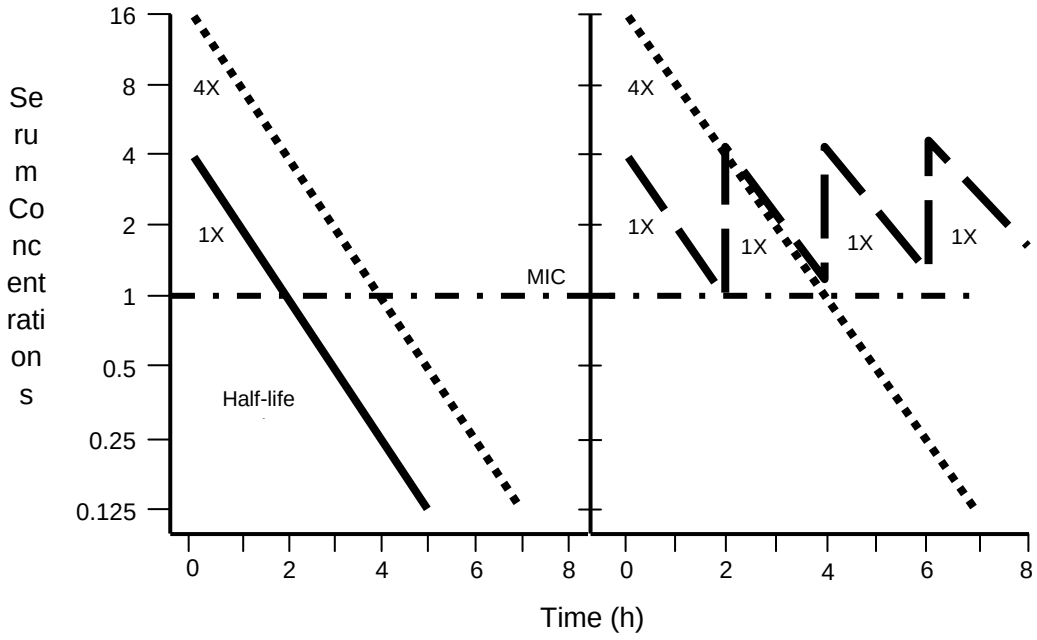
administration of bolus dosing regimen of piperacillin-tazobactam (3.375 g intravenously (IV) for 30 min every 4 or 6 h); was compared with the EI of piperacillin-tazobactam (3.375 g (IV) for 4 h every 8 h). The percentage time above MIC of free non-protein-bound drug % *f* T>MIC was optimized with EI regimen. More importantly, among the most severely ill subset of patients, 14 day mortality was significantly lower among those receiving EI piperacillin-tazobactam (Lodise, Lomaestro et al. 2007). In another retrospective study, the clinical cure rate was significantly improved when meropenem was administered via continuous infusion than intermittent bolus dosing in the treatment of ventilator-associated pneumonia (90.5% versus 59.6%; $P < 0.001$) (Nicolau 2008; Lorente L 2006). Furthermore, continuous infusion of a compound allowed a reduction in total daily dose compared with bolus dosing regimens. (Burgess 1999).

fT>MIC and fT> 4-5X MIC

Reports indicate that maximum killing of pathogens by β-lactams is observed when the concentration of the antibiotic is maintained at a level that is four to five times the MIC of the pathogen for as long as possible (Nicolau 2003; Taccone FS 2010). Achieving such a concentration is highly desired when treating immuno-compromised or critically ill patients. Improved pharmacodynamic parameters (*f*T>MIC) can be obtained by more frequent dosing, extended infusions or by continuous infusions of the β-lactam agents. These methods of administration may be highly useful in critically ill patients who usually develop high glomerular filtration rate (increased CL) and an increased volume of distribution (Vd), or have relatively resistant pathogens for β-lactam antibiotics (Figure 4 & Figure 5). Each approach increases *f*T>MIC.

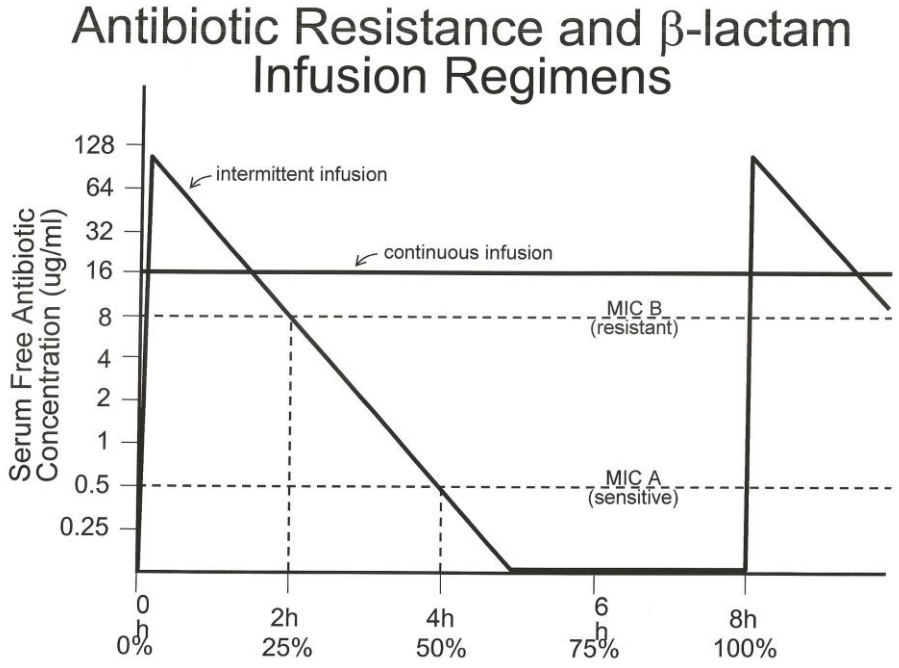
In order to clearly demonstrate the potential utility of any of these approaches, it is necessary to show a clear relationship between survival and time above MIC and/or time above four times the MIC during β-lactam therapy. While several studies have hinted at the potential relationship between microbial efficacy/improved clinical outcome and such PK/PD parameters, none show a clear relationship using a wide range of pathogens and β-lactams.

Figure 4: Effect of Dosing Changes on Pharmacodynamic Parameters.



A four-fold increase in total dose with β-lactam increases *f*T>MIC from 25 to 50% when administered as a single larger dose. However, the same increase can yield 100% *f*T>MIC when the same dose is administered four times more frequently. (Craig et al, 1995)

Figure 5: Antibiotic Resistance and β-Lactam Infusion Regimens.



Continuous infusion can provide a higher *f*T>MIC while reducing toxicity risk. Continuous infusions may be particularly useful when dealing with relatively resistant pathogens (i.e. near or at intermediate susceptibility) (courtesy, A. Kumar, original figure)

1.3 Purpose

To provide evidence to show that critical PD indices for a wide variety of β –lactams are associated with outcome in human septic shock caused by a range of bacterial pathogens.

1.4 Hypothesis

Prior to our investigation, we are aware of minimal clinical data that suggests that outcome of septic shock is associated with any β-lactam-associated pharmacodynamic indices (Lodise, Lomaestro, & Drusano, 2007). In particular, time above MIC of the free drug in plasma has not been shown to be associated with survival. In this project, our primary hypothesis is that % *f*T>MIC for β-lactam is associated with survival in human septic shock caused by bacterial pathogens treated with β-lactam monotherapy.

1.5 Objectives

1) To obtain a wide range of clinical data on cases of bacteremic septic shock treated with appropriate β-lactam monotherapy.

2) To obtain and test blood culture bacterial isolates from bacteremic patients with septic shock for quantitative sensitivity testing (using E-test strips) to the β-lactam antibiotic used for therapy.

3) To estimate individual patient creatinine clearances during the first 24 hours of documented septic shock using available individual clinical data on patient sex, weight, and serum creatinine at several time points.

4) To utilize estimated first 24 hour creatinine clearance and drug-specific volumes of distribution and unbound drug fractions to estimate drug clearance.

5) To use available data to estimate 1st 24 hr fC$_{peak}$ /MIC, fAUC/MIC and fT>MIC and fT above four times MIC and relate these parameters to outcome of septic shock.

2 Materials and Methods

2.1 Materials

2.1.1 Patient selection and data/sample acquisition

A previously developed septic shock database was used to generate a list of eligible bacteremic septic shock cases (without any personal identifying information such as name, address, medical record #'s, etc) with identifying codes for stored bacterial samples associated with their infection. To generate this database, a retrospective review of adult (≥18 year age) patients diagnosed with septic shock had been performed. A waived consent protocol was approved by the Health Ethics Board of the University of Manitoba and at each individual participating center for all aspects of this study. Study sites were selected based on the routine long-term archiving of pathogenic blood culture isolates at the institution. Each potential case was screened to determine if the case met specific criteria for septic shock as described by the revised 2003 Society of Critical Care Medicine (SCCM)/American College of Chest Physicians (ACCP) Consensus Statement on Sepsis Definitions (Levy et al., 2003).

Three hundred and forty two eligible septic shock cases collected from 8 medical institutions (6 from Winnipeg, 1 from Vancouver and 1 from Montreal) in Canada with retrievable monomicrobial isolates were identified for periods between 1996 and 2007. Maintaining complete anonymity, these isolates were retrieved (original samples remain at the primary institution where they are stored) and tested for antibiotic sensitivity (MIC) using standard microbiological techniques (E-test antibiotic strips, AB Biodisk, Inc, Solna, Sweden) in the Health Sciences Centre (HSC) Microbiology Laboratories.

Isolates were tested against the most appropriate initial antibiotics used clinically in the patients with septic shock from whom the isolates were derived.

Only ICU patients with septic shock (defined as sepsis with hypotension) and associated monomicrobial positive blood cultures with aerobic bacteria were included. Primary infections could include primary blood stream infection, or blood stream infection associated with underlying sources such as; catheter-related infections, pneumonia/empyema, complicated UTI including pyelonephritis, soft tissue infections, peritonitis, neutropenic sepsis, etc. For safety reasons, cases of meningococcal septic shock were excluded.

From each eligible case, data including the identity, dose and frequency of antibiotics utilized was retrospectively collected using a uniform data extraction template. Delay in initiation of effective (i.e. appropriate to isolated organism) antimicrobial therapy following onset of recurrent or persistent hypotension was also determined for all cases. An appropriate antimicrobial was considered to one with *in vitro* activity for the isolated pathogen. Appropriate antimicrobial therapy was considered to have been initiated if antimicrobial with *in vitro* activity for the isolated pathogen was received within 6 hours of administration of the first new antimicrobial following onset of recurrent or persistent hypotension. Otherwise inappropriate therapy was considered to have been initiated. A variety of comorbidities, lab values (including serum creatinine) and Acute Physiology and Chronic Health Evaluation (APACHE) II score (a severity of illness scoring system for first 24 hours of presentation) was also collected (Knaus WA 1985).

Renal function, specifically glomerular filtration rate (GFR), is difficult to directly measure in the critically ill. Serum creatinine (S_{cr}) concentration, however, has been used as a surrogate of renal function in the critically ill and can be used to estimate GFR. There are a number of factors that have an impact on $S_{cr.}$ The estimate of GFR in the critically ill is dependent on factors such as age, weight, muscle mass, and whether renal function is at steady-state or is changing acutely. Formulas and constants used for estimating dosing body weight (DBW), volume of drug distribution (Vd), fraction of free drug in plasma (free), elimination half life ($T_{1/2}$ normal), fraction of drug excreted in urine (Fu) and the elimination constants (ke) were obtained from several sources and are shown in Appendix 1. Several additional-conventions used for processing and analysis of individual patient data are listed in Appendix 2. (Power, Forbes, van Heerden, & Ilett, 1998, Manjunath, Sarnak, & Levey, 2001 and, Prigent, 2008).

2.2 Methods

2.2.1 Microbiology

2.2.1.1 Bacterial Culture and Testing

Since our bacteremic septic shock cases were mostly non-fastidious organisms, Mueller-Hinton agar (MH) plates were used for susceptibility testing. In addition, MH broth was supplemented to support the growth of fastidious bacteria. For example, *Haemophilus* Test Medium (HTM) was used as a recommended medium for susceptibility testing of *Haemophilus* spp. Blood was added for testing *streptococci* except *enterococci* (CLSI 2006).

A quality control procedure was followed for this project that includes the quality of the E-test strips used, media, inoculum and procedure used. The goal was to ensure precision (repeatability) and accuracy of antimicrobial susceptibility testing procedures and proper maintenance of performance of materials used in the tests; in addition, the quality control testing may checks any external factors that may play a significant role in susceptibility testing. These goals were achieved by testing of quality control bacterial strains with known susceptibility end points to the designated antimicrobial agents to be tested. American Type Culture Collection bacterial strains (ATCC reference strains with known susceptibility endpoints) were used in this research (CLSI-M7 & M100, 2006). Appendix 3 shows the results of the quality control testing. As was outlined in the manufactures' instruction six different bacterial species were used for quality control measures of E-test strips. These included: *Enterococcus faecalis* (ATCC® 29212), *Escherichia coli* (ATCC® 25922), *Haemophilus influenzae* (ATCC® 49766), *Pseudomonas aeruginosa* (ATCC® 27853), *Staphylococcus aureus* (ATCC® 25923), and *Streptococcus pneumoniae* (ATCC® 49619).

All of the isolates (in small cryo vials) have been stored at HSC Microbiology Lab in -80 °C freezers. These were retrieved, rapidly thawed and a subculture made by inoculating a non-selective solid medium such as blood agar (BAP) to obtain a pure isolated colonies. After appropriate incubation conditions at 35 ± 2 °C (ambient air; 16 to 18 hours), isolated colonies from BAP were subcultured again two more times to make ensure that healthy organisms were used in this study (3 times in total). The last subculture was then used to perform appropriate sensitivity/MIC testing. Reserved skim-

milk vials for each isolate were made for storage at -80 °C freezer at the HSC Microbiology Lab for future research if needed (CLSI 2006).

The direct colony suspension method was used for inoculum preparation since it is compatible with most of the bacterial isolates according to the Clinical and Laboratory Standard Institute (CLSI). This is the recommended method for testing fastidious organisms, such as *Haemophilus* spp., *N. gonorrhoeae*, *N. meningitidis*, *Streptococci* and for testing *Staphylococci species* for potential methicillin resistance. It has also been used frequently when non-fastidious organisms such as Enterobacteriaceae or non-Enterobacteriaceae such as *Pseudomonas* spp. were involved, since fresh (24 hour) colonies as are needed.

The inoculum was prepared by making a normal saline suspension of isolated fresh colonies selected from an 18- to 24-hour nonselective agar plate medium such as blood agar plates (BAP). This was achieved by selecting a 3-5 well-isolated colonies of the same morphologic type from a BAP. A sterile swab was used to transfer the growth into a small glass tube containing 4 to 5 mL of sterile normal saline. To standardize the inoculum density for the required susceptibility testing; Barium sulphate (BaSO4) was used as a turbidity standard equivalent to a 0.5 McFarland standard or its optical equivalent (CLSI-M7, 2006). Then the bacterial suspension was adjusted to obtain a turbidity standard equivalent to the reference 0.5 McFarland turbidity standards by using a photometric device Genesys 20® by Thermo Scientific, (0.08 to 0.1 absorbance units at 625 nm) (CLSI-M7, 2006).

2.2.1.2 MIC Determinations

E-test® strips were used for *in vitro* testing to determine the MIC of clinical isolates in this study. E-test is a quantitative technique "ready to use" plastic reagent strip with a predefined calibrated gradient with a MIC scale in μg/mL of the specific antibiotic tested and a two-letter code of the antibiotic on one side of the strip. A predefined exponential concentration gradient of antibiotic covers the other side of the strip. The underlying dry chemistry technology enables a wide variety of antimicrobial agents to be performed, immobilized (stable) on the predefined calibrated thin, inert plastic strip, measures 5 mm wide and 60 mm long, allowing for accurate assays of antimicrobial sensitivity when done under certain designated conditions such as; specific inoculum, on a specific agar media under specific incubation atmosphere for a specific time. E-test strips must be stored at -20 °C freezers all the time and when used for testing it need to be taken out of the freezers and allowed to be reached to room temperature for about 30 minutes before applying on the MH plates as per manufacture's recommendations.

In clinical microbiology laboratories, antimicrobial susceptibility testing (AST) may be performed by using one of the following quantitative or qualitative methods; disk diffusion, antibiotic gradient strips (E-test), agar dilution and broth micro-dilution or macro-dilution method. Inoculum preparation is an integral part of performing antimicrobial susceptibility testing (AST) including full minimal inhibitory concentration (MIC) assay of microbial organisms. The E-test method used in this study is recognised as a simple method for generating MICs of a wide antibiotic concentration range for a broad range of bacterial pathogens, that include both non-fastidious Gram-negative and Gram-positive aerobic bacteria such as Enterobacteriaceae, *Pseudomonas,*

Staphylococcus and *Enterococcus* species and fastidious bacteria, such as anaerobes, *S. pneumoniae*, other *Streptococcus* and *Haemophilus* species. To minimize variability, a high quality standard and carefully controlled procedure was followed. The test was done in duplicate for each isolate, to ensure the reproducibility of the test which is within ± 1 tow-fold dilution of the end point. The interpretations of the MIC result were based on the CLSI procedure guidelines (High et al., 2001, CLSI-M100,2006).

For testing, the manufacture's recommendations for performing the E-test assay and MIC interpretations as per CLSI guidelines criteria were followed. The appropriate E-test strip was applied to the inoculated agar surface (MH agar) within 15 minutes of inoculation under aseptic techniques and microbial hazard precautions (i.e. as per the manufacturer's instructions). The preformed exponential gradient of antimicrobial agent on the strip diffuses immediately into the agar medium. The antibiotic test was the β–lactam drug used for initial appropriate therapy of the case of septic shock irrespective of how late it was started. After overnight incubation, which was usually 20-24 hours at 35 ± 2 °C in an appropriate atmosphere, a symmetrical inhibition ellipse centered along the strip is develops where the concentration of the β-lactam antibiotic tested completely inhibits microbial growth. The MIC value of the designated drug was readable directly from the scale in terms of µg/mL at the point where the edge of the inhibition ellipse intersects the strip. Where MIC values exceeded the upper limit of the E-test range, a value of twice the upper limit was used (i.e. if the highest listed value was >256 µg/mL, the value used was defaulted to 512 µg/mL). If the test MIC value fell below the lower limit of the E-test strip, that lower limit value was used for calculations (Thomson, 2004& CLSI M7,2006).

2.2.2 Pharmacokinetics (PK)

Serum concentrations of antibiotics during the first 24 hours of therapy were estimated using standard pharmacokinetic modeling based on age, weight, renal function, and dose of antibiotic (all encoded within the ICU sepsis database). Using the MIC and estimated serum antibiotic concentrations, *f*T>MIC and *f*AUC/MIC was determined.

1- Creatinine clearance CL_{CR} was calculated using an adjusted Cockcroft-Gault equation (D'Angio-R 1988) that excluded weight – was normalized to 72 Kg:

$$CL_{CR} = (140 - age)*88.4/ S_{cr}$$

the result of this equation was multiplied by 0.85 if females (Cockcroft 1976), and S_{cr}= serum creatinine (µmol/L).

2- The elimination rate constant (ke_R) for the antibiotics was individualized to the level of each patient's renal function according to the equation:

$$ke_R = ke * [1-Fu*(1- CL_{CR}/100)]$$

where 'ke' is the literature value of elimination rate constant for each antibiotic in normal renal function, and 'Fu' is the literature value for fraction of drug excreted unchanged in the urine.

3- First dose free peak serum concentration (fC_{p1}) was calculated as:

$$fC_{p1} = f * [(D/t') * (1-e^{-keR*t'})] / [ke_R * Vd]$$

where '*f*' is the literature value for percentage free drug in the serum for that antibiotic, D= dose in milligrams, t'= infusion time, and Vd= volume of distribution from the

literature for that antibiotic as 'L/kg' multiplied by the patient's dosing body weight (in kilograms).

Population expected pharmacokinetic parameter values for each of the antibiotics' Vd, Fu, normal half-life (T ½), ke, and free drug fraction are shown in Appendix 1.

4- Steady-state free peak serum concentration (fC_{pss}) was calculated as:

$$fC_{pss} = f * [(D/t') * (1-e^{-KeR*t'})] / [Ke_R * Vd * (1-e^{-KeR*\tau})]$$

where τ = dosing interval for that antibiotic regimen.

5- Steady-state free trough serum concentration (fC_{tss}) was calculated as:

$$fC_{tss} = fC_{pss} * e^{-KeR*(\tau - t')}.$$

6- The free peak concentration after the 1st dose (fC_p1) relative to the MIC was obtained as:

$$fC_{p1}/MIC = f * [(D/t') * (1-e^{-keR*t'})] / [ke_R * Vd]/MIC$$

N.B: This first dose parameter may be important to examine on its own as some patients received only the first dose within the first 24hrs, and had died before getting a second dose of the antibiotic.

7- The percentage of steady-state free time above the MIC was obtained from:

$$\% f_{ss}T>MIC = [t'+\{\ln(fC_{pss}/MIC)/ke_R\}]/ \tau * 100$$

Where $fC_{pss} = [D/t'/(ke*Vd)]*[1-exp^{-keR*t'}]/\{1-exp^{(-keR*\tau)}\}$

8- The percentage of free time steady-state above four times the MIC was obtained from:

$$\%f_{ss}T>4X\ MIC = [t'+\{\ln(fC_{pss}/4*MIC)/ke_R\}]/\tau * 100$$

where $fC_{pss} = [D/t'/(ke*Vd)]*[1-exp^{-keR*t'}]/\{1-exp^{(-keR*\tau)}\}$

9- Area under the free-concentration time curve (fAUC) over MIC for the 1st dose of each antibiotic was calculated as:

$$fAUC/MIC = (fC_{p1} / ke_R)/MIC$$

Where (fC_{p1} / ke_R) represents the first dose to infinity which is the fAUC after a single dose is equivalent to the fAUC for any one dosing interval at steady-state.

10- The steady-state fAUC over MIC for each antibiotic was calculated as:

$$fAUC_{ss}/MIC = AUC_{ss} * (24/\tau)/MIC$$

11-The percentage of time above MIC of free drug after the first dose was calculated as:

$$1^{st}\ \%\ fT>MIC = 100* [t'+ Ln(fC_{p1}/MIC)/ke_R]/\tau$$

If fC_{pss} was less than MIC, then the result was defaulted to 0% T>MIC; and the ratio also by default could not exceed 100%.

12-The percentage of time that free drug concentrations were four times above the MIC after the first dose was calculated as:

$$1^{st}\ \%fT>4*MIC = 100* [t'+ Ln(C_{pf1}/4*MIC)/ke_R] / \tau$$

13-The free area under curve is divided by the MIC for the 1st 24 hours it is calculated as:

$$fAUC_{24h}/MIC = \sum_{n=1}^{n=(24/\tau)} [(Cp_n - Ct_{n+1})/MIC/ke_R]$$

where $Cp_1 = D/Vd$, $Ct_1 = Cp_1 * \exp^{-keR*\tau}$, and

$Cp_2 = Cp_1 + Ct_1$, $Ct_2 = Cp_2 * \exp^{-keR*\tau}$, etc

and where 'n' is the number of doses in 24h

14- $$\%fT>MIC_{24h} = (100/n) * \sum_{n=1}^{n=(24/\tau)} [t' + \{Ln(Cp_n/MIC)\}/ke_R]/\tau$$

where t' is the infusion time.

15- $$\%fT>4*MIC_{24h} = (100/n) * \sum_{n=1}^{n=(24/\tau)} [t' + \{Ln(Cp_n/4*MIC)\}/ke_R]/\tau$$

Values for dosing body weight (DBW), volume of distribution (Vd), fraction of free drug in plasma (free), elimination half life ($t_{1/2}$ normal), fraction of drug excreted in urine (Fu) and the elimination constant (ke) were obtained from several sources are shown in Appendix 1 (Power, Forbes, van Heerden, & Ilett, 1998). (Lodise, Lomaestro, & Drusano, 2007) (MICROMEDEX® 1.0 (Healthcare Series))

Infusion time (i.e. t') used in calculations for all β-lactam antibiotics was 15 minutes. For patients with chronic renal failure on hemodialysis, an estimated creatinine clearance of 5 mL/min/1.73m^2 was used for the calculation of pharmacokinetic parameters in the

first 24 hours since dialysis is rarely implemented within that time frame in the acute care setting of septic shock. Elevated creatinine clearance values were not truncated given recent evidence of augmented renal clearance in highly stressed patients (Udy A, 2009,Udy, Roberts, et al 2010). If actual height and weight were unavailable, sex-specific average values for the subjects from the source institution were used. Sex-specific ideal body weight was used for females, and ideal body weight for heights less than 60 inches was uniformly set at 45 kg. For males, ideal body weights for heights less than 60 inches was uniformly 50 kg.

2.2.3 Statistical Analysis

The primary outcome variable of interest was survival to 30 days inclusive of discharges to chronic health care facilities (nursing homes, etc). Mortality over 28 days was assessed using a Cox proportional hazard model. The calculated PD indices (e.g. *f*T>MIC) were the independent variables. Data is expressed as mean ± standard deviation unless otherwise indicated.

Logistic regression modeling was used to examine survival to hospital discharge as a function of T above MIC and T above four times the MIC as a continuous variable. The same effect was stratified over the entire group and specific subgroups. A multiple logistic regression model was used to examine the independent impact of a variety of clinical and therapeutic variables including delay in effective antimicrobial initiation on survival to hospital discharge. The delay was imputed to a zero value if the appropriate antimicrobial was given before onset of hypotension. Correlations with delay in effective antimicrobial administration were examined using Pearson coefficients. In addition, Chi-square testing was used to assess *f*T>MIC as discrete categories (<30%, 30-60%, 60-

90% and >90%). The confidence limits and p-values reported reflect a α level of 0.05. All statistical analyses were performed using SAS 9.0.

3 Results

3.1 Characteristics of patient cohort

342 eligible bacteremic patients with septic shock were determined to have been treated with a β-lactam as the only appropriate antimicrobial (β-lactam monotherapy). Of these, males accounted for 59.1% of the total cases and 40.9% were females. The average (±standard deviation) age of patients was 63.9 ± 15.6 years. Average Acute Physiology and Chronic Health Evaluation II (APACHE II) (Knaus WA 1985) was 27.0 ± 8.8.

Descriptive statistics for the overall subject cohort are shown in (Table 1). Notably, the average creatinine used for estimation of the normalized creatinine clearance was elevated at 170 ± 98 μmol/L consistent with the presence of septic shock. Consequently, the estimated creatinine clearance using the creatinine value closest to 24 hours after documentation of hypotension was reduced at 37.4 ± 23.2 ml/min for the overall group.

The distribution of comorbidities is displayed (Table 2). Diabetes mellitus and substance abuse were the most common co-morbidities and were present in a total of 28.9% and 18.1% of cases (insulin-requiring in about 1/3 of total diabetic patients) respectively. Community-acquired infections accounted for 64.6% of cases with a survival rate of 50.7%. Nosocomial infections accounted for the remaining 34.5% of cases and had a survival rate of 30.5%.

Table 1: Descriptive statistics for the overall subject cohort.

Parameters	Measures
Average age (SD) Year	63.9 (± 15.6)
Sex(%) male/female	202 (59.1) /140 (40.9)
APACHE II score (SD)	27.0 (± 8.8)
Median actual weight (IQR) kg	77.3 (67.1-85)
Median IBW (IQR) kg	70.2 (53.5-73.3)
Median DBW (IQR) kg	71.1 (60-76.4)
Serum creatinine at shock (median, IQR) μmol/L	165 (113-273)
Serum creatinine at 24 hours post shock (median, IQR) μmol/L	171(98-271)
Maximum serum creatinine in first 24 hrs post-shock (median, IQR) μmol/L (includes baseline and 6 hr post-shock values)	170 (98-269)
Creatinine clearance estimated from serum creatinine closest to 24 hours post shock onset (median, IQR) μmol/L/min	37.1 (23.3-63.1)
Time to 1st antibiotic (median, IQR) hrs	3.67 (1.0-7.5)
Microbiologically inappropriate initial empiric therapy	15.2%

342 eligible bacteremic patients with septic shock were determined to have been treated with β-lactam monotherapy. The average (±SD) age of patients was 63.9 ± 15.6 years. Average Acute Physiology and Chronic Health Evaluation II (APACHE II) was 27.0 ± 8.8. Elevated creatinine clearance at 170 ± 98 μmol/L was consistent with the presence of septic shock. Inappropriate initial empiric therapy was 15.2%. Time to 1st antibiotic hrs was 3.67 (1-7.5).IBW: ideal body weight, DBW: dosing body weight, IQR: interquartile range.

Table 2: Distribution of comorbidities associated with septic shock patients of the cohort.

Comorbidity	%
DM (oral medication dependent)	22.1
Hypertension	18.6
Substance abuse (alcohol or IV abuse)	18.1
Immunosuppression	14.7
Chronic renal failure (creatinine >150 umol/L)	13.9
Elective surgery	11.2
COPD (medication or oxygen requiring)	10.5
Liver failure/cirrhosis	10.3
Metastatic CA	9.1
Emergency surgery	8.8
Neutropenia (<1000/uL)	8.0
Leukemia	7.4
DM (insulin-dependent)	6.8
Dialysis	5.3
Lymphoma	2.9
Heart disease	1.8
AIDS	1.8

Comorbidities are commonly found in most sepsis patients. Epidemiologic studies have shown that, more than 50% of the severe septic patients have at least one chronic comorbid medical condition. Diabetes, obstructive pulmonary disease, congestive heart failure, liver and kidney disease, hypertension, and cancer, each can be found in 10-20% of sepsis patients. Notably, >90% of septic shock patients have one or more of these major comorbidities conditions AIDS: Acquired immune deficiency syndrome, COPD: chronic obstructive pulmonary disease, DM: diabetes mellitus, IV, intravenous.

3.2 Microbiological features of the cohort

The distribution of clinical infections is shown (Table 3). Pneumonia accounted for the largest clinical subset of patients (28.4% of total cases). Intra-abdominal infections and urinary tract infections also accounted for a large fraction of patients in the cohort (23.1% and 17.8% respectively). Urinary tract infections and skin/soft tissue infections had particularly high survival rate at 68.85% and 64.7% respectively. In contrast, primary blood stream infections exhibited survival rates of 21.1%. Survival in other groups of >10 cases ranged from approximately 47% to 64%.Overall, survival to ICU and hospital discharge was 60.6% and 47.9%, respectively. Thirty day survival was 48.8% (167/342).

Isolated pathogens are listed (Table 4). *E. coli* was the single most common isolate accounting for 27.5% of cases. *S. aureus* was the 2nd most common pathogen at 20.2% of total cases. *Klebsiella species* (10.2%), *S. pneumoniae* (9.0%) and *P. aeruginosa* (7.3%) represented the other individual pathogens isolated in >5% of cases. Gram-positives accounted for 44.4% of the total cases while Gram- negatives accounted for 55.6%.

Patients receiving penicillins and penicillin/penicillinase-inhibitor combinations accounted for 45.9% of the cases of β-lactam monotherapy. Cephalosporins were used in 37.1% of cases and carbapenems (meropenem and imipenem-cilastin only) in 17.0%. The breakdown of specific antibiotics used in this cohort is shown (Table 5). Piperacillin/tazobactam (31.6% of the total), cefotaxime (23.1%) and meropenem (13.2%) comprise the largest groups within the β-lactam monotherapy cohort. The breakdown of antimicrobials utilized within nosocomial and community-acquired

Table 3: The distribution of clinical infection sites among the septic shock patients of the cohort (n=342)

Type of underlying Infection	No. of patients	% of total	No. of survivors (hospital discharge)	% of survivors (fraction of group)
Pneumonia	98	28.4	31	32.0
Intra-abdominal infection IAI	81	23.1	32	40.5
Urinary tract infection UTI	61	17.8	42	68.9
Primary blood stream infection PBSI	38	11.1	8	21.1
Catheter-related infection	19	5.6	9	47.4
Skin and soft tissue infection (other than wound)	17	5.0	11	64.7
Septic arthritis	11	3.2	6	54.5
Central nervous system infection	5	1.5	1	20.0
Wound infection	4	1.2	2	50.0
Cardiovascular	4	1.2	2	50.0

Regarding the site of infections associated with sepsis syndromes, the greatest percentage (25-50%) are due to respiratory tract infections, with IAI, UTI, and PBSI they represent together about 80% of the total cases in this study. IAI, Intra-abdominal infection; UTI, Urinary tract infection; SSTI, Skin and soft tissue infection.

Table 4: Distribution of isolated pathogens for the cohort

Organism	Number of organisms	% Total
Gram-positive organisms	**152**	**44.4 %**
Staphylococcus aureus	69	20.2 %
Streptococcus pneumoniae	31	9.1 %
Enterococcus faecalis	14	4.1 %
Streptococcus viridans	11	3.2 %
Non-A/ B-streptococci	10	2.9 %
Group A β-hemolytic *Streptococci (GAS)*	10	2.9 %
Enterococcus faecium	5	1.5 %
Other β-hemolytic streptococci	4	1.2 %
Bacillus species	1	0.3 %
Corynebacterium jeikeium JK	1	0.3 %
Gram-negative organisms	**190**	**55.6 %**
Escherichia coli	94	27.5 %
Klebsiella species	35	10.2 %
Pseudomonas aeruginosa	25	7.3 %
Enterobacter species	12	3.5 %
Citrobacter species	6	1.8 %
Serratia species	5	1.5 %
Proteus species	4	1.2 %
Acinetobacter species	2	0.6 %
Haemophilus influenzae	1	0.3 %
Morganella morgane	1	0.3 %
Stenotrophomonas maltophilia	1	0.3 %

Only monomicrobial isolates were utilized in this study (n=342). Gram-positives accounted for 44.4% of the total cases while Gram- negatives accounted for 55.6%.

Table 5: Distribution of β-lactams monotherapy for the cohort (n=342).

Antibiotic	No.	% total
Penicillins	**157**	**45.9**
Piperacillin-tazobactam	108	31.6
Cloxacillin	25	7.3
Ticarcillin-clavulanate	10	2.9
Ampicillin	7	2.0
Piperacillin	5	1.5
Penicillin	3	0.9
Cephalosporins	**127**	**37.1**
Cefotaxime	79	23.1
Cefuroxime	18	5.3
Ceftazidime	12	3.5
Cefazolin	8	2.3
Ceftriaxone	5	1.5
Cefoxitin	4	1.2
Carbapenems	**58**	**17.0**
Meropenem	45	13.2
Imipenem/cilastatin	13	3.8

Breakdown β-lactams monotherapy showing the number of patients treated with the designated antibiotics and the percent of total utilization of the antibiotic in the cohort.

infection groups and among the 4 major clinical syndromes (pneumonia, IAI, UTI and PBSI) are shown in (Table 6).

3.3 Clinical outcomes

Survival to hospital discharge in relation to free T>MIC is shown in (Table 7). A clear stepwise increase in survival is noted. Similar results were found with free T above four times the MIC and with 30 day survival.

Clinical outcomes in relationship to age, severity of illness (APACHE II score), creatinine clearance and time to appropriate antimicrobial therapy are shown in (Table 8). P-values for these relationships (as continuous variables) are shown in table 8. Each of these variables has been shown to be linked to the outcome of septic shock in other studies.

Only a weak relationship between age and outcome was shown. This may be due to some unexpected variability in outcomes over age quartiles. However, overall, older patients had inferior outcomes compared to younger when comparing the younger half to the older half of patients (p=.025, Chi-Square analysis).

An inverse relationship was demonstrated between APACHE II score and the patient outcome (survival rate). An increase in the APACHE II was associated with a decrease in the survival rate, dropped from 70.7% when APACHE II was <21, to only 15.3 % when APACHE II score was >32 (p<.0001 as a continuous variable, logistic regression).

A higher survival rate was shown with higher 1st 24 hour estimated creatinine clearance (CrCl); survival was 58.8% with >63.5 μmol/L/min CrCl but only 35.7 % of

the patients survived when CrCl was >23 μmol/L/min (p=.0045 as a continuous variable, logistic regression).

Table 6: Distribution of β-lactam monotherapy used for the common infection syndrome groups.

Antimicrobial Agents Used in Treatment	Nosocomial Infection	Comm. Infection	Major Underlying infections (**n=278**)			
			Pneumonia	IAI	UTI	PBSI
Penicillins (%)	**63(52.1)**	**94(42.5)**	**40(40.8)**	**36(44.4)**	**29(47.5)**	**20(52.6)**
Piperacillin-tazobactam	42	66	27	27	23	13
Cloxacillin	13	12	8	0	0	5
Ticarcillin-clavulanate	1	9	3	4	3	0
Ampicillin	4	3	1	2	3	0
Piperacillin	3	2	0	3	0	1
Penicillin	1	3	1	0	0	1
Cephalosporins (%)	**33(27.3)**	**94(42.5)**	**47(48.0)**	**20(24.7)**	**25(41.0)**	**12(31.6)**
Cefotaxime	18	61	24	12	21	8
Cefuroxime	7	11	14	1	0	1
Ceftazidime	7	5	5	2	2	3
Cefazolin	0	8	2	1	0	0
Ceftriaxone	0	5	2	0	2	0
Cefoxitin	1	3	0	4	0	0
Carbapenems (%)	**25(20.7)**	**33(15.0)**	**11(11.2)**	**25(30.9)**	**7(11.5)**	**6(15.8)**
Meropenem	17	28	10	17	6	5
Imipenem/cilastatin	8	5	1	8	1	1
TOTAL (%)	**121(35.4)**	**221(64.6)**	**98(28.6)**	**81(23.7)**	**61(17.8)**	**38(11.1)**

The 4 major underlying infection groups (pneumonia, IAI, UTI, PBSI) shown above represent 81.3 % of the total (n=278/342). IAI: Intra-abdominal infection, PBSI: Primary blood stream infection, UTI: Urinary tract infection.

Table 7: Survival to hospital discharge according to *f*T>MIC (%).

*f*T>MIC (%)	No. survivors	No. of cases	Survival (%)
0.0-29.9 %	15	55	27.2
30-59 %	15	38	39.5
60-89 %	21	47	44.6
>90 %	100	202	49.5

A clear stepwise increase in survival is noted with increasing the percentage of free time above MIC. P-value for improved survival with increased *f*T>MIC % quartiles = .0035 (Chi-square analysis).

Table 8: Quartile breakdown of; Age, APACHE II Score, creatinine clearance, and antibiotic initiation delay.

Parameter		No. of patients	No. of survival	% of survival
Age (year)	>76	91	40	44.0
	65-76	84	27	32.1
	54-65	87	47	54.0
	<54	80	37	46.3
p-value		**p=.044**		
APACHE II	>32	85	13	15.3
	26-32	89	40	44.9
	21-25	86	40	46.5
	<21	82	58	70.7
p-value		**p<.0001**		
CrCl (µmol/L/min)	>63.5	85	50	58.8
	37.4-63.4	85	36	42.4
	23.1-37.3	88	35	39.8
	<23	84	30	35.7
p-value		**p=.0045**		
Antibiotic Initiation Delay (hr)	>7.4	84	20	23.8
	3.67-7.39	84	32	38.1
	1-3.66	85	48	56.5
	<1	83	52	62.7
p-value		**p<.0001**		

Only a weak relationship between age and outcome was shown. An inverse relationship was demonstrated between APACHE II score and the patient outcome

(survival rate). A higher survival rate was shown with higher 1st 24 hour estimated creatinine clearance (CrCl). Shorter delays from initial documentation of hypotension to initiation of appropriate antimicrobial therapy were also associated with a stepwise improvement in survival (from hypotension documentation).

Shorter delays from initial documentation of hypotension to initiation of appropriate antimicrobial therapy were also associated with a stepwise improvement in survival. Survival was 23.8% among those in whom appropriate antimicrobials took >7.3 hrs but 63.7% among those whose where appropriate antimicrobials were initiated within the first hour of hypotension documentation ($p<.0001$ as a continuous variable, logistic regression).

Patients who received appropriate antibiotics only after the onset of hypotension accounted for 87.4% (299/342) of patients compared to12.6% (43/342) of patients who were treated with an appropriate antibiotic before documentation of hypotension. Survival in the two groups was 42.8% (128/299) and 53.5% (23/43) respectively ($p=0.19$). Patients received initially appropriate antimicrobial therapy in (290/342) 84.8% of cases; survival was 50% in such cases. Only 15.2% (52/342) of cases received inappropriate initial antimicrobial therapy; survival in this group was 11.5% (6/52). Only 12 (3.5%) of patients never received appropriate antimicrobial therapy. Receipt of initially inappropriate therapy had a substantial adverse impact on survival on Chi-square analysis (OR 0.145, 95% CI 0.061-0.304, $p<0.0001$) (Table 9). In addition, the delay in administration of the first dose of appropriate antimicrobial therapy was associated with worse outcome (OR for survival 0.912 per hour delay, 95% CI (0.873- 0.946), $p<.0001$) in univariate analysis (Table 10).

3.4 Statistical analysis of epidemiological factors

Epidemiologic factors were associated with outcome in univariate analysis. These included age, APACHE II score, renal function (creatinine), and a variety of comorbidities (Table 9 & Table 10).

Table 9: Univariate analysis of the cohort

Parameter	Odds ratio (95% CI) for survival with unit increments	p-value
Age(year)	0.986 (0.972-1.000)	0.044
Sex (reference female)	0.944 (0.611- 1.457)	0.7934
APACHE II score (points)	0.885 (0.855-0.913)	<.0001
Baseline (at shock)SCr (µL/L)	1.000 (0.998- 1.001)	0.7948
24 hrs Creatinine	0.999 (0.997- 1.000)	0.1261
Cr(max) within 24hrs	0.998 (0.996- 1.000)	0.022
CrCl at 24hrs post-shock	1.007 (1.003- 1.013)	0.0045
Time to 1st antibiotic (hr)	0.912 (0.873- 0.946)	<.0001
Inappropriate initial empiric therapy (reference appropriate)	0.145 (0.061- 0.304)	<.0001

Age, Apache II score, maximum serum creatinine (Cr max) in the first 24 hours of shock, creatinine clearance at 24 hours post-shock, time to 1st appropriate antibiotic following documentation of hypotension and the initial use of inappropriate empiric antibiotics each had a significant association with survival in univariate analysis. CI, confidence interval; OR, odds ratio

Table 10: Univariate analysis of cohort showing comorbidity associated with outcome.

Comorbidity	OR (95% CI) for Survival	p-value
AIDS	0.200 (0.010- 1.258)	0.1438
Lymphoma	1.025 (0.280- 3.748)	0.9697
Leukemia	0.124 (0.029- 0.367)	0.0008
Metastatic CA	0.718 (0.333- 1.507)	0.3842
Immunosuppression	0.181 (0.080- 0.369)	<.0001
Neutropenia (<1000/uL)	0.209 (0.069- 0.525)	0.0021
Liver failure/cirrhosis	0.372 (0.165- 0.779)	0.0115
Heart disease	0.506 (0.070- 2.629)	0.4353
Hypertension	1.961 (1.120- 3.498)	0.02
COPD (medication or oxygen requiring)	0.705 (0.344- 1.411)	0.3274
Chronic renal failure (creatinine >150 umol/L)	0.596 (0.312- 1.111)	0.1077
Dialysis	0.636 (0.229- 1.657)	0.3624
DM (oral medication dependent)	2.089 (1.236- 3.591)	0.0066
DM (insulin-dependent)	0.774 (0.322- 1.811)	0.5566
Elective surgery	1.157 (0.588- 2.291)	0.6733
Emergency surgery	2.190 (1.014- 5.023)	0.0521
Substance abuse (alcohol or IV abuse)	0.606 (0.326- 1.117)	0.109

Different varieties of comorbidities of the cohort were associated with outcome (survival) in univariate analysis. OR and p-value for survival have been calculated for the major comorbidities of the cohort. CI, confidence interval; OR, odds ratio

3.5 Pharmacodynamic Indices and outcome of septic shock

Logistic regression analysis demonstrated that 1st 24 hour time above MIC (OR 1.175 95% CI 1.073-1.288 per 10% increment, p=.0005) (Table 11) and 1st 24 hour time above four times the MIC (OR 1.127 95% CI 1.057-1.203 p=.0003) were both strongly associated with improved survival of septic shock. Neither the 1st 24 hour fC_{p1}/MIC (p=.0762) or 1st 24 hour fAUC/MIC (p=.0872) achieved significance in relation to outcome in logistic regression analysis. Logistic regression of subgroups (Table 11) suggested that T>MIC was associated with survival most strongly for Gram-negatives including enterobacteriaceae among the organism groups. No specific organism grouping (e.g. *S. aureus*, *E. coli*, etc.) achieved significance in logistic regression. The effect was most pronounced for cephalosporins with a similar trend for carbapenems but not for penicillins.

In logistic regression with time to antimicrobial therapy, the overall effect of time above MIC remained highly significant (Table 12). Gram-negatives demonstrated that both time to appropriate antimicrobial therapy and time above MIC were independently associated with outcome. However, the same did not hold for Gram-positives. For Gram-positives, the time to appropriate antimicrobial initiation remained a significant correlate of outcome but the time above MIC did not. No specific organism grouping (e.g. *S. aureus*, *E. coli*, etc.) achieved significance in logistic regression (data not shown).

Similar logistic regression for penicillins, cephalosporins and carbapenems demonstrated that all groups demonstrate superior outcomes with faster time to antimicrobial therapy from documentation of hypotension (Table 12). However, only

Table 11: Logistic regression analysis of PD indices and outcome of septic shock

	No.	OR survival	95% Lower CI	95% Upper CI	p-value
all organisms/all β-lactams	**342**	**1.175**	**1.073**	**1.288**	**0.0005**
All Gram-negative	**190**	**1.268**	**1.071**	**1.592**	**0.015**
Enterobacteriaceae	151	1.354	1.097	1.837	0.017
E. coli	94	1.109	0.839	1.525	0.47
All Gram-positive	**152**	**1.081**	**0.954**	**1.235**	**0.23**
S. aureus	69	1.061	0.882	1.252	0.53
all β-lactams					
Penicillins	**157**	1.059	0.921	1.231	0.43
Piperacillin/Tazobactam	108	1.154	1.058	1.531	0.212
Cephalosporins	**127**	1.229	1.053	1.481	0.016
Cefotaxime	79	1.255	1.046	1.590	0.028
Carbapenems	**58**	1.192	0.951	1.664	0.152
Meropenem	45	1.188	0.942	1.594	0.19

Logistic regression was used to examine the impact of *f*T>MIC (in 10% increments) on survival to hospital discharge for the entire cohort and subgroups. Over all significant improved in survival was seen with all organisms/all β-lactams (OR 1.175, p-value = 0.0005).

Table 12: Multiple logistic regression analysis of the cohort

	No.	OR survival	95% Lower CI	95% Upper CI	p-value
All organisms/β-lactams	**342**				
*f*T>MIC		1.163	1.053	1.295	.0038
Antibiotic delay		0.922	0.885	0.955	<.0001
Gram-negative	**190**			1.679	
*f*T>MIC		1.324	1.112	344.468	.0059
Antibiotic delay		0.939	0.895	0.976	.0048
Gram-positive	**152**				
*f*T>MIC		1.057	0.921	1.215	.4313
Antibiotic delay		0.869	0.791	0.936	.0012
Penicillin	**157**				
*f*T>MIC		1.059	0.910	1.239	.4577
Antibiotic delay		0.942	0.895	0.978	.0020
Cephalosporins	**127**				
*f*T>MIC		1.239	1.060	1.496	.0129
Antibiotic delay		0.894	0.812	0.964	.0067
Carbapenems	**58**				
*f*T>MIC		1.241	0.938	1.732	0.1196
Antibiotic delay		0.870	0.761	0.955	0.0178

A multiple logistic regression model was used to examine the independent impact of a delay in appropriate antimicrobial initiation and *f*T>MIC (in 10% increments) on

survival to hospital discharge for the entire cohort, Gram-stain (+/-) grouping and β-lactams groups. A delay in appropriate antibiotic initiation is the most single factor associated with decreased survival.

cephalosporins yielded significant advantage to higher *f*T>MIC with respect to outcome although carbapenems trend in that direction.

Analysis of the impact of time above MIC was also assessed in logistic regression using categorical values (*f*T>MIC of 0.1-30, 30-59.99, 60-89.99 and ≥90%) with antimicrobial delay included in the analysis. Results are shown (Table 13). Overall, significant differences in outcome were detected among the different *f*T>MIC groups (p=.0398). Logistic results were similar whether or not antimicrobial delay was included in the analysis (p=.0127 *f*T>MIC category and <.0001 antibiotic delay). Similar results were found for T above four times the MIC and antimicrobial delay.

In separate logistic regression models which included antimicrobial delay, Apache score and age, both T above MIC and T above four times the MIC remained significantly associated with outcome (Table 14).

Table 13: Relationship of Chi-square analysis to survival

Reference Time *f*T>MIC **>90% (n=260)**	No.	Survival OR	Lower 95% CI	Upper 95% CI	p-value
*f*T>MIC 0.0-29.9%	25	0.210	0.077	0.578	0.0025
*f*T>MIC 30-60%	17	0.589	0.217	1.596	0.0952
*f*T>MIC 60-90%	40	0.561	0.285	1.106	0.2982
*f*T>MIC 0.0-29.9%	25	0.310	0.104	0.927	0.0362
*f*T>MIC 30-60%	17	0.472	0.262	2.354	0.0373
*f*T>MIC 60-90%	40	0.785	0.233	0.957	0.666
Time to 1st appropriate antibiotic (per hr delay)		0.912	0.873	0.946	<.0001

Chi-square testing of 1st 24 hr *f*T>MIC as discrete percentage of time categories in relation to survival (with and without time to first appropriate antibiotic included in equation). This group (n=82) were compared to the reference group (n=260) for whom >90 % *f*T>MIC was covered. OR is improved (0.912) with *f*T>MIC especially when time to 1st antibiotic delay was included in the analysis.

Table 14: Multivariate analysis for *f*T>MIC and *f*T>4X MIC.

Model elements	Survival OR	Lower 95% CI	Upper 95% CI	p-value
***f*T>MIC**	1.126	1.048	1.295	.0067
Antibiotic delay	0.900	0.857	0.939	<.0001
APACHE score	0.873	0.839	0.905	<.0001
Age	0.834	0.703	0.983	.0326
***f*T>4X MIC**	1.130	1.044	1.226	.0027
Antibiotic delay	0.899	0.856	0.939	<.0001
APACHE score	0.872	0.839	0.904	<.0001
Age	0.836	0.706	.985	.0350

Logistic regression model which included *f*T>MIC and *f*T>4X MIC, antimicrobial delay, Apache score and age, was used as a continuous variable to examine survival to hospital discharge is shown, all elements remained significant to outcomes.

4 Discussion

This study demonstrates that hospital survival in bacteremic septic shock patients treated with microbiologically appropriate β-lactam monotherapy is associated with the duration of time (relative to the dosing interval) that the estimated free blood concentration is above the MIC/4X MIC of the isolated pathogen during the first 24 hours of therapy. A *f*T>MIC or *f*T>4X MIC value of >90% was associated with optimal survival with lower values associated with decreasing survival. Similar results were seen with 30 day survival (data not shown). Neither the peak concentration of antibiotic in the first 24 hours or the area under drug concentration curve in the first 24 hours divided by the MIC of the isolated pathogen achieved significance in their relationship to survival. In subgroup analysis, the beneficial effect of an increased *f*T>MIC appeared to found primarily in septic shock associated with Gram-negative pathogens and/or treated with cephalosporins. Patients treated with carbapenems also trended towards significance in this regard. Patients treated for septic shock associated with Gram-positive pathogens and/or those treated with penicillins did not appear to benefit significantly.

The significance of *f*T>MIC and *f*T>4X MIC was supported in multivariate regression models when other important prognostic factors were included in the model. These prognostic factors included age and APACHE score. Both age and severity of illness as reflected by APACHE score are well accepted as risk factors for death in septic shock (Knaus WA 1985; Kumar, Roberts et al. 2006; Kumar, Ellis et al. 2009). Other studies of the impact of time-dependent PK indices of antimicrobial activity have not controlled for such risk factors.

Time from initial documentation of hypotension to the first administration of appropriate β-lactam antibiotic was also included in the model. This has a substantial impact on the clinical validity of the observation of the importance of *f*T>MIC as a key PD variable in outcome of septic shock. Other studies of the impact of time-dependent PD indices on outcome of severe infections have not included time to appropriate antimicrobial therapy in the analysis despite the fact that this variable has an extremely strong relationship to outcome in serious infections and that it, like antimicrobial PK indices, can be potentially manipulated (Kumar, Roberts et al. 2006; Funk DJ 2011). Assessment of the parent database used in this study suggests that a significantly longer time to antimicrobial therapy among nosocomial infections (compared to community-acquired infections) is likely responsible for the significantly higher mortality in such cases.

The results of this study are more notable given the inclusion of only patients who received the most microbiologically "appropriate" therapy defined as β-lactam antibiotics to which the isolated pathogen was either fully or intermediately susceptible (wherever such as agent was used). This transfers predictive power from the time-dependent PD index (i.e. *f*T>MIC) to the time to antibiotic variable. In comparison to using time to any (including microbiologically inappropriate) antibiotic therapy, this approach eliminates almost all zero % *f*T>MIC values of microbiologically inappropriate therapy in favor of the *f*T>MIC value of the appropriate β-lactam, irrespective of the later start of that antibiotic. Since 0% *f*T>MIC values (i.e. microbiologically inappropriate therapy) are anticipated to be associated with worse clinical outcomes, the effect of this approach is to transfer this predictive power to the time to appropriate antibiotic parameter. Longer

delays in initiation of antimicrobial therapy have also been shown to be associated with poor survival (Angus, Linde-Zwirble et al. 2001; Kumar, Roberts et al. 2006; Kumar, Ellis et al. 2009; Martin, Brunkhorst et al. 2009; Funk DJ 2011). This approach is important from a clinical point of view since without including time to appropriate antibiotic therapy in the model, significant findings in favor of a role of time-dependent PD indices in survival could potentially be driven by the inappropriate therapy group, a group that has been shown to have very poor survival in septic shock (Kumar, Ellis et al. 2009).

The lack of a significant relationship between concentration-related PD indices and survival in septic shock is consistent with previous work in regard to bacterial killing with β-lactams. Numerous studies have suggested that for β-lactams, time-related indices such as *f*T>MIC or *f*T>4X MIC are more closely associated with bacterial clearance than are concentration-related indices such as fC_{peak}/MIC or *f*AUC24/MIC in both *in vitro* and animal models (Craig 1998; JD. 1998). This difference exists despite the mathematical relationship between all these indices.

Although the time-dependent PD indices were significantly associated with outcome for the overall group encompassing a wide variety of organisms and β-lactam antibiotics, subgroup analysis suggested the effect was substantially limited to Gram-negative pathogens and isolates treated with cephalosporins (though a trend towards significance also existed with carbapenems). One potential explanation for the absence of a significant time-dependence of survival in Gram-positives is that the *f*T>MIC threshold for maximal β-lactam-driven bacterial kill of Gram-positive infections is relatively low (approximately 50-60%) compared to Gram-negatives (90-100%) due to the post-

antibiotic effect seen with Gram-positives and β-lactams (Vogelman B 1988; Craig 1998). Given the lower threshold, it is possible that too few *f*T>MIC values in the low/intermediate susceptibility range existed in our dataset to generate a significant relationship (i.e. clustering of the Gram-positive isolates above the maximum killing threshold would result in a near uniform high clearance so that no time-dependency would be found). It is notable that the concentration of free drug in this study was maintained above the pathogen MIC for >90% in 76.0 % (260 of 342) of the cohort. Because penicillins are often used for empiric treatment of potential Gram-positive infections, this group may be affected for the same reason. A similar issue of clustering of individual *f*T>MIC at 100% in a study of meropenem PD indices with lower respiratory tract infection has been noted in at least one previous study (Li, Du et al. 2007). This clustering was proposed as a cause for a limited ability to discern a stronger relationship of *f*T>MIC with outcome.

These observations are noteworthy for a number of reasons. Our study involves, for the first time, a high mortality human infection syndrome (i.e. septic shock) with a wide, clinically relevant range of pathogens, anatomic infection sites and pathogenic organisms in the context of the most important endpoint, survival to hospital discharge. Previous studies have tended to be limited to substitute endpoints in spontaneous human disease or survival in experimental animal models; few studies have directly examined the impact of β-lactam PD parameters in human survival despite the fact that available data suggests that early β-lactam levels may be suboptimal in sepsis and septic shock (Taccone FS 2010). In addition, previous human studies have not controlled for the severity of illness

or time to appropriate antimicrobial therapy, both of which have a major impact on survival from septic shock.

Eagle and colleagues were the first to demonstrate that β-lactams (specifically, penicillin) was most effective in clearance of *Streptococcus species* in animal models when administered with greater frequency rather than higher dose (Eagle H 1950; Eagle H 1950; Eagle H 1953). Since then many others have made similar observations of a wider list of Gram-positive pathogen in animal models of pneumonia, peritonitis and neutropenic thigh infection (Schmidt LH 1949; Schmidt LH 1951; Vogelman B 1988; Craig 1998; JD. 1998). Further animal studies have demonstrated the primacy of time-related PD indices (e.g. *f*T>MIC) as predictors of microbiologic efficacy for penicillins and cephalosporins in the treatment of experimental *E.coli*, *Klebsiella spp* and *P. aeruginosa* infections in a variety of animal models (Roosendaal R 1985; Vogelman B 1988; Leggett JE 1989; Bakker-Woudenberg IA 1990; Fantin B 1991; Craig 1998; JD. 1998).

With respect to humans, several studies have examined substitute endpoints in relation to β-lactam time-dependent PD indices. Schentag and colleagues examined the time to clearance of pathogens from the tracheal secretions of patients with nosocomial pneumonia treated with cefmenoxime (Schentag JJ 1984). The dynamic response concentration (DRC) is analogous to MIC. The speed of pathogen clearance was associated with both time above DRC and the area under curve divided by DRC. The key subset of patients in whom a fixed dose of drug was maintained demonstrated better correlation to bacterial clearance with the time-related rather than concentration-related PD indices (Schentag JJ 1984; JD. 1998). More recently, the same group has shown that a

*f*T>MIC of 100% in 76 patients enrolled in comparative RCTs of ceftazidime and cefepime for sepsis with bacteremia, lower respiratory tract infection or complicated urinary tract infection was associated with better bacterial eradication and clinical cure rate than patients with an *f*T>MIC<100% (McKinnon PS 2008). Similarly, others have shown that *f*T>MIC<60% was associated with inferior microbiological response in patients with non-urinary tract sensitive pseudomonas infection (Crandon, Bulik et al. 2010). A clinical study conducted by Li et al (2007) examined the relationship of meropenem PD indices and outcome in patients with lower respiratory tract infection. A *f*T>MIC maintained above 54% was a significant correlate of a better microbiological response although only trough free concentration of meropenem divided by MIC (fC_{min}/MIC) above 5 predicted clinical response (Li, Du et al. 2007).

Continuous and extended infusion of β-lactams provides a way to increase time-related PD indices in clinical practice. If such indices are linked to increased bacterial clearance, then they may also affect clinical outcome. In one study, continuous infusion (which generates 100% *f*T>MIC for sensitive pathogens) rather than intermittent cefamandole (both with intermittent carbenicillin) was resulted in improved clinical cure in the neutropenic (ANC<100) and cefamandole sensitive pathogen groups of a group of 235 randomized patients (Bodey GP 1979). The use of continuous infusion as opposed to intermittent administration of piperacillin has also been shown to be associated with a more rapid decrease in APACHE II at days 2-4 of the ICU stay in another randomized trial of 40 septic critically ill patients with serious infections (Rafati, Rouini et al. 2006). Extended infusion of drugs is another way to increase *f*T>MIC. Among the sickest of the approximately 200 patients (with an APACHE II score of ≥17), extended infusion of

piperacillin/tazobactam (4 hr rather than a standard 30 min bolus) for serious pseudomonas infections has been found to be associated with a shorter hospitalization and lower 14 day mortality (Lodise, Lomaestro et al. 2007). This finding is particularly noteworthy in that a mortality effect was noted in only the most critically ill patients; presumably, a large proportion of these would have had septic shock. Similarly, others have shown that continuous infusion of meropenem, piperacillin/tazobactam and ceftazidime are each associated with a higher rate of clinical cures in high risk Gram-negative ventilator-associated pneumonia than is intermittent dosing (Lorente L 2006; Lorente L 2007; Lorente L 2009). This applied particularly to organisms with higher MIC values including *P. aeruginosa*.

For β-lactams antimicrobials, it has been proven experimentally in animal studies that unbound drug does not have to exceed the MIC for the entire dosing interval to reach the effect clinical cure (Craig 1998). In fact, a bacteriostatic effect appears sufficient to treat serious infections in immuno-competent animals (Drusano 2004). In such situations, the concentration of free drug merely has to exceed the MIC of the pathogen for 35-55% of the dosing interval for cephalosporins, 30-35% for penicillins and 20-25% for carbapenems in order to effect a cure (WA. 1993; WA. 1995; Craig 1998; JD. 1998). Clinically, achieving a static effect is likely to be sufficient for the treatment of relatively minor community-acquired infections with the intact immune mechanisms of the immuno-competent host contributing to the clearance of the microbial pathogen. Similarly, achievement of a $fT>MIC$ of 60–70%, 50% and 40% coverage, respectively for cephalosporins, penicillins and carbapenems appears sufficient to effect clinical cure of some experimental bacterial infections in neutropenic animals (Craig 1998). However,

in neutropenic thigh infection and pneumonia models, a *f*T>MIC of 90-100% is needed for maximum bacterial clearance for β-lactam/organism combinations without a PAE although 50-60 % *f*T>MIC suffices for those combination that are associated with a PAE (Craig 1998; JD. 1998). In order to achieve optimal outcomes, the generation of maximal bactericidal effects may be optimal for β-lactam treatment of neutropenic patients with serious infection (Drusano 2004; Ariano, Nyhlén et al. 2005). Patients with septic shock may be similar; such patients have well defined deficits of leukocyte function (Williams MA 1998; Wenisch C 2001; Holzer K 2002; Chishti AD 2004). In addition, given that cidality is an index of speed of bacterial clearance, a bactericidal effect may be helpful in the same way that more rapid administration of antibiotics is useful (Kumar, Roberts et al. 2006).

A knowledge of the mechanism of action of β-lactam drugs is useful to help understand the nature of the relationship between the time-related PD indices (e.g. *f*T>MIC) and the microbiological (bacteriostatic/bactericidal) effect (Craig 1998). β-lactams cause acylation of the target site penicillin binding proteins (PBPs). A maximum percent of PBPs can be acylated over a period of time. Once maximal acylation is achieved, the cidal effect cannot be increased any further. This explains why the killing rates for free β-lactam drugs are maximal at a low multiple of the MIC (*f*T> 4X MIC) and why β-lactam free drugs do not have to exceed the MIC for the whole dosing intervals in most circumstances (Drusano 2004).

Our data suggest that optimal outcomes in β-lactam monotherapy of septic shock is achieved with maximal *f*T>MIC and *f*T>4X MIC values. *f*T>MIC values of >90%

appear to yield maximal survival in a broad range of patients with a variety of clinical infection sites, pathogenic organisms and selection of β-lactam therapy. In this regard, septic shock patients may be similar to neutropenic patients where maximal cidality is desirable. This relationship to survival in human septic shock is a novel observation.

4.1 Limitation

There are significant limitations to our study and potential results. This is a retrospective study. Subjects are not chosen in advance. They are critically ill patients with septic shock that were treated during their ICU admission. This means that we cannot directly manipulate the research question but must assess the issue through observational methods without experimentation. For that reason, many research variables can only be estimated rather than directly measured. . In particular, blood antibiotic levels are not directly measured but rather calculated from estimated physiologic indices. This includes most components related to renal function (creatinine clearance) which is a key element in determining drug clearance. A similar problem exists with volume of distribution of antibiotics which can be substantially altered in the critically ill (Roberts, Hons et al. 2007). Although values derived from critically ill patients were used wherever available, this was not always possible. As a result, some of our data may overestimate pharmacodynamic performance (% fT>MIC) in the study population. Further, we are limited in the range of septic shock patients that can be assessed since we can only use those with positive blood cultures; anatomic infection site cultures could potentially be of use but these are not normally stored for a prolonged period making them very difficult to access. Thus the population under assessment is limited and may not entirely reflect the broader group of patients who have septic shock (typically only about 1/3 of septic shock

patients are blood culture positive (Kumar, Roberts et al. 2006; Roberts, Webb et al. 2009). Also, the population under assessment is restricted to those patient who had antibiotic monotherapy for the isolated pathogen, a result which may reflect non-random choice on the part of the physician or may reflect a high degree of resistance in the pathogen (i.e. combination therapy may have been intended but failed due to resistance to one of the two or more antibiotics administered).

4.2 Conclusion

Sepsis and septic shock due to infection in critically ill patients are a major cause of morbidity and mortality in intensive care units (ICUs) globally. Despite the fact that major improvements have been made in the field of sepsis therapy, treatment of patients with septic shock remains one of the major challenges to the ICU clinicians. The evidence available to date suggests that the source control of the pathogen and an early administration of appropriate antimicrobial therapy remain the most important and powerful clinical intervention. However, given that mortality of septic shock remains extremely high, other approaches to maximizing microbial clearance using antimicrobials may be very important.

Our study shows that critical time-related PD indices for β-lactams are associated with outcome in a broad range of human septic shock. This study suggests that even after adjusting for time to appropriate antimicrobial therapy, the most powerful determinant of outcome of septic shock (Kumar, Roberts et al. 2006; Kumar A 2006) and severity of illness, time-related β-lactam PD indices remain important correlates of survival. This suggests that there is potential value in using PK/PD parameters as guides for

establishing optimal dosing regimens of β-lactam therapy for the treatment of septic shock patients.

4.3 Future work

Additional work is needed to provide a deeper understanding of the pathophysiology and treatment of sepsis and septic shock. A larger cohort of patients and isolates may be helpful to more closely examine subgroups where the relationship between time-related β-lactam PD indices and outcome was weak (carbapenems and penicillin-treated patients and gram positive infections). Our study could also be potentially expanded to examine vancomycin and fluoroquinolone monotherapy. Further, validated pharmacokinetic approaches to examining combination therapy now exist. Given that a substantial subset of patients with septic shock treated with β-lactam monotherapy received combination therapy (frequently with fluoroquinolones or aminoglycosides); pharmacokinetic of this group is warranted. One, less labor intensive approach to address issues related to the limited study population could be Monte Carlo simulations. Finally, our results should encourage the consideration of a randomized controlled trial where β-lactam therapy is manipulated to compare outcomes of standard dosing vs optimized time-related PD index dosing.

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Appendix 1:

Antibiotic	Vd	Fu	$T_{1/2}$ normal	ke	free
Ampicillin	0.3	0.9	1.2	0.58	0.75
Cefazolin[1]	0.14	1	1.8	0.39	0.2
Cefotaxime[1]	0.4	0.5	1.2	0.58	0.6
Cefoxitin	0.11	0.88	0.85	0.82	0.25
Ceftazidime[1]	0.35	0.9	1.75	0.4	0.85
Ceftriaxone[2]	0.16	0.49	7.3	0.09	0.075
Cefuroxime	0.2	0.9	1.25	0.55	0.6
Cloxacillin	0.14	0.75	0.5	1.39	0.05
Imipenem[2]	0.23	0.69	0.9	0.35	0.6
Meropenem[1]	0.36	0.9	1	0.69	0.98
Penicillin	0.47	0.8	0.5	1.39	0.4
Pip/Tazo	0.2	0.8	1.12	0.62	0.8
Piperacillin	0.2	0.8	1.12	0.62	0.8
Ticarcillin/Clav	0.15	0.69	1	0.6931	0.55

Dosing Body Weight (DBW) was calculated as follow; DBW=IBW+0.3*(ABW-IBW)

Where ABW is the actual body weight and IBW is the ideal body weight.

1- Power, Forbes, van Heerden, & Ilett, 1998.

2- Goodman, Gilman. The Pharmacological basis of Therapeutics, 10th Ed.

3- MICROMEDEX® 1.0 (Healthcare Series), Copyright © 1974-2010 Thomson Reuters

Appendix 2:

Additional-conventions Used

1) Chronic renal failure CRF on Hemo/ Peritoneal Dialysis HD/PD; CL_{CR} listed as "0", use 5 mL/min/1.73m^2.
2) Creatinine clearance CL_{CR} calculated as >100 mL/min/1.73m^2, use calculated value.
3) If no listed height or weight, used sex-specific -"average"- value of all bacteremic septic shock pts from that institution.
4) For female heights <60 inches, use 45kg as IBW.
5) For male heights <60 inches use 50kg as IBW.
6) Infusion time for β-lactams was assumed to be (t') =0.25h, , the institutional recommended duration
7) For antibiotic given once, %T > MIC was calculated for 1st 24h period;
8) AUC24/MIC value used AUC for doses in first 24h.
9) If Cpeak<CMIC, %TMIC was listed as "0"
10) For %T>MIC calculated as greater than "1" (i.e. >100%), value changed to "1.00" MIC greater than highest value on E-test strip (i.e. MIC listed as e.g. >256 μg/mL) changed to one dilution higher; e.g. >256 μg/mL becomes 512 μg/mL etc.
11) Low, off scale MIC's e.g. <0.016 μg/mL left unchanged as 0.016 μg/mL (ref. JAC (1998) 42;321 Warnock et. al.)

Appendix 3

E-test® Performance and Quality Control Ranges

QC Strains /Antibiotics tested	Antibiotic Code	QC Test Result	Acceptable MIC Range µg/mL
S. aureus **ATCC® 25923**			
Azithromycin	AZ	1.0	0.5-2
Ciprofloxacin	CI	0.38	0.125-0.5
Clindamycin	CM	0.064	0.032-0.125
Oxacillin	OX	0.38	0.125-0.5
Rifampicin	RI	0.006	0.004-0.016
Vancomycin	VA	1.5	1-4
E. coli **ATCC® 25922**			
Amikacin	AK	1.5	0.5-4
Cefotaxime	CT	0.064	0.032-0.125
Ceftriaxone	TX	0.064	0.032-0.125
Cefuroxime	XM	4	2-8
Gentamicin	GM	0.38	0.25-1
Imipenem	IP	0.25	0.064-0.25
Levofloxacin	LE	0.032	0.008-0.064
Meropenem	MP	0.032	0.008-0.064
Piperacillin/Tazobactam	PTC	1.5	1-4
P. aeruginosa **ATCC®27853**			
Cefepime	PM	1	1-4
Imipenem	IP	1	1-4
Meropenem	MP	0.25	0.125-1
Piperacillin/Tazobactam	PTC	4	1-8
H. influenzae **ATCC® 49766**			
Cefuroxime	XM	0.5	0.25-1
Meropenem	MP	0.032	0.032-0.125
E. faecalis **ATCC® 29212**			
Vancomycin	VA	2	1-4
S. pneumoniae **ATCC® 49619**			
Ceftriaxone	TX	0.016	0.032-0.125

Appendix 4

Antibiotics	Total	MIC			1st 24 T>MIC (%)			1st 24 Cpeak/MIC			1st 24 AUC/MIC		
		Median	25 %	75 %	Median	25 %	75 %	Median	25 %	75 %	Median	25 %	75 %
Cloxacillin	25	1	0.75	1.25	76.2	60.6	85.8	9.2	6.6	13.0	69.8	41.3	88.8
Piperacillin/ Tazobactam	108	2	1	6.625	100	100	100	78.3	30.7	136.6	782.4	368.6	1801.7
Ticarcillin/ Clavulanate	10	1.5	0.625	2.6875	100	100	100	114.6	71.8	253.3	734.6	460.6	1870.5
Cefazolin	8	2.5	0.5	6	100	100	100	21.7	3.6	45.8	210.0	56.9	648.2
Cefuroxime	18	2.25	1.125	4	100	68.5	100	12.2	6.2	52.9	133.7	54.5	474.8
Cefotaxime	79	0.094	0.047	0.5	100	89.7	100	304.5	63.3	835.3	1752.9	410.9	5188.0
Ceftazidime	12	0.86	0.19	8	100	77.3	100	90.0	7.7	254.8	1068.6	59.7	3014.4
Imipenem	13	0.25	0.25	0.75	100	84.6	100	57.4	36.8	69.8	498.8	241.0	800.0
Meropenem	45	0.064	0.047	0.19	100	100	100	301.7	100.3	656.1	2839.9	738.0	5943.5

Summary data for key pharmacodynamic indices per β-lactam antibiotics of the cohort are shown. Median (25-75h percentile)

Values restricted to antibiotics used for a minimum of 8 patients.