FACULTY OF SCIENCE, ENGINEERING AND COMPUTING

School of Pharmacy and Chemistry

Optimising Antibiotic Therapy for Inpatient and Outpatient Settings

Submitted by

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CONTENTS

ABSTRACT

Background: Not only have antibiotics saved countless patients' lives but they have also played a crucial role in supporting major advances in modern medicine. However, precipitously emerging resistant bacterial strains jeopardise the remarkable advances achieved with antibiotics. In the past, the development of new antibiotics was an effective strategy to combat resistant bacteria. However, with the discovery of new antibiotics diminishing, optimising the administration of currently available antibiotics has become a necessity. A strategy of particular interest involves applying pharmacokinetic and pharmacodynamic concepts to optimise time-dependant antibiotics dosing regimens. The latter is a growing area of interest for reducing the development of antibiotic resistance, and it involves differential dosing regimens such as prolonged or continuous infusions of beta-lactam antibiotics.

Aim: The overarching aim of this research is to optimise antibiotic therapy for inpatient and outpatient use.

This thesis consists of literature-based, practice-based, and laboratory-based research.

Literature-based: The aim of the literature-based research category was to review existing literature to compare the clinical outcomes of continuous vs intermittent infusion beta-lactam antibiotics and appraise the strengths and the weaknesses of current evidence. Overall, literature-based research demonstrated a wealth of studies in terms of systematic reviews, meta-analysis as well as primary studies. Despite the literature exhibiting favourable outcomes towards prolonged/continuous infusions, the literature review and systematic reviews conducted support the need for better conducted, definitive trials and systematic reviews given the variability in scope of the available studies.

Practice-based: The aim of the practice-based research category was to provide a snapshot of beta-lactam antibiotic use in clinical practice. The first study was single-centre retrospective cohort practice review conducted to Investigate the prescribing patterns of beta-lactam antibiotics in critical care wards. The second study was a cross sectional survey investigating nurse's knowledge, perceptions, and experiences regarding differential antibiotic dosing. Findings show that prolonged/continuous infusions as dosing strategies are implemented in practice to improve patient outcomes, however, healthcare

professionals implementing this practice have not received sufficient training to support the administration of differential antibiotic dosing. This was evident from both practice based studies that disclose beta-lactam antibiotics are not used to their full potential or are inaccurately used. There is a need for tailored education and training to improve health care professional's knowledge of prolonged/continuous infusions.

Laboratory-based: Despite the advantages that prolonged/continuous infusions beta-lactam antibiotics offer, in order to use these dosing regimens efficiently, infusion solutions should remain stable for the preparation, storage and infusion time. Concerns regarding stability present a challenge in practice as most stability information is based on administration via bolus injection or an intermittent infusion. Therefore, the aim of the laboratory-based research category was to determine the feasibility of prolonged/continuous infusion beta-lactam antibiotics for hospital and outpatient settings. Findings from the conducted studies aid in ameliorating current dosing regimens to optimise antibiotic efficacy. Results obtained from stability studies assist in resolving challenges experienced in practice in terms of preparation, storage, and administration as they indicate the effects of temperature, diluent, and pre-preparation of infusion solutions. Studies demonstrated that stability data generated in all studies are an improvement to the stability data presented in the British, American, and European pharmacopoeias.

Conclusion: Findings of this PhD research are supportive of the beneficial role of differential antibiotic dosing. Overall, the gathered data indicate that prolonged/continuous infusions are feasible, advantageous and could potentially improve patient clinical outcomes.

Keywords: antibiotic resistance, beta-lactam antibiotic, beta-lactamase inhibitor, differential antibiotic dosing, stability, continuous infusion, prolonged infusion, intermittent infusion, piperacillin, tazobactam, amoxicillin, clavulanic acid.

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me laugh when I didn’t even want to smile. I adore you and wish for success to be there with you in every walk of life.

ABBREVIATIONS

ADME	Absorption, distribution, metabolism, excretion
AMS	Antimicrobial stewardship
API	Active pharmaceutical ingredient
AR	Antibiotic resistance
AUC	Area under curve
AVR	Average
BL	Beta-lactam
BLA	Beta-lactam antibiotic
BLE	Beta-lactamase enzyme
BLI	Beta-lactamase inhibitor
BLING	Beta-Lactam InfusioN Group
BLISS	Beta-Lactam Infusion in Severe Sepsis
BSAC	British society of antimicrobial chemotherapy
CAP	Community acquired pneumonia
CC	Clinical cure
CI	Continuous infusion
CS	Colorectal surgery
P/CI	Prolonged/continuous infusion
COPD	Chronic obstructive pulmonary disease
CRCL	Creatinine clearance
CRP	C-reactive protein
CS	Colorectal surgery
CT	Combined therapy
DD	Daily dose
DNA	Deoxyribonucleic acid
DST	Drug stability testing
EP	Elastomeric pump
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FDA	Food and drug agency
GPR	Good practice recommendation

HAP	Hospital acquired pneumonia
HCP*	Health care professionals
HPLC	High performance liquid chromatography
IC	Intensive care
ICH	International conference of harmonisation
ICU	Intensive care unit
IE	Infective endocarditis
II	Intermittent infusion
IM	Intramuscular
IV	Intravenous
IVB	Intravenous bag
LD	Loading dose
MA	Meta analysis
MBC	Minimum bactericidal concentration
MCS	Monte Carlo simulation
MDR	Multidrug resistant
MHRA	Medicines and Healthcare products Regulatory Agency
MIC	Minimal inhibitory concentration
MT	Monotherapy
NAG	*N*-acetylglucosamine
NAM	*N*-acetylmuramic acid
NHS	National health service
NP	Nosocomial pneumonia
NR	Not recorded
NRT	Non-randomised trials
OPAT	Outpatient antimicrobial therapy
OS	Observational studies
PBP	Penicillin binding protein
PD	Pharmacodynamic
PI	Prolonged infusion
PK	Pharmacokinetic

PS	Prospective studies
PVC	Polyvinyl chloride
RCT	Randomised control trial
RS	Retrospective studies
RT	Randomised trials.
SD	Standard deviation
SGH	St Georges Hospital
SIM	Stability indicating method
SR	Systematic review
SrCr	Serum creatinine
USP	United states pharmacopeia
UV	Ultraviolet
VAP	Ventilator acquired pneumonia
WBC	White blood cell
WHO	World Health Organisation
YCD	Yellow covered document

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CHAPTER 1

INTRODUCTION

1.1 Introduction to Infectious Diseases

It is often too difficult to comprehend how frequently humans and microbes interact. Every aspect of life and the natural world, for better or for worse, is influenced by the actions of these organisms. The worst come in the form of disease. An infectious disease is caused by microbes, usually microscopic in size, such as bacteria, viruses, parasites, or fungi (1–3).

Around 39 trillion microbial cells live on or within the human body (**Figure 1**); with the colon harbouring the densest microbial habitat (> 98%). Most microbes are harmless and aid in digestion, protection against infection, maintaining good reproductive health as well as synthesise certain vitamins including vitamins B (4) and K (5). Microorganism species populations significantly vary between individuals and are originally determined by one's DNA, environmental exposures and diet (6–8).

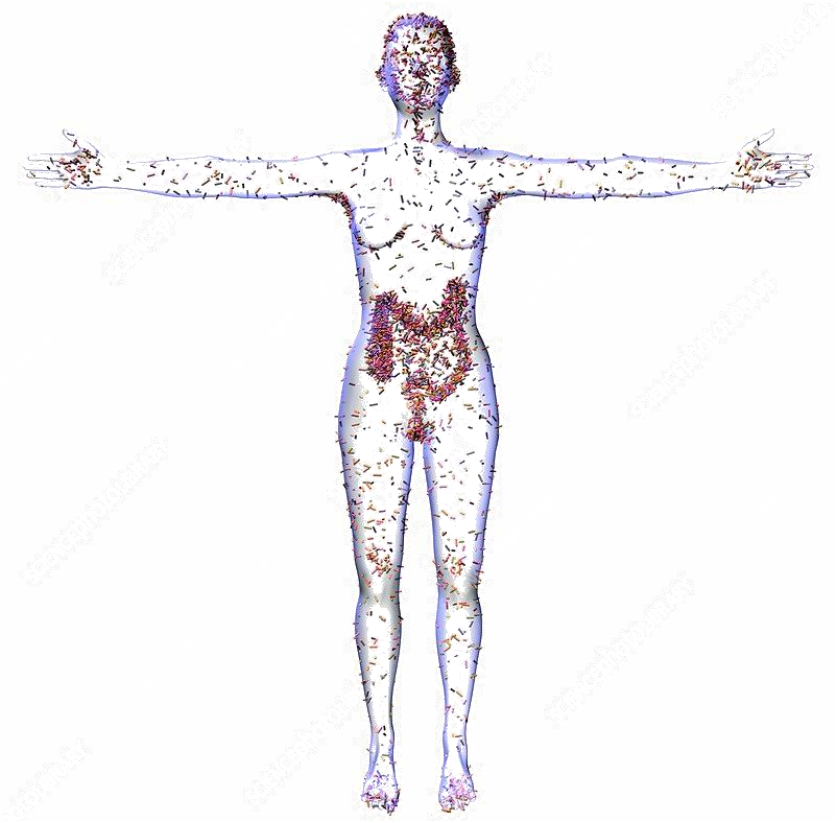


Figure 1 Conceptual illustration of the human microbiome. Up to 100 trillion microbial cells make up the microbiota, with >98% occupying the gut [4], [5], [7].

The spectrum, complexity and variability of microorganism communities that colonise humans is referred to as the human microbiome (**Figure 1**). The microbiome works in harmony with various organs in the body. However, its relationship to and associations with disease is a relatively new and rapidly evolving field of study. Although microbes are an

integral component of human health, the microbiome consists of microbes that are potentially harmful, causing illness in the form of infectious disease (6–8).

Historically, up until the 1940's, infectious diseases were the leading cause of death (9,10). They have continually played a role in influencing human history, e.g., in the 13th century the bubonic plague pandemic claimed the lives of roughly a quarter of the world's population (Figure 2). Fortunately, improvements in nutrition, hygiene, antibiotics, immunisation, and food safety have led to a significant reduction in infections (11). However, despite these advances, harmful pathogenic bacteria and viruses are mutating faster than the establishment of innovative antibiotics and vaccines to treat them, resulting in novel strains (1–3).

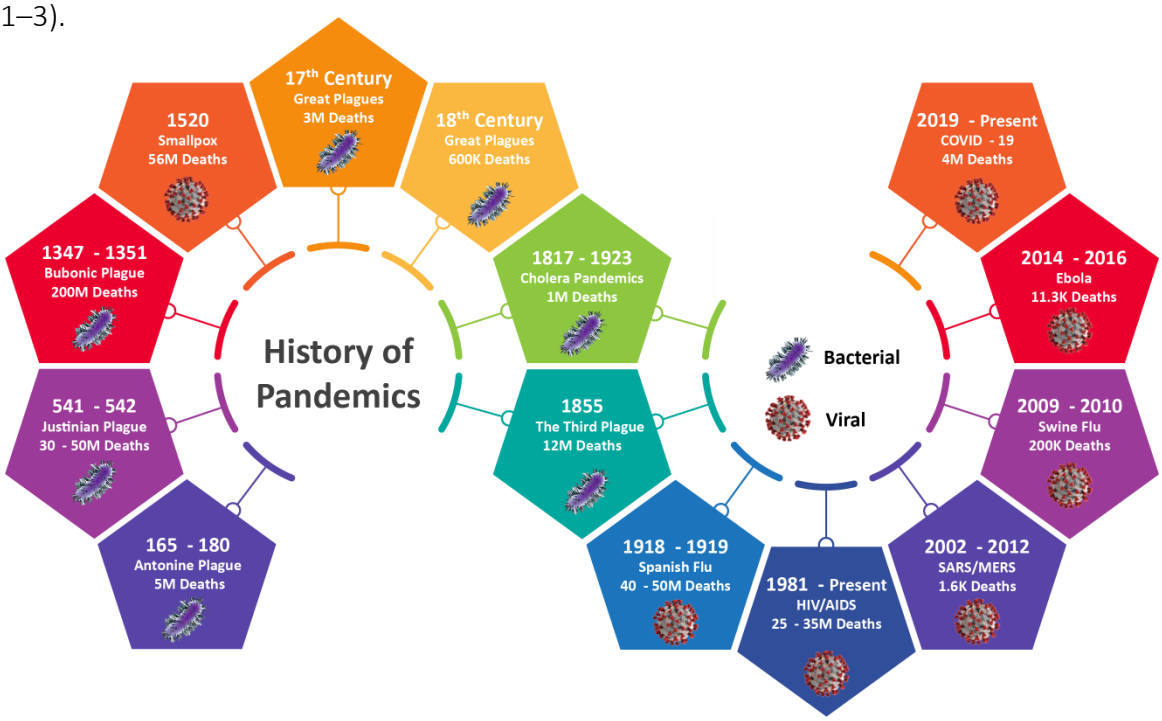


Figure 2 Timeline showing the history of pandemics caused by bacterial and viral infectious diseases.

Bacteria can infect any site on or within the body (2). A bacterial infection is caused by transmission and proliferation of a harmful pathogenic strain of bacteria (3). In 2019, three bacterial infectious diseases - lower respiratory tract diseases, diarrheal diseases, and tuberculosis – were ranked in the top ten causes of death worldwide by the World Health Organisation (WHO) causing 4 million, 2 million and 1.5 million deaths a year, respectively (12).

Bacterial diseases arise from the interaction of an infectious agent, a host, and its environment (13,14). Transmission of diseases occur when the infecting agent leaves its reservoir (human, animal, or the environment) or the host through a portal of exit and by some mode of transmission enters through a portal of entry to infect a susceptible host. This cycle is known as the chain of infection (Figure 3) (11,14,15).

Figure 3 Chain of Infection; public health control and prevention efforts aim on breaking the links in the chain to stop the spread of infectious diseases [13], [14].

Bacterial infections can be transmitted by vertical or horizontal transmission mechanisms (16,17). Vertical transmission occurs when the pathogen is transferred from parent to offspring either through the placenta or breastmilk (e.g., human immunodeficiency syndrome (HIV)). Horizontal transmission arises when the pathogen is transferred from an infected individual to another either by direct or indirect transmission (Figure 4).

Direct transmission occurs when an infectious agent is transferred from a reservoir to a host by direct contact (human-to-human contact) or droplet spread. Direct contact involves skin contact, injection, or through sexual intercourse (direct physical contact with blood or bodily fluids) (e.g., pink eye and chicken pox). Droplet spread involves a healthy individual being exposed to infected droplets from short-ranged aerosols produced by an infected individual sneezing and coughing (e.g., influenza). Indirect occurs when an infectious agent is transferred from a reservoir to a host via airborne transmission, vehicles such as food and water (e.g., food poisoning, hepatitis and E-coli) or vectors such as mosquitoes, fleas, and ticks (e.g., malaria, bubonic plague and Lyme disease) (14,15).

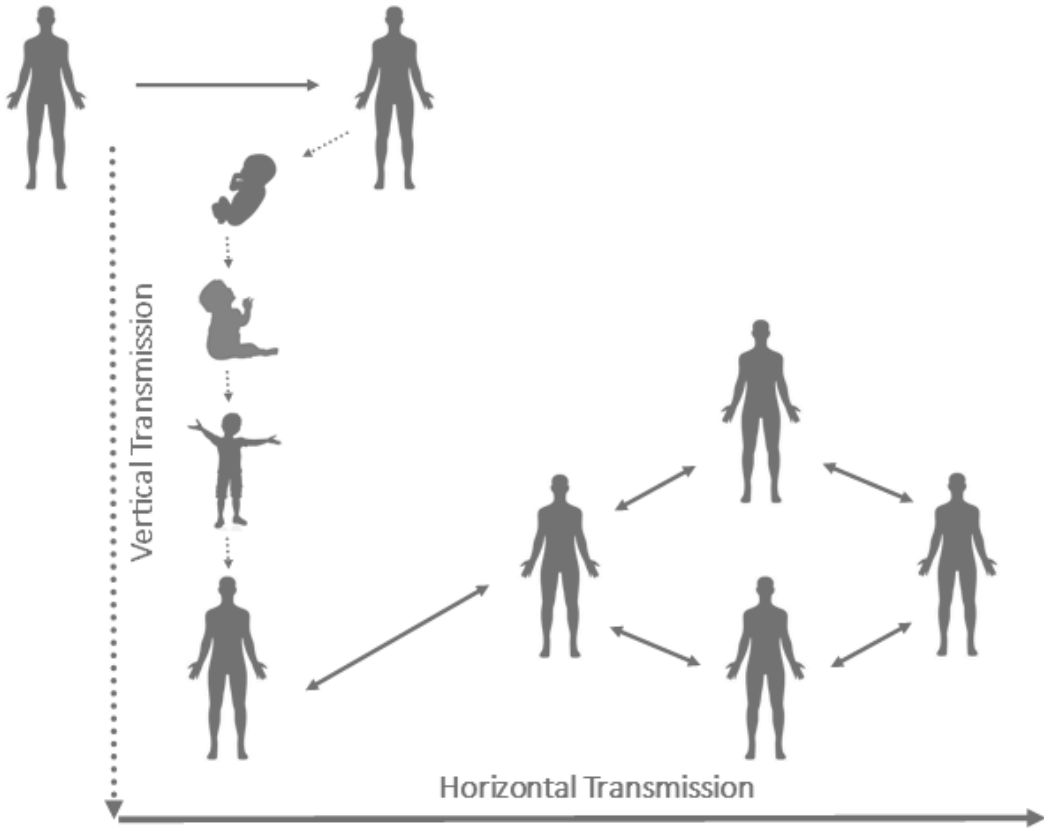


Figure 4 Transmission of infection in humans. Solid lines represent horizontal transmission and dashed lines represent vertical transmission

Infections can be local (e.g., ear or tooth infection) or systemic (e.g., bloodstream infection) depending on the site and spread of the infection. Infections can be classified as either acute (e.g., influenza), chronic (e.g., hepatitis B or HIV), or latent (e.g., herpes simplex virus). Definitions for the different types of infection are shown in **Figure 5** (3,18). The interval from when bacteria are introduced into the susceptible host until clinical symptoms appear is known as the incubation period. The length of this period is dependent on the pathogen growth rate and/or health of the host and ranges from a few days to weeks or months. The onset of illness occurs after the incubation period and is known as the prodromal phase, when symptoms first become apparent. At this stage, the symptoms may not be specific to the infection and include fatigue, body aches, and headaches. The pathogen population continues to grow to reach full toxicity by multiplying and colonising the site of infection. In the period of invasion more severe symptoms including fever, cough, rash, diarrhoea and/or swelling appear and an immune response is initiated. As the immune system or antibiotics fight off the infection the illness progresses to the decline phase. At this stage, the symptoms start to subside, and the pathogen population begins to decline. When symptoms disappear,

the decline phase transitions into the convalescent period (a phase of recovery) where the host's health is regained (19,20).

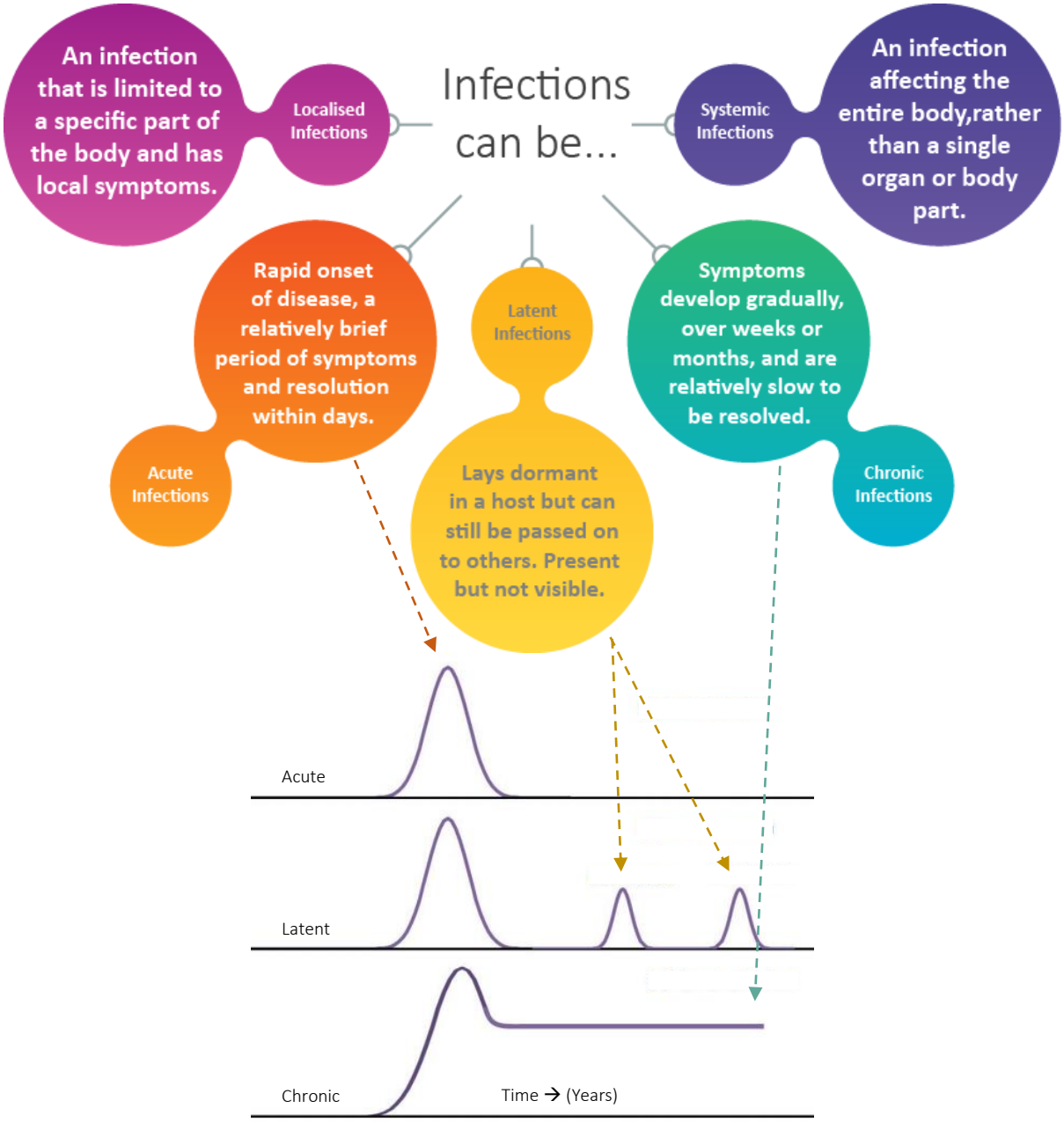


Figure 5 Definitions for the types of infection (localised or systemic) and the pattern of infection (acute, latent, or chronic) with schematic diagram showing the pattern of infection [3], [17].

1.2 Introduction to Bacteria

Bacteria are prokaryotic, single cells that form colonies (2,21). They do not have a nucleus; hence, their genetic material is contained within the cell membrane. Bacteria can be separated into categories based on their shape and cell wall structure. They are typically a

few micrometres in length and have numerous shapes, including spheres, rods, and spirals (**Figure 6**) (21). Another classification involves establishing whether the bacteria responsible for an infection are gram-positive or gram-negative. Though both groups of bacteria cause infection, they often require different treatments (11).

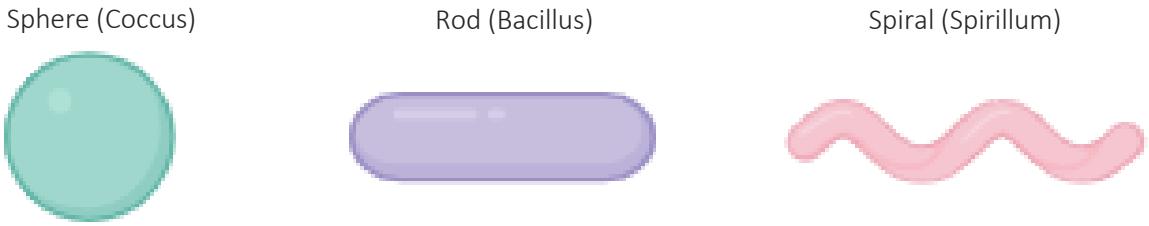


Figure 6 Bacterial cell shapes. The shape is determined by the rigid cell wall and the cytoskeleton of the organism [20].

1.2.1 Basic Anatomy of Bacterial Cell Walls

Classifying bacteria based on the properties of their cell wall is particularly important in medicine in terms of determining an effective treatment plan. Bacterial cell walls are made up of a large molecule known as the peptidoglycan (22). It is composed of glycan strands consisting of alternating -N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) - connected by β-(1,4)-glycosidic bonds. NAG and NAM disaccharides are cross-linked with short polypeptide chains. The polypeptide chains are interconnected by enzymes, known as transpeptidase (penicillin binding proteins (PBP)), to form rigid cell walls (**Figure 7**) (23,24). The peptidoglycan layer is a complex, mesh-like structure that is essential for maintenance (giving structural support) of the cell shape in terms of strength and rigidity and protecting the cell from osmotic and mechanical stress (25,26).

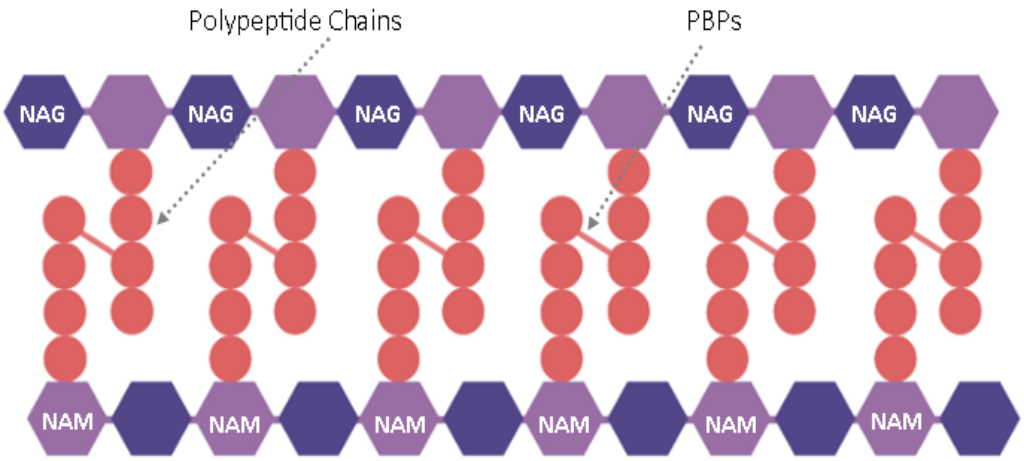


Figure 7 Structure of a typical bacteria peptidoglycan.

A Danish bacteriologist, Hans Christian Gram, developed the Gram staining technique in 1884 by which bacteria are classified based on their reaction to Gram stain. This staining procedure identifies bacteria as either Gram positive or Gram negative based on the physical properties of their cell walls (27). The process involves staining cells with a crystal violet dye followed by the addition of a mordant (iodine) to fix the dye. Next, a decolouriser (95% alcohol) is added. Due to the differences in thickness of the peptidoglycan layer, the crystal violet dye is either retained or not retained. Then, a secondary stain (safranin) is added. After which, only the decolourised cells are stained red (**Figure 8**) (27–29).

Gram-positive bacteria have a thick cell wall (up to 30 layers of peptidoglycan), that surrounds a monoderm (single plasma membrane). Gram-negative bacteria have a much thinner cell wall (consisting of a single layer of peptidoglycan), that is located between two lipid bilayer membranes (a diderm) (11). Following Gram staining, Gram-positive bacteria have a distinctive purple colour due to the retention of crystal violet stain in the thick peptidoglycan layer, whereas Gram-negative bacteria show up as a pale red colour as the cell-wall is unable to retain crystal violet stain, so it is coloured by the safranin (**Figure 8**).

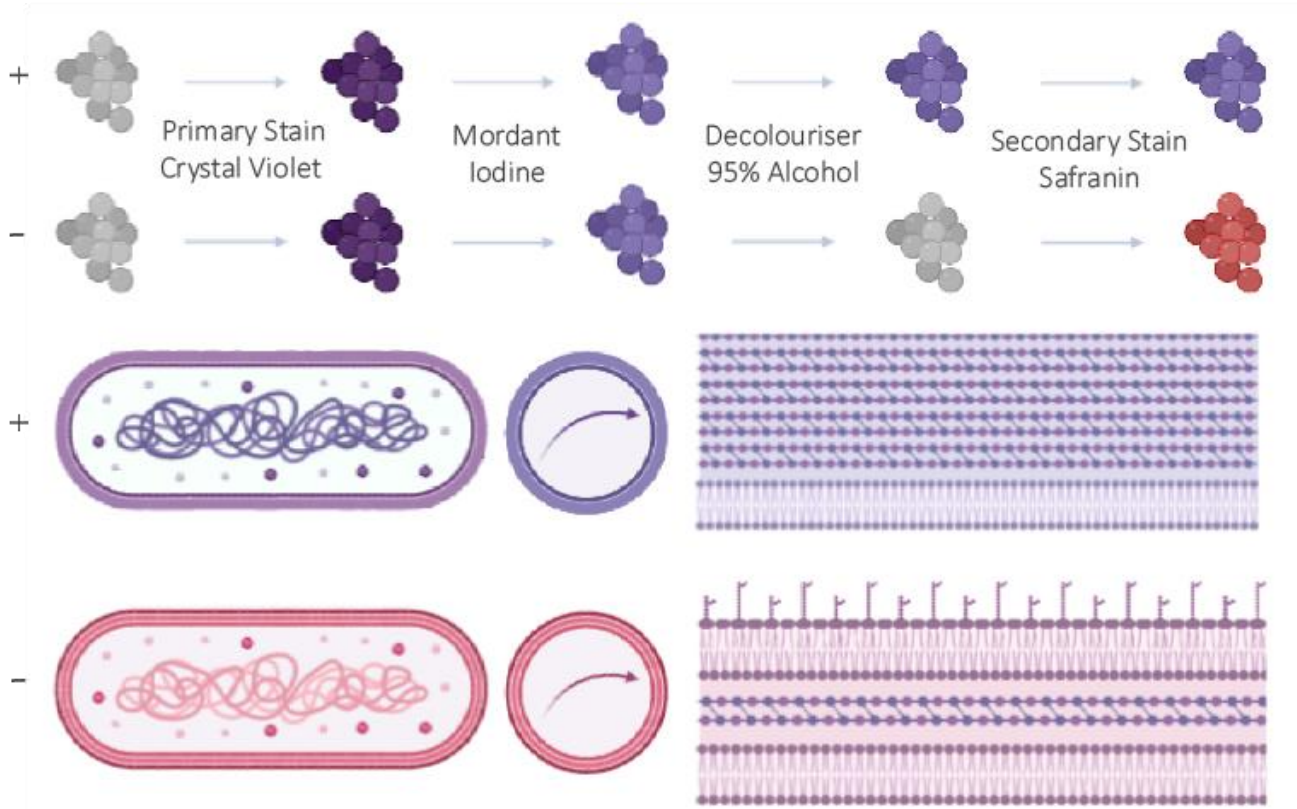


Figure 8 Gram staining procedure for the determination of whether bacteria of interest are Gram-positive or Gram-negative. Also, showing the bacterial cell wall structure and differences between Gram-positive and Gram-negative bacteria wall compositions [26] – [28].

Based on the Gram stain, the type of bacteria isolated will guide physicians to diagnosis as well as influence targeted antibacterial therapy (27–29).

1.3 Introduction to Antibiotics

'Antimicrobial' is an umbrella term for any substances that destroy or inhibit the reproduction of microbial cells including antibiotics, antivirals, antifungals and antiseptics. Antibiotics, also referred to as antibacterials, are substances that kill or inhibit the growth of a bacterium on or within the body, thus, antibiotics are used to treat infectious diseases caused by bacteria (30).

1.3.1 Antibiotic Discovery

Before the 1940's, infectious diseases were a leading cause of death (**Figure 9**). The accidental discovery of penicillin by Sir Alexander Fleming, a Scottish scientist, in 1928, made a big impact on human history. He discovered that a fungus contaminating his petri dishes had released a diffusible extract that had antibacterial activity against surrounding staphylococcal bacteria (31). Not only did Sir Alexander Fleming's discovery change the course of medicine, but it also led to a cure for bacterial infections that were once deadly.

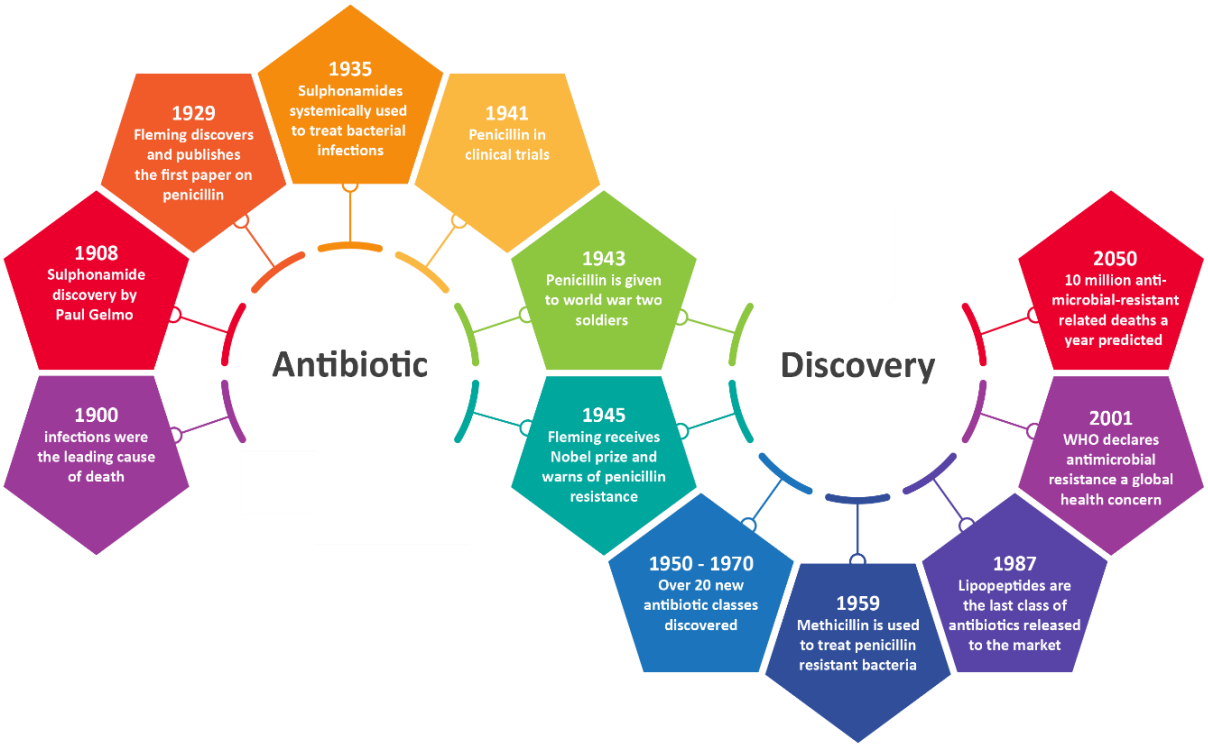


Figure 9 Timeline of antibiotic discovery.

Prior to the introduction of penicillin, there was no effective treatment for most fatal infections caused by bacteria. Thus, infections that are currently curable (e.g., pneumonia, meningitis, gonorrhoea) were previously the leading cause of human death (32–35).

Around two decades later, after years of intense co-operation between scientists, manufacturers and government agencies, penicillin was made broadly available in 1945. In 1946, Dorothy Hodgkin, a biological chemist who won the Nobel Prize (in 1964) in Chemistry for solving the structure of molecules, determined the structure of penicillin G using X-ray crystallography. Establishing the structure of penicillin fuelled the quest to synthesise the drug. In 1952, a form of penicillin suitable for oral use, Penicillin V, was developed and was produced synthetically for the first time in 1957, laying the foundation for the development and synthesis of new penicillin antibiotics. Since Penicillin's discovery, over a hundred new compounds have since been developed (**Figure 9**) (31,36).

1.3.2 Availability of New Antibiotics

Previously, bacterial mutations were outpaced by replacing increasingly ineffective antibiotics with new agents. However, the discovery of new antibiotics has slowed down due to economic and regulatory obstacles (37,38). Antibiotic development is no longer considered to be an economically wise investment for pharmaceutical industry and antibiotic research conducted in academia is diminishing as a result of funding cuts (36). The accessibility, simplicity of use, and usually low cost of antibiotics has also led to a perception of low value among funding bodies. Another factor responsible for the lack of antibiotic development is microbiologists and infectious-disease specialists warning that when new antibiotics are eventually used, the cycle of bacteria rapidly acquiring resistance is inevitable (36).

1.3.3 Antibiotic Categorises

There are 12 classes of antibiotics (**Figure 10**) which differ in their spectrum of activity, effect on bacteria, mechanism of action, pharmacokinetics (PK) and pharmacodynamics (PD) as well as clinical efficacy.

1.3.3.1 Spectrum of Activity

An antibiotic's spectrum of activity refers to the range of microorganisms an antibiotic is usually effective against (30). They are classified as either narrow-spectrum or broad spectrum. Narrow spectrum antibiotics are more specific and only active against a limited group of bacteria, whereas those that are broad spectrum act against a wider range of bacteria and tend to be active against both gram-positive and gram-negative organisms. The spectrum of activity may narrow with acquisition of resistance genes (39,40).

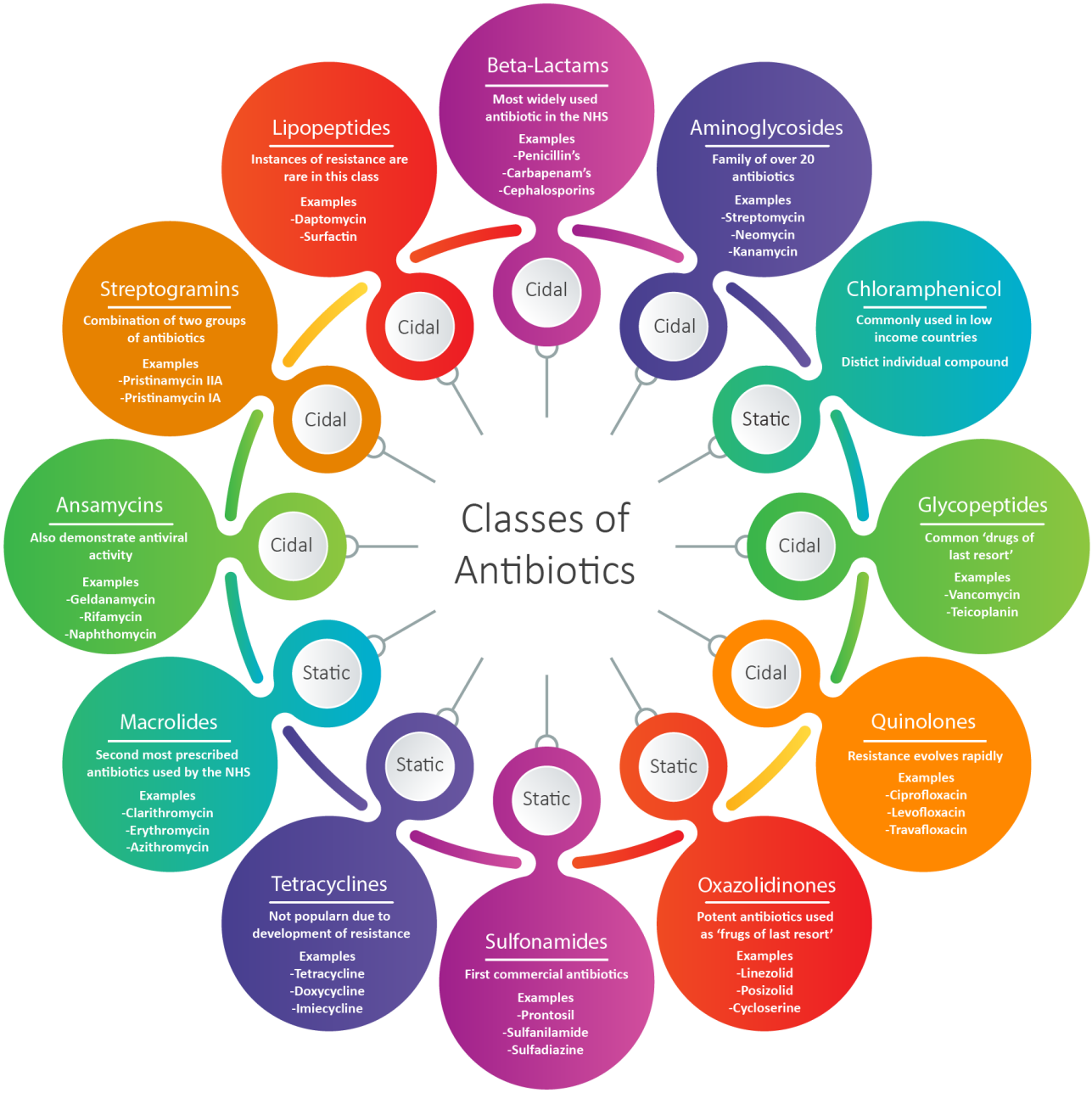


Figure 10 An overview of different classes of antibiotics with examples; suffix's 'cidal' and 'static' for bactericidal (restricting growth and reproduction) and bacteriostatic (causing bacterial cell death) agents, respectively [40].

1.3.3.2 Effect on Bacteria

There are two main mechanisms by which antibiotics affect pathogens: inactivation (inhibition) or actual death of the bacteria. Bacteriostatic antibiotics inhibit or delay the growth and replication of the bacterium without killing it (**Figure 10**). These agents are usually effective in treating infection as they allow the patient's immune system to "catch up" and kill of the organisms. Bactericidal antibiotics kill the target organism without or with minimal support from the immune system. Some agents can be both bacteriostatic and bactericidal, depending on the pathogen, the dose and the duration of exposure (**Figure 10**). For most infections, bacteriostatic and bactericidal agents inhibit/kill pathogens at the same rate and so this distinction need not be a factor when selecting antibiotics (30).

1.3.3.3 Mechanisms of Action

Antibiotics can be categorised according to their mechanism of action. Antibiotics have different modes of action depending on their structure and degree of affinity to target sites within the bacterial cell (**Figure 11**). In general, there are five basic mechanisms of antibiotic action, these include inhibition of: cell wall synthesis, protein synthesis, nucleic acid synthesis, cell membrane function or antimetabolite activity (30,41) (**Figure 12**).

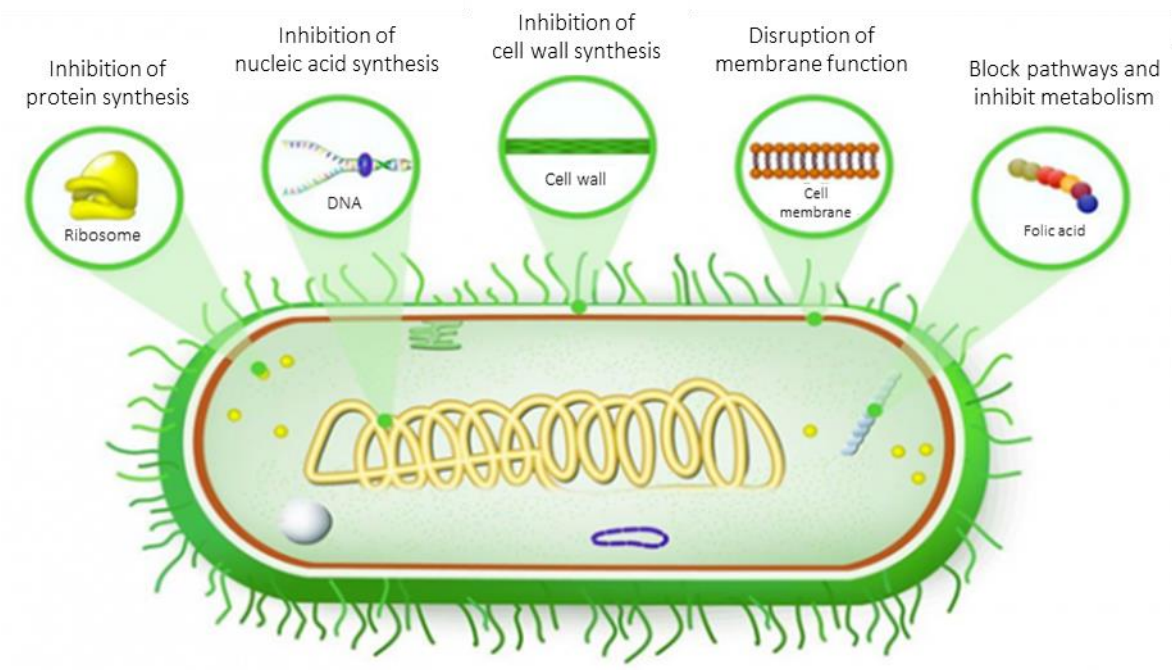


Figure 11 Typical antibiotic target sites in bacterial cells.

Many of the cellular functions targeted by antibiotics are most active in multiplying cells. Since there is often cellular function overlap between prokaryotic bacterial cells and

eukaryotic cells, numerous antibiotics (e.g., doxorubicin, daunorubicin bleomycin and mitomycin) have been found to be useful as anticancer agents as they work in all phases of cell cycle. These above mentioned agents have been found to promote cancer apoptosis, inhibit cancer growth, and prevent cancer metastasis (42,43).

Figure 12 Antibiotic mechanisms of action with examples. Different antibiotics exhibit distinct modes of action depending on their structure and degree of affinity to target sites within the bacterial cell [29], [41].

1.3.3.4 Antibiotic Pharmacokinetics and Pharmacodynamics

PKs is the study of the effect the body has on the drug. Questions relating to PK include: How does the drug get into the body? Where does the drug go? What does the body do to the drug? How does the body get rid of the drug? Understanding the PK of an antibiotic is crucial to its effectiveness in practice. For example, there is no benefit of a patient taking an antibiotic against a specific bacterium if the antibiotic does not reach the site of infection at

a significant concentration. Antibiotic PKs describe how antibiotics are administered, where the antibiotic goes once inside and how it is eliminated. The phases of PKs are termed as absorption, distribution, metabolism, and excretion (ADME) (**Figure 13**).

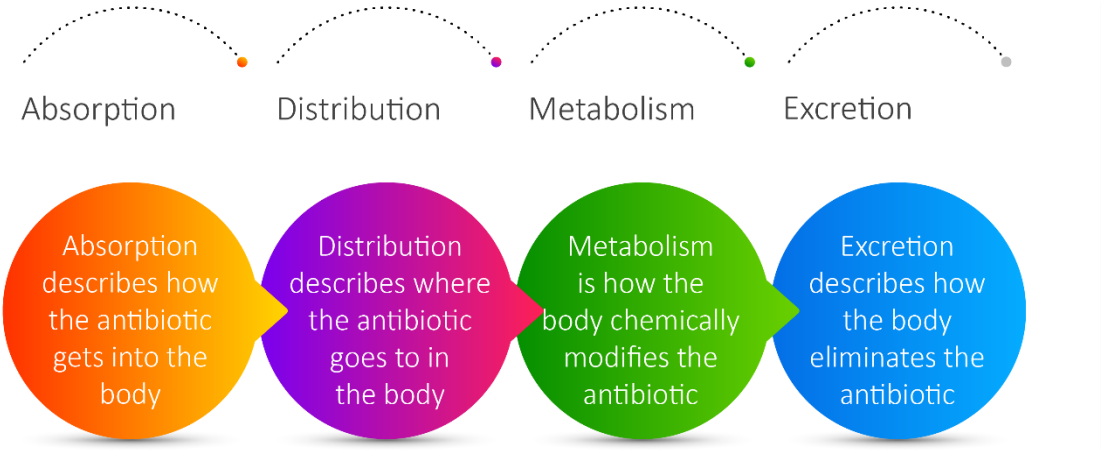


Figure 13 The main processes involved in pharmacokinetics; ADME.

PDs is the study of the effect the drug has on the body. Antibiotic pharmacodynamics is the way antibiotics interact with bacteria. Questions relating to PD include: Does the antibiotic inhibit the growth of or kill the bacteria? High dose of the antibiotics once a day or low dose administered frequently throughout the day? (30,44).

Antibiotic prescribing in terms of dose and frequency is commonly determined using PK as it describes the relationship between an antibiotic dosage regimen and concentration in serum and/or at the site of infection. However, PK alone does not provide an understanding of an antibiotic's desired and undesired pharmacological effects. PK does not correlate the concentration of antibiotic at the site of infection with the antibiotic's biological effect. PD describes the relationship between antibiotic concentration at the site of infection and its biological effect on the bacteria (44,45).

The most commonly used PD measures of *in vitro* antibacterial activity against pathogens are the 'minimum inhibitory concentration' (MIC) or the 'minimum bactericidal concentration' (MBC). The MIC is the lowest concentration of antibiotic necessary to inhibit visible growth of bacteria under specifically prescribed conditions whereas MBC is the lowest concentration of antibiotic needed for complete bacterial death, thus, the closer the MIC is to the MBC, the more effective the antibiotic treatment (45–47).

Although MIC and MBC are good predictors of antibiotic potency against an infecting organism, they do not take into account the time course of antimicrobial activity, nor do they predict physiologic conditions such as:

1. The intermittent administration of an antibiotic to a patient which results in the target pathogen being subjected to constantly changing concentrations of the drug,
2. The effects of antibiotic concentrations below the MIC (sub-MIC effect) and,
3. The post antibiotic effect which is the persistent inhibition of bacterial replication after the removal of antibiotic from the system.

The MIC is used as a potency measure of antibiotic- bacteria interactions and three PK/PD indices used to predict an antibiotic's response. These indices are: (1) ratio of maximum free drug concentration to MIC (C_{max} : MIC) , (2) MIC ratio of free area under the concentration-time curve (AUC) to the MIC (AUC : MIC) and, (3) the duration of time where free drug concentration remains above the MIC (time (T) > MIC) (**Figure 14**) (48,49).

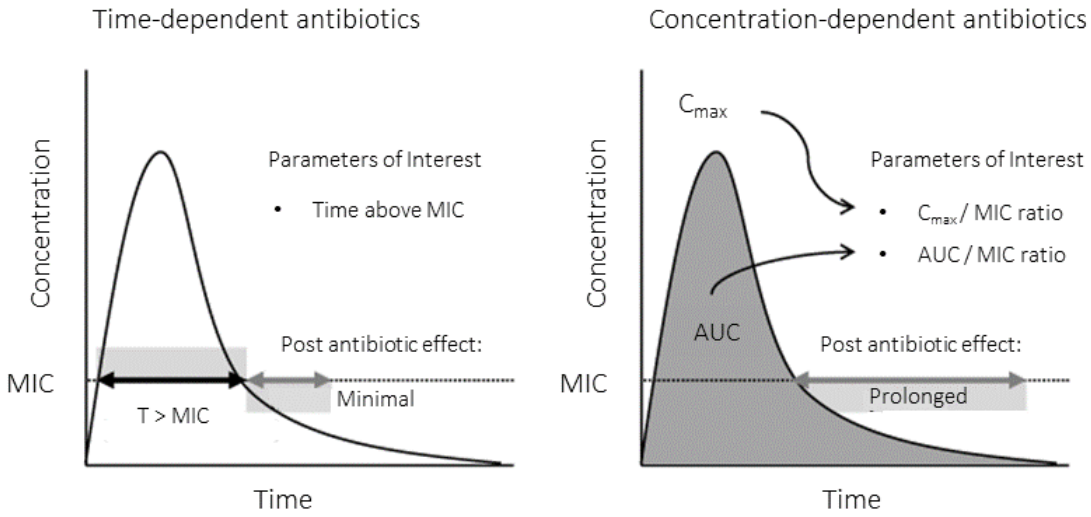


Figure 14 Principal PK/PD characteristics of antibiotics.

In general, antibiotics are categorised using two major determinants of bacteria inhibition/killing: concentration and the time the antibiotic remains above the MIC, thus, antibiotics exhibit either concentration-dependent or time-dependent activity (48). Concentration-dependent antibiotics are effective to a greater extent with increasing concentration, with best responses occurring when the concentration is ≥ 10 times the MIC of the infecting organism. Time-dependent antibiotics are effective over the time at which

the concentration is maintained above the MIC and optimal responses occur when concentrations are above the MIC for ≥ 50% of the dosing interval (**Figure 14**) (44,46).

1.3.3.4.1 Antibiotic Breakpoint Concentration

Antibiotic breakpoints are the concentrations at which bacteria are susceptible to successful treatment with an antibiotic (recommended standardised breakpoints are set by EUCAST). These antibiotic concentrations (μg/L) define whether the infecting pathogen is susceptible or resistant to the antibiotic. If the MIC of the pathogen is less than or equal to the breakpoint concentration, the infecting bacteria is considered susceptible to the antibiotic. If the MIC is greater, the bacteria is considered resistant.

An antibiotics breakpoint and the MIC of the infecting organism reflect the most paramount PD measures for antibiotics. However, this value simply demonstrates the potency of an agent. The incorporation of information regarding PK in terms of bioavailability will determine the percentage of the antibiotic dose that reaches the systemic circulation without any change in characteristics for its therapeutic effects.

Other major factors affecting the bioavailability of antibiotics are their formulation and their route of administration.

1.4 Introduction to Antibiotic Administration

1.4.1 Antibiotic Formulations

Antibiotic formulation is the process by which the active pharmaceutical ingredient (API) is combined with other chemical components (e.g., excipients and diluents) to form a final pharmaceutical product. To ensure that the medicine is effective, safe and selective in its mode of action, factors such as particle size, polymorphism, pH and solubility must be considered.

The formulation of an antibiotic can influence its bioavailability (proportion of antibiotic that is successfully absorbed into the systemic circulation) and distribution at the intended site of action. There are several factors that need to be considered when selecting the method

by which a drug is administered to achieve a therapeutic effect, including: (1) chemical properties of the drug, (2) onset of action required, (3) convenience and, (4) cost.

Antibiotic formulations are available in various forms: solid (tablets and capsules), semisolid (creams and ointments) and liquid (syrups, gargles and parenteral solutions). Many APIs can be delivered via several formulations hence the antibiotic's route of administration is dependent on the dose, the target location and the time required for the onset of antibacterial activity. The delivery method can vary from patient-to-patient depending on the individual and should be determined case-by-case.

1.4.2 Routes of Administration

The way in which an antibiotic is administered will influence its clinical benefit as it affects whether the antibiotic reaches its intended site of action on or within the body (50). An antibiotics route of administration is dependent not only on patient convenience and compliance but also on the drugs properties and its PK and PD profile. Antibiotic administration routes are often classified by the location at which the drug is administered such as topical, enteral (e.g., oral) and parenteral (e.g., intravenous (IV) or intramuscular (IM)). The advantages and disadvantages of the most commonly used routes are shown in **Figure 15** (51).

Topical: This route involves applying antibiotics that are in cream or ointment form directly to the site of infection on the skin. Thus, the antibacterial effect is achieved only in the infected area, reducing the risk of systemic side effects caused by other routes of administration (51,52). Topical antibiotics are currently used for many dermatological infections, including acne, rosacea and impetigo (52).

Oral: The most common route of antibiotic administration is by oral ingestion into the gastrointestinal system where the antibiotic absorbed and distributed to the target site of infection. The oral route has good penetration to almost every organ and is used in respiratory, gastrointestinal, and dermatological infections. In general, oral therapy is the most convenient route as it is well tolerated, provides adequate therapeutic effect, and is cost effective (51).

Figure 15 Advantages and disadvantages of the most common antibiotic routes of administration.

Parenteral: This route of administration is generally interpreted as injecting an antibiotic directly into the body. The most common parenteral routes are IV and IM. Common formulations administered via this route include any reconstituted solution, suspension, or emulsion formulation. IV involves the administration the antibiotic directly into the bloodstream either by direct injection or infusion. The antibiotic reaches the site of action faster and at a higher concentration than oral antibiotics. The IM route involves the direct inoculation of antibiotic into the muscle tissue that is released gradually into the bloodstream (51).

Other routes of antibiotic administration include otic and intraperitoneal. Also, antibiotic-containing collagen sponges (53) and antibiotic-impregnated cement beads (54) are used for the treatment of localised or systemic acute and chronic bone infections. These infections typically involve sequestration of dead bone with minimal blood supply, if any.

Therefore, systemic antibiotics may not reach the area of greatest need as well as exhibiting poor penetration of bone, even vascularised bone (50,54).

Antibiotic-containing collagen sponges (fleeces) are used to provide a prophylactic local haemostatic effect for the treatment of bone infections (53). They work by gradually releasing antibiotics locally and are available in a variety of sizes and antibiotic types. Internal fixation of fleece/s is carried out prior to closure of the wound and are used in conjunction with IV antibiotics (55,56). There is no need for secondary surgery for the removal of these antibiotic carriers since they are bioabsorbable (57).

Antibiotic-impregnated cement beads are used for antibiotic release to the site of infection. They vary in size, amount of antibiotic and type of bone cement used and are used in conjunction with IV antibiotic therapy (54). The beads are placed within the wound or bone allowing for high levels of antibiotic bathing of the wound. Once the infection is controlled; the beads are surgically removed. One complication of this administration route includes difficulty with bead removal when beads are left in place too long (54,58).

Typically, patients receive topical and oral antibiotics in outpatient settings, whereas administration via the parenteral routes (IV and IM) require a specialised healthcare professional and most commonly administration takes place in hospital settings. For many infections, oral antibiotics can be as effective as IV antibiotics. Hence, IV antibiotics are recommended for more severe, life-threatening and deep-seated infections.

1.4.3 Antibiotic Therapy Approaches for the Treatment of Bacterial Infections

The treatment of infectious diseases falls into three general categories: prophylaxis, empiric use and definitive therapy:

1. Prophylactic Therapy: Antibiotic prophylactic therapy is the treatment given to prevent an infection that has not yet developed. It involves the use of antibiotics before surgery or a dental procedure to prevent bacterial infection. The use of prophylactic therapy is not widespread and is usually limited to patients that are at a high risk of developing an infection during surgery, patients on immunosuppressive therapy and those with cancer (30,59).

2. Empiric Therapy: This approach is taken when patients have an infection or suspected infection, but the responsible pathogen has not yet been identified. In inpatient and outpatient practice, most antibiotics are initiated without or prior to identifying the pathogen and its susceptibility to antibiotics. Healthcare professionals assess the likelihood of an infection based on a physical examination, symptoms and experience and predict which antibiotic will be most effective against the likely cause of infection. Prior to initiating empiric therapy, samples should be taken for culture and Gram staining (**Figure 16**) (30,59).

3. Definitive Therapy: Once blood culture and sensitivity results are obtained (this process usually takes several days), definitive therapy treatment can commence. Unlike empiric therapy, definitive therapy is given after receipt of culture and susceptibility results, when infective pathogens are known, and the treatment is based on identifying the antibiotics that work against them. In this phase of treatment, antibiotics that are narrow in spectrum should be chosen as there is no need to target organisms not causing the infection (**Figure 16**) (30,59).

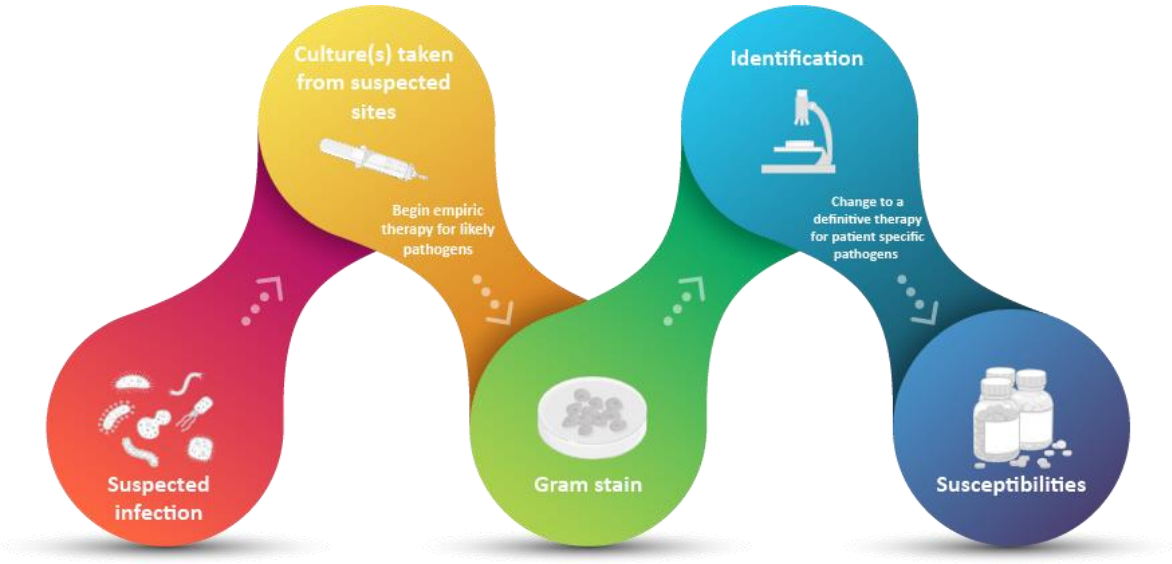


Figure 16 General approach to infectious diseases. Initiation of empiric antibiotic therapy is vital in a case of serious infection. However, when the microbiological information become available, antibiotic therapy is appropriately adjusted [29], [59].

In practice, initial antibiotic treatment for infections is often empiric therapy, guided by a patient's symptoms and a practitioners' experience, as microbiological results commonly require between 24-72 hours. To avoid microbiological failure of empirical therapy, broad

spectrum antibiotics are prescribed, which in turn promotes the evolution of resistant organisms.

1.5 Introduction to Antibiotic Resistance

The accidental discovery of penicillin began the 'era of antibiotics' and is recognized as one of the greatest advances in therapeutic medicine (60). Nonetheless, the world is on the cusp of a 'post antibiotic era'. Alexander Fleming himself predicted the rise of antibiotic resistance (AR), stating in his Nobel Prize acceptance speech in 1945 that, ''…there is the danger that the ignorant man may easily under-dose himself and that by exposing microbes to non-lethal quantities of the drug will make them resistant'' (61).

AR is a natural evolutionary phenomenon. However, the widespread exploitation of antibiotics is accelerating this process. AR occurs when the bacteria causing infection develop resistance to one or more antibiotics to which they were originally sensitive. Resistance is defined, taking into account the PK/PD criteria, by an increase in the MIC of the antibiotic against bacteria in relation to its previously established MIC threshold (62). Therefore, if the MIC for a bacterium is above the pre-defined threshold, it is classified as resistant. The number and types of bacteria developing resistance have dramatically increased due to the overuse and misuse of antibiotics in recent years, as well as the lack of new antibiotic development (36). The full impact of antibiotic resistance is unknown as there is no system in place to track antibiotic resistance globally.

1.5.1 Scope of the Problem

AR is increasing to alarmingly high levels worldwide, to the point that the emergence of resistance mechanisms threatens HCPs ability to treat common infectious diseases. This leads to longer hospital stays, higher medical costs, and increased mortality. Almost every human infecting pathogen has acquired resistance to at least one class of antibiotics in clinical use. Both Gram-positive and Gram-negative (particularly Gram-negative) pathogens, are developing resistance to almost all the antibiotic options available, creating situations reminiscent of the pre-antibiotic era (36). If antibiotics stop working, common infectious diseases could become fatal and it is predicted that drug resistant infections could kill more than 50 million people a year worldwide by 2050 if no action is taken (63).

1.5.2 Types of Resistance

Resistant bacteria can infect humans and animals and spread between them through food and the environment. Bacteria have evolved several resistance mechanisms (**Section 1.5.3**) against currently utilised antibiotics. It is noteworthy that these resistance mechanisms can be intrinsic or acquired. Intrinsic resistance is when some bacterial species are naturally resistant to a certain antibiotic or class of antibiotics as they intuitively lack the antibiotic target structure required (64). Examples of bacteria with intrinsic antibacterial resistance include: all Gram positive bacteria having intrinsic resistance to aztreonam and all Gram negative bacteria having intrinsic resistance to glycopeptides (65).

Extrinsic resistance results from the acquisition of mutations. Mutations that confer resistance can be transmitted either vertically when the bacterium reproduces (from parent cells to offspring) but also horizontally from one bacterial cell to another using mobile genetic elements such as plasmids (via conjugation, transformation, and transduction) and bacteriophages (**Figure 17**). This means that a bacterial strain can share its AR with other bacterial strains, even if they are only distantly related bacterial species. Horizontal transfer is a major mechanism underlying the spread of AR among bacterial species (30).

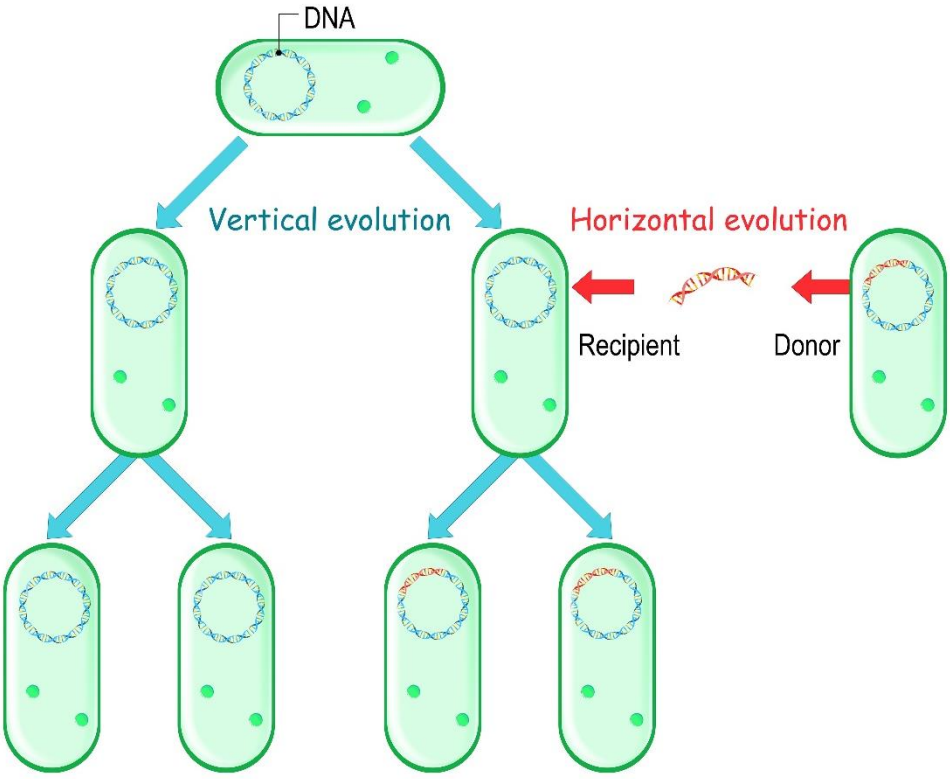


Figure 17 Schematic representation of horizontal and vertical gene transmission.

1.5.3 Mechanisms of Resistance

The main mechanisms by which resistance occurs include:

1) Inactivation of enzymes – antibiotic modifying enzymes synthesised by the bacteria destroy the antibiotic by an enzymatic reaction to give an inactive form of the drug. This enzymatic inactivation mechanism degrades the beta-lactam antibiotic class (**Figure 18**).

2) Efflux pump –the bacterium expresses a membrane protein that rapidly pumps the antibiotic out of the cell. As the antibiotic is removed (most common in the tetracycline antibiotic class), the bacterial cell is left unaffected (e.g., Pseudomonas aeruginosa) (**Figure 18**).

3) Target modification – bacterium modifies the antibiotic target so it can no longer bind to the ribosome and so protein synthesis and cell growth continue unaffected. Antibiotics modification mainly occurs in aminoglycoside, chloramphenicol, and beta-lactams (**Figure 18**).

4) Blocked penetration – a bacterium (e.g., Pseudomonas aeruginosa) modifies its membrane permeability, preventing the antibiotic from entering the cell. Reduction in permeability plays a key role in quinolones and aminoglycoside resistance (**Figure 18**) (66).

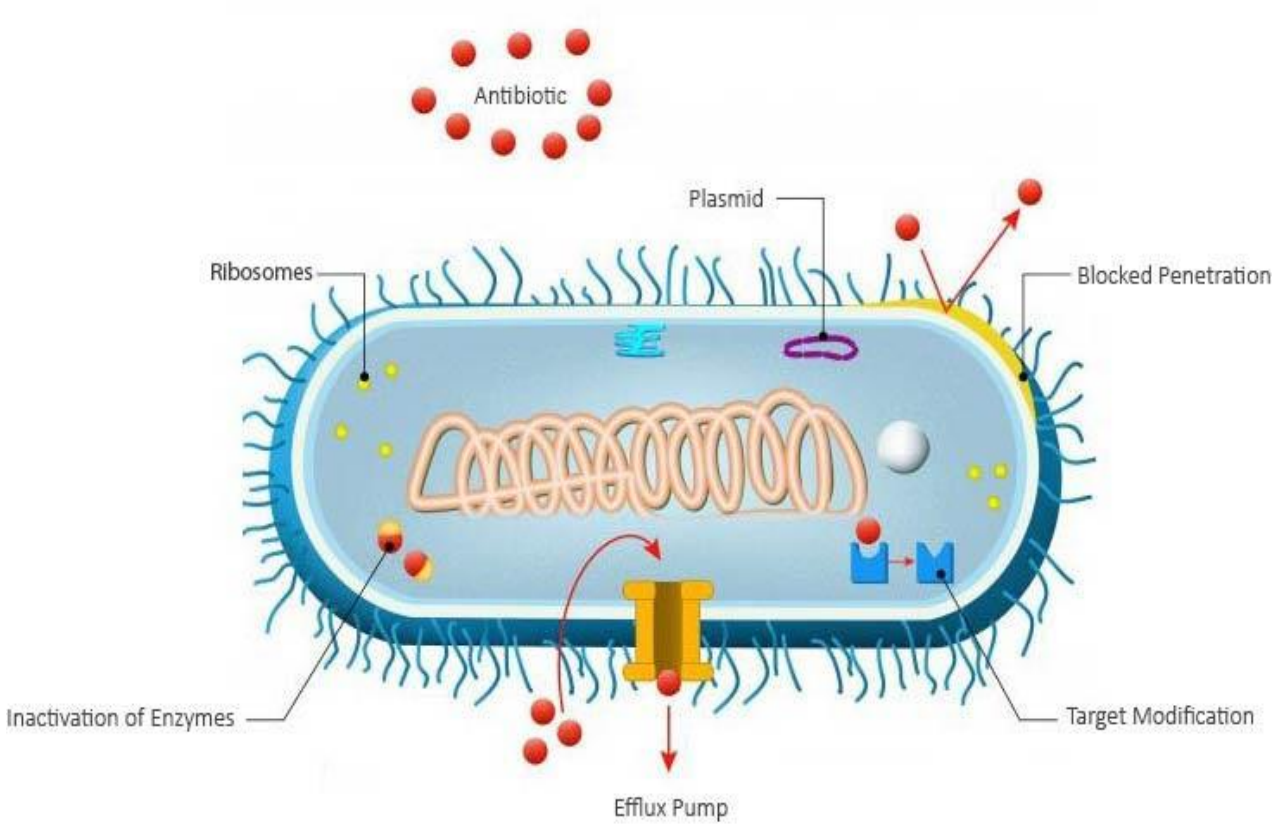


Figure 18 Illustration highlighting the four main mechanisms by which resistance occurs.

1.5.4 Prevention and Control

Antibiotics are a class of drug for which their use in one person can affect the effectiveness in another person since bacteria are transmissible. Judicious use of antibiotics (**Figure 19**) is necessary to control the spread of resistance. Studies have demonstrated that reducing antibiotic use, from a patient to hospital to country level, reduces antibiotic resistance. However, each of these levels have diverse perspectives. For example, patients seek antibiotic treatment for cure, hospitals are concerned with patient cure and costs and countries lack diagnostic certainty increasing empiric antibiotic use (67–70).

Infection control in healthcare facilities is vital as infections can spread rapidly if precautions are not taken. Ways to prevent the spread of infection include proper sterilisation of equipment, use of appropriate personal protective gear, isolation of infected patients, strict hand washing practices, regular cleaning and having a good hospital surveillance system so that infections are recognised and thoroughly contained (71).

1.5.5 Antimicrobial Stewardship

The antimicrobial stewardship (AMS) is defined as a rational, coordinated organisational or healthcare system-wide approach to the use of antimicrobial agents in order to achieve optimal outcomes and reduce the incidence of AR. AMS provides guidance for organisations, prescribers and health and social care staff on establishing a programme that promotes prudent, effective prescribing through optimization of antimicrobial selection, dosage, duration of treatment, and route of administration (**Figure 19**). It is now a requirement that organisations adopt an AMS programme that aims to improve antibiotic prescribing, minimize harm, reduce AR, and promote cost-effective prescribing while taking into consideration: monitoring and evaluating feedback to individual prescribers, education and training for health and social care staff, and integrating audits into existing quality improvement programmes (72).

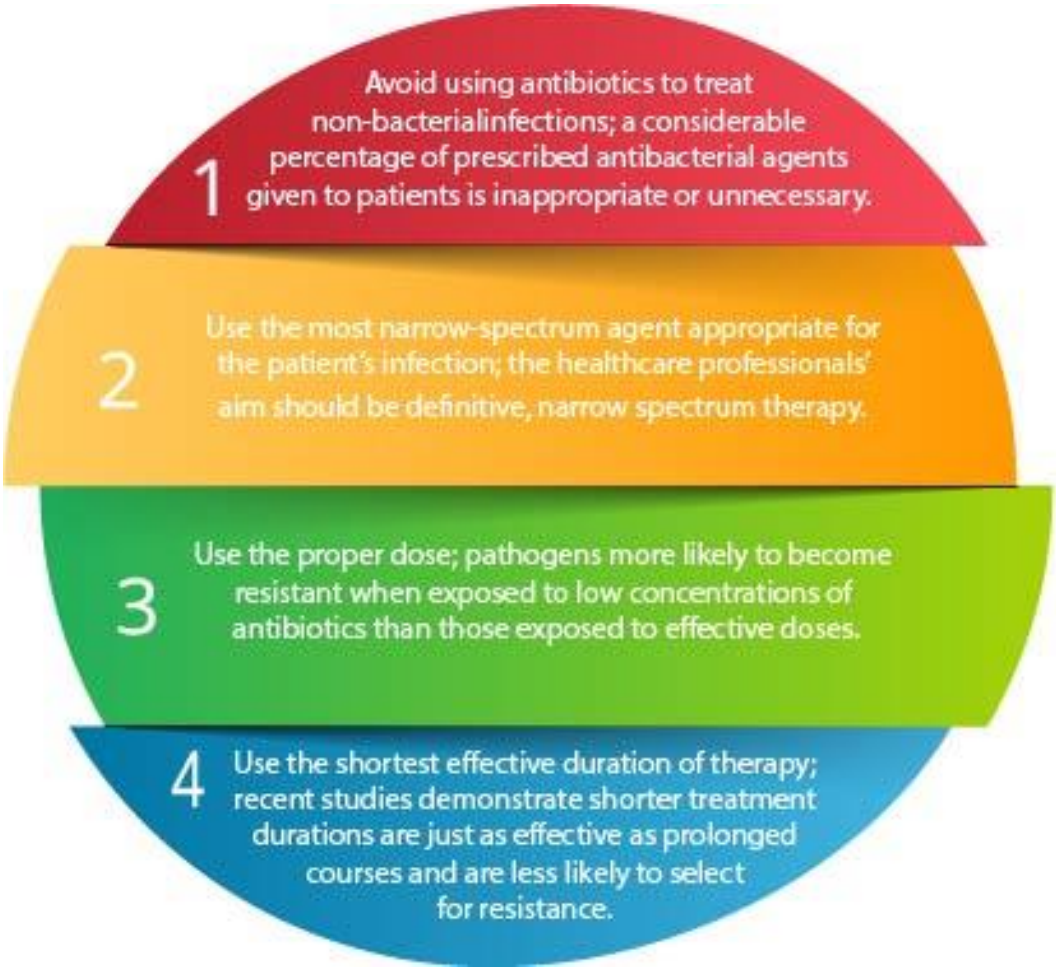


Figure 19 General guidelines for antibiotic use to aid in reducing antibiotic exposure without affecting quality of care in terms of patient outcome as well as reducing the risk of adverse events and antibiotic resistance [71], [72].

1.5.6 Global Strategies Proposed to Decelerate the Spread of Resistance

The prevalence of multi-drug resistant (MDR) bacteria and the lack of new innovative antibiotics has directed the global prioritisation to preserving the efficacy of antibiotics. The increased threat of drug resistance is now imminent, and it is in the interest of all health providers/systems to tackle it. The Centres for Disease Control and Prevention, USA, propose a global strategy that involves creating and implementing a comprehensive plan based on improving antibiotic use. A collaborative worldwide approach to detect, respond, contain, and prevent resistant infections across healthcare settings, food supplies and communities is required (72,73).

The National Prescribing Service, Australia, have proposed practice measures that will aid in slowing down the prevalence of antibiotic resistance. These include (1) careful consideration of whether the health condition is self-limiting before prescribing antibiotics, (2) prescribing the narrowest-spectrum antibiotic at the appropriate dose and duration and (3) educating patients on the appropriate use and disposal of prescribed antibiotics (74).

Recently, the Department of Health, UK, set out a strategy to slow the development and spread of antimicrobial resistance. This approach focused on three strategic aims, including: (1) improving knowledge and understanding of resistance, (2) stimulating the development of new antibiotics, diagnostics and novel therapies and (3) conserving and optimising the effectiveness of existing antibiotics (75).

The latter aim is a growing field of study. One strategy currently employed to aid in reducing the development of AR involves optimising the administration of existing time-dependent antibiotics by extending their infusion time, thus, maximising the duration of free drug above the MIC (37,76–80).

The optimisation of existing antibiotics therapeutic potential has become a necessity for the management of severe infections caused by multidrug resistance, especially with the lack of new antibiotics in clinical practice. Although intermittent administration of time-dependent antibiotics is the universal dosing regimen in practice globally, clinicians and researchers are interested in investigating the advantages of differential antibiotic dosing to potentially further improve the clinical effectiveness of antibiotics (81).

1.6 Introduction to Antibiotic Dosing Regimens

Injectable drugs are either readily available in the form of a premixed solution (specified dosage) or a dry powder. Injectable antibiotics are commonly marketed as dry powders which require reconstitution and dilution prior to administration.

1.6.1 Antibiotic Dosing Regimens

At present, there are four methods of IV antibiotic administration:

1) IV Bolus

IV bolus, also referred to as IV push injection involves the administration of more concentrated IV medications. It constitutes the delivery of an antibiotic in a small volume of diluent, delivered directly into the bloodstream over a period of 3-5 minutes using a needle and syringe. This route of delivery is the quickest and most effective way to reach optimal serum drug levels (82). It is used when the maximum serum concentration of the antibiotic is required or when antibiotics cannot be further diluted for pharmacological reasons such as antibiotic plasma concentration. Bolus injections are the fastest way to induce adverse drug reactions as rapid administration can result in toxic levels or anaphylaxis. Therefore, the rate at which the antibiotic is given may depend on its toxicity (83). Administration via bolus dosing may give undesired erratic peaks and low troughs in plasma concentration, resulting in concentrations falling below the MIC between dosing intervals (**Figure 20**).

2) Intermittent Infusions

Intermittent infusion (II) involves administration of small volume infusions (50-250mL) over a period of 20 minutes to 2 hours. This route of antibiotic delivery is administered using infusion containers and devices at repeated intervals over a 24-hour period and is the most common method for IV antibiotic therapy (82,83). Administration via II is standard clinical practice and has numerous advantages, including: better utilisation of intravenous access, little concern about drug degradation over time and drug compatibility concerns (77). Although II are easier to perform than extended infusions, patient PK profiles are less predictable, a higher daily dose is required to reach target concentrations and concentrations may fall below the MIC between dosing intervals (**Figure 20**) (82,83).

3) Prolonged Infusions

Prolonged infusions (PI), also referred to as extended infusions, involve IV administration over a period of 3-4 hours. The use of PI as well as continually infusing antibiotics is particularly important for optimising T > MIC for time-dependent antibiotics to improve microbiological and clinical cure rates due to their superior improvement in PK/PD properties (77). For antibiotics administered via PI, the volume of diluent is usually increased by 1.5 or 2-fold compared to II antibiotics. One advantage of this route of administration over continually infused antibiotics is that it allows intermittent time between doses where other prescribed IV medications may be administered via the same single IV catheter. Continuous administration requires a dedicated IV catheter (84).

4) Continuous Infusions

Continuous infusions (CI) involve constant IV administration over a 24-hour period. This method of administration is used when medication needs to be delivered in a highly dilute form or when maintaining a constant plasma concentration is required (e.g., vancomycin). Often, the release of CI antibiotics is via an infusion device to ensure an accurate flow rate and delivery of the medicine. As patients are continually connected to a line, antibiotics must have a dedicated IV access to avoid risks associated with incompatibility (82,83). An advantage of CI is that serum levels are constantly maintained above the MIC facilitating more predictable PK profiles and so lower daily doses of antibiotics may be possible. However, CIs are more complex to perform and are associated with a higher risk of drug incompatibility problems when administered through the same IV line as well as stability concerns (Figure 20).

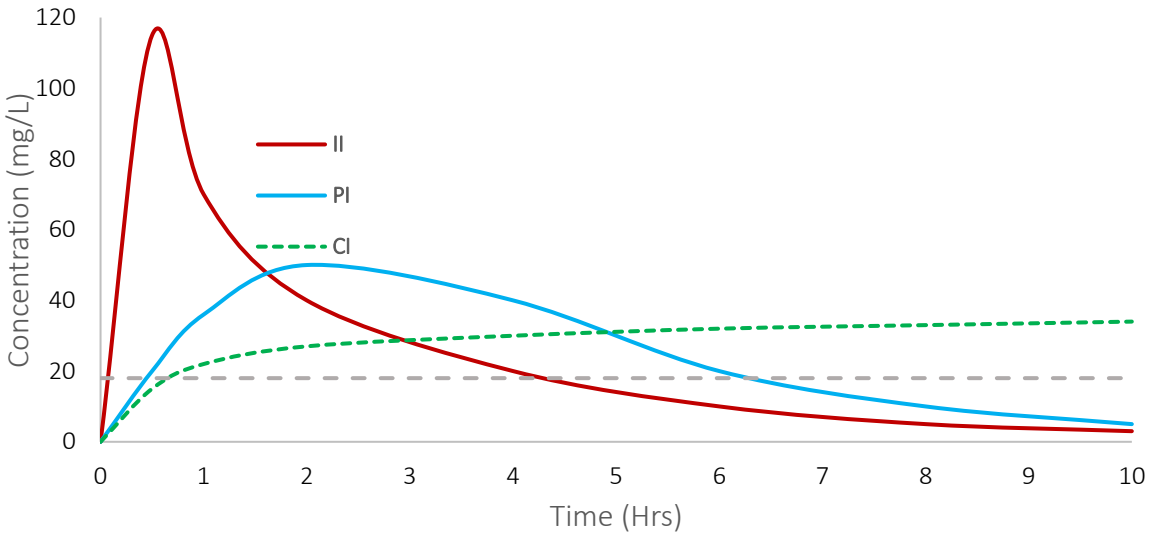


Figure 20 Schematic plot demonstrating the effects of CI beta-lactam dosing regimens on the concentration curves and time above the MIC compared with traditional IV bolus and II dosing regimens.

1.6.2 II vs CI Antibiotics

In 1999, Georges *et al*., compared the efficacy of CI vs II cefepime in critically ill patients with gram-negative rod infections. 9 patients were assigned to each of the regimen groups. Patients in the CI group received 4g cefepime daily dose (DD) via CI for 24 hours whereas patients in the II group received 2g cefepime every 12 hours. T > MIC was significantly higher in patients receiving CI. However, no significant difference in duration of therapy, recovery rate and duration of mechanical ventilation was found. Although no significant differences were found, CI achieved more favourable clinical outcomes in terms PK/PD attainments (**Table 1**) (85).

In 2000, Hanes *et al*., assessed the adequacy of CI and II ceftazidime therapy in critically ill trauma patients with nosocomial pneumonia (CI = (n = 14) vs II = (n = 17)). Patients in the CI group received 2g ceftazidime loading dose (LD) followed by DD of 60mg/kg via CI for 24 hours whereas patients in the II group received 2g ceftazidime every 8 hours. Results showed that clinical cure was achieved in 56% and 71% of patients in the CI and II groups, respectively. Clinical failure was also observed to be higher in CI patients (CI = 44% vs II = 29%). They concluded that both dosing regimens were equally effective and emphasised that more studies are needed to define the advantages of CI (86).

In 2000, Angus *et al*., studied the PK profiles for ceftazidime given by either CI (n = 10) or conventional 8 hourly II (n = 11) in septicaemic melioidosis patients. Patients in the CI group

received 12mg/kg ceftazidime LD followed by DD 96mg/kg via CI for 24 hours and patients in the II group received 40mg/kg ceftazidime via II every 8 hours. This study found that there was no significant difference in the dosing regimens in terms of peak plasma concentration. However, there was a significant difference between mortality rates (CI = 30% vs II = 82 %). Also, CI was found to be advantageous in terms of cost-effectiveness. This study concluded that II could result in ceftazidime concentration falling below the MIC of infecting organism but this would be unlikely with CI (87).

A 2001 study compared the two dosing modalities, CI and II vancomycin to determine which is more efficient, safer, easier and more cost effective. 119 patients with severe staphylococcal infections were enrolled on the study by Wysocki *et al*., (CI = (n = 61) vs II = (n = 58)). Results showed that treatment durations were similar in both groups (CI = 13 ± 5 days vs II = 14 ± 6 days). Despite the more rapid target concentration attainment in the CI group, there was not a statistically significant ascendency in microbiological or clinical superiority of CI (Table 1). CI was found to be 23% more cost effective than II. Wysocki concluded that CI achieved more favourable outcomes in terms of acquiring target concentration, had less variability in daily dose adjustments and lower cost (88).

A study by Buijk *et al*., compared PK profiles in patients with intra-abdominal infections receiving CI and II ceftazidime. A total of 12 patients were enrolled in the randomised comparative part of the study (CI = 6 vs II = 6). Results obtained showed that CI eliminates the undesired high peak and subtherapeutic trough concentrations found with II as well as giving more predictable serum concentrations, allowing for accurate dosage adjustment. Also, mortality rates were lower in the CI group. Buijk concluded that CI ceftazidime resulted in more favourable outcomes from a PK and PD perspective in terms of serum concentration attainment compared to II in critically ill patients with intra-abdominal infections (89).

Lorente *et al*., compared the clinical efficacy of ceftazidime administered via CI or II in the treatment of patients with ventilator acquired pneumonia (VAP) caused by Gram-negative bacilli. Patients assigned to CI therapy (n = 56) received 1g ceftazidime LD followed by DD 4g via CI 24 hrs whereas patients assigned to II received 2g ceftazidime over 30 min II every 12 hrs. Ceftazidime administration via CI proved superior to II achieving 89.3% and 52.3% clinical cure rates, respectively. The study concluded that CI was more effective than II in the treatment of patients with VAP (90).

The influence of vancomycin administration on renal function and nephrotoxicity was studied by Hutschala *et al*., Renal failure is associated with an increased mortality, thus, they evaluated the nephrotoxic side effects of CI (n = 119) vs II (n = 30) of vancomycin in critically ill patients after cardiac surgery. They found that both modes of administration were associated with deterioration of renal function. Although, there was not a statistically significant difference in terms of nephrotoxicity between CI and II, the data indicated that CI was less nephrotoxic compared with II. Mortality was significantly lower in CI therapy patients compared with II therapy patients (CI = 24.3% vs II = 56.7%). It was concluded that CI showed the tendency to be less nephrotoxic than II of vancomycin (91).

Table 1 Summary of studies that previously investigated CI vs II antibiotics.

Study (country)	Patient Population	No of Patients		Antibiotic Dosage		Duration of Treatment		Clinical Cure (%)		Clinical Failure (%)		Mortality (%)	
		CI	II	CI	II	CI	II	CI	II	CI	II	CI	II
Georges *et al*., 1999 (85) (France)	Nosocomial pneumonia and bacteraemia	9	9	4g cefepime DD via CI for 24 hrs	2g cefepime via II every 12 hrs	NR	NR	NR	NR	22	22	NR	NR
Hanes *et al*., 2000 (86) (USA)	Critically ill trauma patients	17	14	2g ceftazidime LD followed by DD of 60mg/kg via CI for 24 hrs	2g ceftazidime via II every 8 hrs	Up to 14 days	Up to 14 days	56	71	44	29	NR	NR
Angus *et al*., 2000 (87) (Thailand)	Septicaemic melioidosis	10	11	12mg/kg ceftazidime LD followed by DD 96mg/kg via CI for 24 hrs	40mg/kg ceftazidime via II every 8 hrs	< 10 days	< 10 days	NR	NR	NR	NR	30	82
Wysocki *et al*., 2001 (88) (France)	Severe staphylococcal infections	61	58	15mg/kg vancomycin LD followed by DD 30mg/kg via CI for 24 hrs	15mg/kg vancomycin over 60 min II every 12 hrs	13 ± 5 days	14 ± 6 days	NR	NR	21	19	18	12
Buijk *et al*., 2002 (89) (Netherlands)	Intra-abdominal infections	12	6	1g ceftazidime LD followed by DD 4.5g via CI 24 hrs	1.5g ceftazidime via IV bolus every 8 hrs	< 10 days	< 10 days	NR	NR	NR	NR	25	33
Lorente *et al*., 2007 (90) (Spain)	Ventilator-associated pneumonia	56	65	1g ceftazidime LD followed by DD 4g via CI 24 hrs	2g ceftazidime over 30 min II every 12 hrs	NR	NR	89.3	52.3	NR	NR	NR	NR

Hutschala *et al*., 2009 (91) (Australia)	Cardiac surgery infection	119	30	20mg/kg vancomycin LD followed by DD 20-25mg/kg via CI for 24 hrs	20mg/kg vancomycin over 60 min II every 12 hrs	9 ± 6	8.5 ± 7	NR	NR	NR	NR	24.3	56.7
Huang *et al*., 2014 (92) (China)	Neurosurgical intracranial infections	34	34	0.5g cefepime LD followed by DD 4g via CI for 24 hrs	2g cefepime over 30 min II every 12 hrs	6.6 ± 1.9	.8 ± 2.6	100	100	0	0	0	0
Hong *et al*., 2015 (93) (USA)	Neurosurgical infections	65	65	20mg/kg vancomycin LD followed by DD 15-40mg/kg via CI for 24 hrs	15mg/kg vancomycin via II every 8-24 hrs	10.4 ± 7.8	14.1 ± 8.8	59.1	55.5	NR	NR	15.4	20
Bissell *et al*., 2018 (94) (USA)	Critically ill trauma patients	75	75	20mg/kg vancomycin LD followed by DD 15-30mg/kg via CI for 24 hrs	15mg/kg vancomycin via II every 12 hrs	AVR 3.83 days	AVR 6.8 days	60	40	1.3	5.3	9.3	17.3

AVR = average, CI = continuous infusion, DD = daily dose, II = intermittent infusion, IV = intravenous, LD = loading dose, NR = not recorded.

A study by Huang *et al*., in 2014 evaluated the clinical efficacy of CI (n = 65) vs II (n = 65) in patients with neurosurgical infections by measuring cefepime concentrations in plasma. The plasma concentration of cefepime remained 4 times higher than the MIC throughout CI therapy, whereas concentrations fell below the MIC in patients receiving II therapy. No adverse effects were observed in any of the patients. Huang concluded that CI cefepime is clinically more effective and consistently generated concentrations above the MIC compared with II therapy (92).

In 2015, Hong *et al*., studied whether CI vancomycin improved clinical outcomes compared with II in patients with neurosurgical infections. Both administration regimen groups had 65 patients. Time to goal serum concentration was significantly lower in the CI group in comparison to the II group (CI = 1.96 days vs II = 3.76 days) and achievement of target serum concentrations was twice as likely in CI patients (CI = 40% vs II = 21.5 %). Although the study found no significant difference between the two dosing regimens in terms of nephrotoxicity, a lower mortality rate was observed in CI patients (CI = 15.4% vs II = 21.5%). Hong concluded that CI resulted in improved PD optimisation as well as fewer dose adjustments. However, additional research is needed to determine the applicability of CI for other antibiotics and patient populations (Table 1) (93).

A 2018 study by Bissell *et al*., compared the effects of CI and II vancomycin administration on clinical outcomes and adverse events. 75 patients were included in each of the therapy groups. This study supports the use of CI as it was found that patients within the II group had a significantly higher frequency of subtherapeutic levels. Nephrotoxicity was also significantly reduced in patients receiving CI and a significantly shorter duration of therapy was required in the CI group compared with the II group. It was concluded that CI achieved more favourable clinical outcomes. However there is a need for larger studies to validate the outcomes (94).

Overall, antibiotic administration via CI has potential benefits including equal or better PD profiles (antibiotic concentration remained above the MIC for a longer duration), improved tolerability, and more convenient administration. The mentioned studies also emphasised that CI may be a more cost-effective mode of treatment when compared to conventional II therapy.

Further research on the efficacy and safety of potential CI agents may lead to the development of new delivery devices for inpatient and outpatient use. Similar developments have occurred for hormones (e.g., insulin and progesterone).

CI antibiotics may also have applicability in outpatient settings, especially in patients requiring prolonged treatment (81). There is an increasing emphasis on improving patient care by reducing the length of inpatient hospital stay and where possible, avoiding admissions completely. In recent years, a service that provides outpatient parenteral antibiotic administration has been introduced to treat medically stable patients who require continued IV antibiotics therapy to receive treatment in outpatient settings.

1.7 Introduction to Outpatient Parenteral Antibiotic Therapy

Hospitalisation for no other reason than to carry out parenteral antibiotic therapy is expensive and inconvenient to the patient (95). After treatment in the hospital and once the medical problem has stabilised, patients can receive parenteral antibiotics as outpatients, facilitating early discharge or potentially avoiding admission (96). Intravenous antibiotic administration outside hospital settings (i.e., at outpatient infusion centres or at home) is termed outpatient parenteral antibiotic therapy (OPAT). OPAT is particularly useful in situations where patients are not severely unwell but do require prolonged antibiotic treatment. OPAT was first described by Rucker and Harrison in 1974 for the management of paediatric patients with cystic fibrosis. Since its introduction, OPAT has been reported to be safe, effective and cost-effective (97). OPAT has become increasingly common for infectious diseases where IV antibiotic administration is the only barrier to discharge from hospital (98). OPAT has widely been proven to improve quality of life and is associated with high levels of patient satisfaction. However, as there is less clinical supervision, governance arrangements associated with patient care must comply with published guidelines (99).

1.7.1 UK Guidance on OPAT Delivery Service

The OPAT working service published guidelines on how the UK OPAT service could be delivered in 1998 (99). These guidelines address questions such as: (1) which diseases are amenable to OPAT? (2) which patients are appropriate? and how they are selected? (3) what are the pharmacy issues? (4) how can OPAT be delivered? (5) how are patients monitored

during therapy and (6) what follow up arrangements are necessary? (100). The suggestions made by the OPAT working service were later supported in the practical OPAT guidelines by the Infectious Disease Society of America in 2004 (99). Amended UK good practice recommendations (GPRs) were published in 2012 for adult patients (101) and in 2015 for paediatric patients (102). These proposals were an update to the consensus statement on OPAT published in 1998. The modifications were based on national and international guidelines to provide pragmatic guidance on the development and delivery of OPAT services with the aim of providing high-quality, low-risk care (101). Since the publication of the GPRs (101,102), numerous UK OPAT guidelines and recommendations that encompass attributes of service design including delivery of care, outcome monitoring and quality assurance have been published to provide appropriate guidance to OPAT services across a range of healthcare settings (103–105).

1.7.2 The OPAT Process

The OPAT process involves six distinct phases (**Figure 21**). The first four entail identifying, confirming the suitability of, and accepting the patient for the service. The patient is identified by the OPAT microbiology team, educated about their infectious disease and the information regarding antibiotic therapy is sent to the pharmacist. Process five incorporates the initiation of treatment and continuing treatment. Once the treatment is initiated, the first antibiotic dose is administered in a supervised setting where the patient is monitored closely for adverse effects and blood tests are carried out to check for any abnormalities. Depending on the OPAT delivery model the patient is assigned to, OPAT nurses both train and assess the patients. The OPAT pharmacists remain in contact with the patient to co-ordinate the delivery of continuing prescriptions. Patients are reviewed daily and have weekly blood tests to ensure there are no complications with the line or medication and to monitor their clinical response to antibiotic therapy. The final process involves assessing the efficacy of the IV OPAT therapy. At this stage, the patient has attended a consultation and a decision is made regarding discontinuation of the treatment or the need for ongoing treatment or concluding the therapy (99).

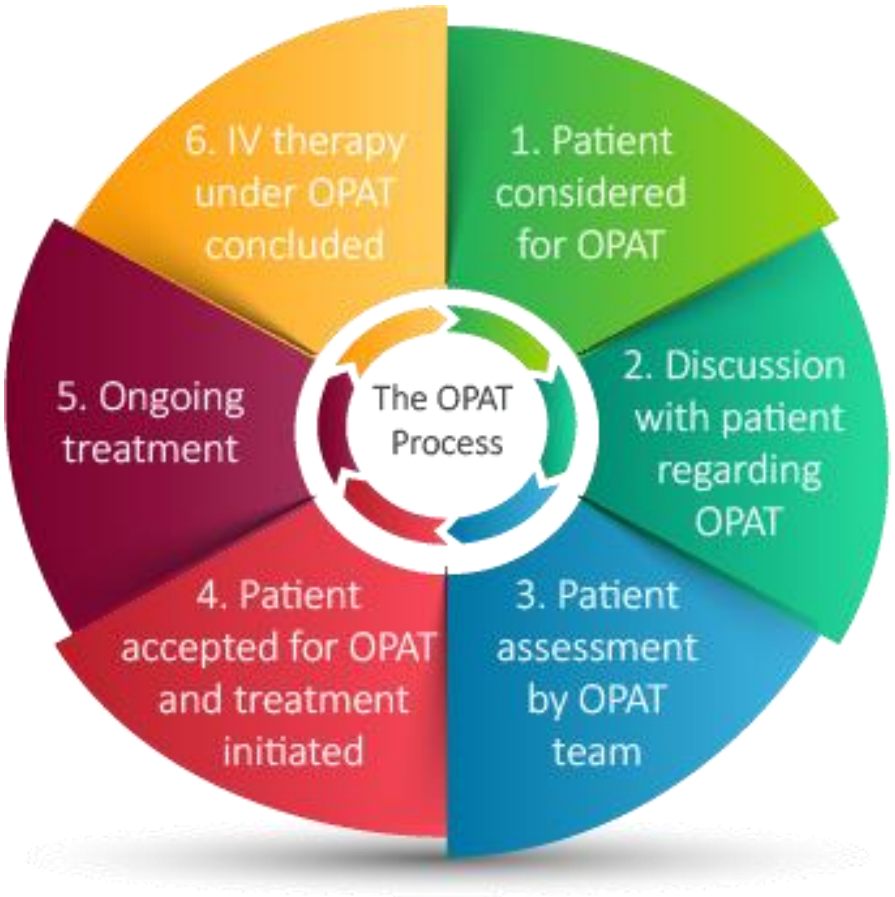


Figure 21 The OPAT process. All six stages require communicating between the patient and the OPAT service HCPs [99].

1.7.3 OPAT Service Delivery Models

Outpatient services can be considered either after a period of hospital assessment and stabilisation (for 'high risk' infections such as meningitis and endocarditis) or directly, without hospital inpatient admission (for 'low risk' skin/soft tissue infections and cystic fibrosis) (100). Two crucial factors to be considered prior to assigning a patient to an OPAT programme include: (1) the methods by which the antibiotic is administered and (2) how patient is monitored during therapy (106). A wide range of OPAT treatment models have been adopted worldwide, but in general three models of administration are required:

1) Infusion centre delivered (H-OPAT): Patient attends an outpatient health care facility (hospital clinic or A&E) where the therapy is administered by a healthcare professional.

2) Delivery in patients' home (C-OPAT): Antibiotic therapy is administered by a visiting nurse at the patient's home.

3) Self or carer administration (S-OPAT): The patient or family member administers OPAT at home (96).

Table 2 outlines the advantages and disadvantages of the three models listed above.

Table 2 OPAT delivery models: advantages and disadvantages

Model	Advantages	Disadvantages
H-OPAT	Supervised administration where medical staff are available. Access to medication and devices	High cost of facility Patients must travel to clinic
C-OPAT	Supervised administration Convenience to patients	High cost of nurse time Nurse must travel to patient's home
S-OPAT	Reduced staff costs Reduced facility cost	Patient/carer training Unsupervised administration and patient compliance

Each hospital develops and manages their own system for providing OPAT. The choice of delivery model depends on the experience and resources available. The antibiotic to be used may also affect which delivery model is used. For example, if an antibiotic is dosed to be administered more often than every 8 hours, the most reasonable model is S-OPAT (107).

1.7.4 Antibiotics and Infections Treated via OPAT

OPAT is mostly suitable for patients with skin infections (like cellulitis) or infections that require prolonged parenteral treatment such as bone infections (like osteomyelitis) and those in joints or heart valves. Other suitable infections include pyelonephritis, lung abscesses, cerebral abscesses, and empyema. Currently, the most frequently used antibiotics for OPAT are those that are administered once daily (e.g., ertapenem) or those associated with few adverse events and which have a long serum half-life (ceftriaxone and vancomycin) (108). Other commonly used beta-lactam antibiotics such as penicillin G, cefazolin and oxacillin are used in the UK for OPAT (Table 3). In some cases, concentrated antibiotics are infused into the patient via portable pumps after discharge from hospital. In practice, some of the most used antibiotics are insufficiently stable for use as OPAT medications as they must be pre-prepared and stored prior to administration. Poor drug stability may result in patient receiving sub-therapeutic doses, leading to extended treatment durations, treatment failure, readmission to hospital and antibiotic resistance. Stability should be maintained throughout the pre-preparation and infusion time and is a critical factor when deciding whether an antibiotic is appropriate for OPAT use (109).

Table 3 Properties of commonly prescribed parenteral antibiotics for OPAT

Drug	Half-Life (h)	Optimal Dilution (mg/mL)	Duration of stability by storage temperature	
			5°C	25°C
Cefazolin	1 - 2	10 - 20	10 Days	1 Day
Ceftazidime	1.4 - 2	1 - 40	21 Days	2 Day
Ceftriaxone	5.4 – 10.9	10 - 40	10 Days	3 Day
Clindamycin	2 - 3	6 -12	32 Days	16 Days
Ertapenem	4	20	1 Day	6 Hours
Nafcillin	0.5 – 1.5	2 – 40	3 Days	1 Day
Oxacillin	0.3 – 0.8	10 – 100	7 Days	1 Day
Penicillin G	0.4 – 0.9	0.2	14 Days	2 Days
Vancomycin	4 - 6	5	63 Days	7 Days

1.7.5 Clinical Effectiveness

OPAT is preferred by patients as it is efficient, safe and offers care closer to home. It has been shown to be both clinically and cost effective in NHS settings (110). In 2009, Chapman *et al*., examined the clinical efficacy of OPAT services in a large Sheffield teaching hospital over a two-year period. A total of 296 patients were enrolled on OPAT after meeting predefined criteria (in which skin and soft tissue infections accounted for most illnesses. Over the course of the study, 87% of patients enrolled were either clinically cured or improved on completion of their IV therapy. Also, the total number of bed days saved through the OPAT service was 4034. It was concluded that OPAT is safe, clinically effective, has low levels of complication and high levels of patient satisfaction (111).

Similarly in 2017, Durojaiye *et al*., published a study that examined the clinical effectiveness of the OPAT service in 3004 patients over a 10-year period. Antibiotics were administered by the three OPAT pathways H-OPAT, C-OPAT and S-OPAT. The majority of patients enrolled, were diagnosed with either a skin or soft tissue infection. 88% of the patients experienced a successful outcome (cure or improvement). When cure was not recorded after OPAT, most patients continued oral antibiotics after discharge. A total number of 49854 bed days were saved over the 10 years. The study concluded that OPAT is suitable for a vast number of infections (e.g., Gram-positive and Gram-negative infections) in increasingly complex patient groups (e.g., patient that acquired pathogens with high MIC breakpoint) (112).

The use of OPAT is associated with a reduction in the length of hospital stay and improved rates of admission. A UK study conducted by Hitchcock *et al*., in 2009 assessed the types of infection, management strategy and outcomes for patients referred to OPAT services at St Marys Hospital, over a 3.5-year period. Patients who had a serious infection that required parenteral antibiotic therapy but were well enough to leave hospital were enrolled. Outpatient treatment was found to be well tolerated with a mean treatment length of 24 days (range 1-165 days). Hitchcock reported that patients found the service highly satisfactory and that it improved their quality of life during the treatment period. Additionally, 7394 bed days were saved over the study period (113).

1.7.6 Cost-Effectiveness of OPAT

OPAT is not only clinically effective, with low rates of complications and high levels of patient satisfaction, it also represents a cost-effective service, compared with inpatient care in healthcare settings (111). Several studies have now confirmed that OPAT is a cost-effective, safe alternative to inpatient care and recommended that it should become a routine recommendation for eligible patients (111,113–115). A 2009 UK retrospective study by Chapman *et al*., conducted an economic evaluation of OPAT in which the delivery of OPAT services was compared with conventional inpatient care. Total costs were calculated using actual costs of staffing (nursing, medical and clerical) and running (drugs, consumables, and equipment) the service over a 2-year period. Results obtained from this study indicated that OPAT results in lower cost when compared with inpatient care in the UK. Chapman concluded that OPAT reduced inpatient costs by 47% (111).

A systematic review of OPAT economic analyses by Psaltikidis in 2017 reported that 33/35 studies showed that OPAT was less expensive than inpatient therapy. Considering all 35 studies included in the review, the average OPAT economic saving was 57.19% (115).

Although these studies report substantial cost savings compared with inpatient hospital stay, they lack fiscal analysis (116). Economic evaluations are usually based on specific homogenous populations. However, OPAT is highly complex with no internationally standardised methods for costing and involves different patient ages, diagnostics, therapeutic plans and self-care capacities (115). Nevertheless, cost-effectiveness is only one

dimension of potential benefit OPAT offers; patient satisfaction is another important quality outcome indicator that was satisfied.

1.7.7 Patient Satisfaction

Approximately 4% of inpatients are solely in hospital to receive IV antibiotics. Outpatient therapy offers patient the choice of admission avoidance or early discharge while maintaining high quality care (110). Studies evaluating patient satisfaction with OPAT treatment have been positive. In 2002, Goodfellow *et al.*, conducted a health-related quality-of-life assessment for patients treated at an OPAT unit and established that there was a significant improvement in physical functioning, bodily pains and mental health score after discharge from hospital and enrolment on OPAT (117). Also, a telephone survey conducted by Montalto verified that 97% of patients would choose OPAT again if the occasion arose (118).

Mansour *et al.*, conducted a OPAT patient experience survey to assess overall patient satisfaction, to compare OPAT satisfaction between H-OPAT and S-OPAT patients and identify barriers to patient satisfaction. They found that overall patients were satisfied with the OPAT service. Patient satisfaction was higher for patients who received home infusions (S-OPAT) than those who attended outpatient healthcare facilities (H-OPAT). However, several patient satisfaction barriers were identified, including errors in medical management, lapses in communication and organisation, a lack of attention to symptoms and gaps in infection prevention. He concluded that in order to improve the patient experience, OPAT programs need to better engage with patients in both H-OPAT and S-OPAT to improve communication and care delivery (119).

Another study by Saillen *et al.*, in 2017 investigated patient satisfaction in terms of OPAT, predominantly focusing on H-OPAT patients who self-administer antibiotics via elastomeric devices. A questionnaire consisting of 16 open and closed ended questions was given to 188 patients, of whom 112 returned it completed. Results from this study showed that there was a low rate of treatment failures and complications and that a large majority of patients achieve clinical cure or improvement. This study concluded that patients were very satisfied overall with the care received and were particularly positive about treatment via elastomeric devices. They also appreciated having been given responsibility for their own care (120).

1.7.8 Provider Preference

Although many studies have explored patient preference and satisfaction in relation to OPAT, few studies have investigated its practitioner acceptability. Four studies (121–124) have shown that health care professionals also find OPAT beneficial but encountered organisational barriers in managing its funding, compounded by poor leadership and inadequate links between primary and secondary care (121,122,124,125).

In a 2016 study by Hamad *et al*., infectious disease physicians were surveyed to gain insight into their perspectives relating to outpatient therapy and to identify barriers to the safe care of OPAT patients. The results indicated that most respondents felt OPAT services: (1) were not adequately supported financially (64.6%), (2) were not valued by leadership (58.4%) and, (3) struggled to effectively communicate with OPAT providers (54.4%). Other barriers to safe OPAT care reported were retrieval of laboratory results in a timely fashion (58.5%) and the large volume of laboratory results that must be reviewed (47.8%) (126).

Furthermore, a survey conducted to obtain opinions of general practitioners' perceptions regarding OPAT found that many respondents (94%) believed that patients benefited from treatment in a familiar environment. However, a large number of respondents considered OPAT presented no advantages to general practitioners (74%) and many believed that treatment away from hospital increased their workload (70%) and was challenging for self-administering patients (123).

Another barrier reported by providers was the health care technology used in OPAT. This technology presented a challenged to both patients and HCPs, limiting the care that they could deliver (127). Nurses had difficulties with the technical nature of the devices used when providing OPAT and also in dealing with patient concerns and questions regarding the technology and its associated risks (121)(128).

1.8 Introduction to Infusion Containers and Devices

IV antibiotic therapy requires:

1) solution containers (syringes, bags, bottles, and vials)
2) an administration set (sterile tubing)

3) if necessary, an infusion device (infusion pump).

IV Infusion containers are tools made from medical grade materials containing infusion solutions. ''Medical grade'' refers to the materials biocompatibility and safety. Although insufficiently studied, the characteristics (e.g., toughness, flexibility and sterility) of the container may influence factors associated with administration such as the total volume of antibiotic infusion solution delivered to the patient, which impacts the amount of drug received (129,130).

Infusion containers can be categorised as either open or closed. Open infusion containers are made from rigid (glass, non-collapsible plastic) or semi-rigid (semi-collapsible plastic) materials. Although these systems require venting to empty content, which increases the risk of extrinsic microbial contamination and air embolism for the patient. Open containers are still widely used around the world. Closed infusion containers consist of flexible, fully collapsible plastic (typically PVC bag). Since these containers are collapsible, no external venting (air filter or needle) is required during the solution delivery to the patient, preserving sterility and reducing the potential for air embolism.

1.8.1 Infusion Containers

Prescribed antibiotics are commonly prepared in syringes for administration via IV push/bolus or in an IV bag (IVB) where administration is by either II, PI or CI.

1.8.1.1 Syringes

A syringe is a rigid infusion container composed of a sliding plunger that fits tightly into a cylindrical barrel. Pressure is used to operate the syringe whereby the plunger is pulled or pushed linearly through the barrel, allowing the syringe to withdraw or expel the medication through a discharge orifice at the tip of the tube where the needle is attached. Syringes are available in different sizes with volumes ranging from 0.25 – 45 mL to conveniently deliver the required dose of medication. Syringes should not be filled to capacity, therefore, a

syringe whose capacity is slightly larger than the volume to be measured should be selected as each syringe will be graduated in the smallest possible increments (**Figure 22**) (131).

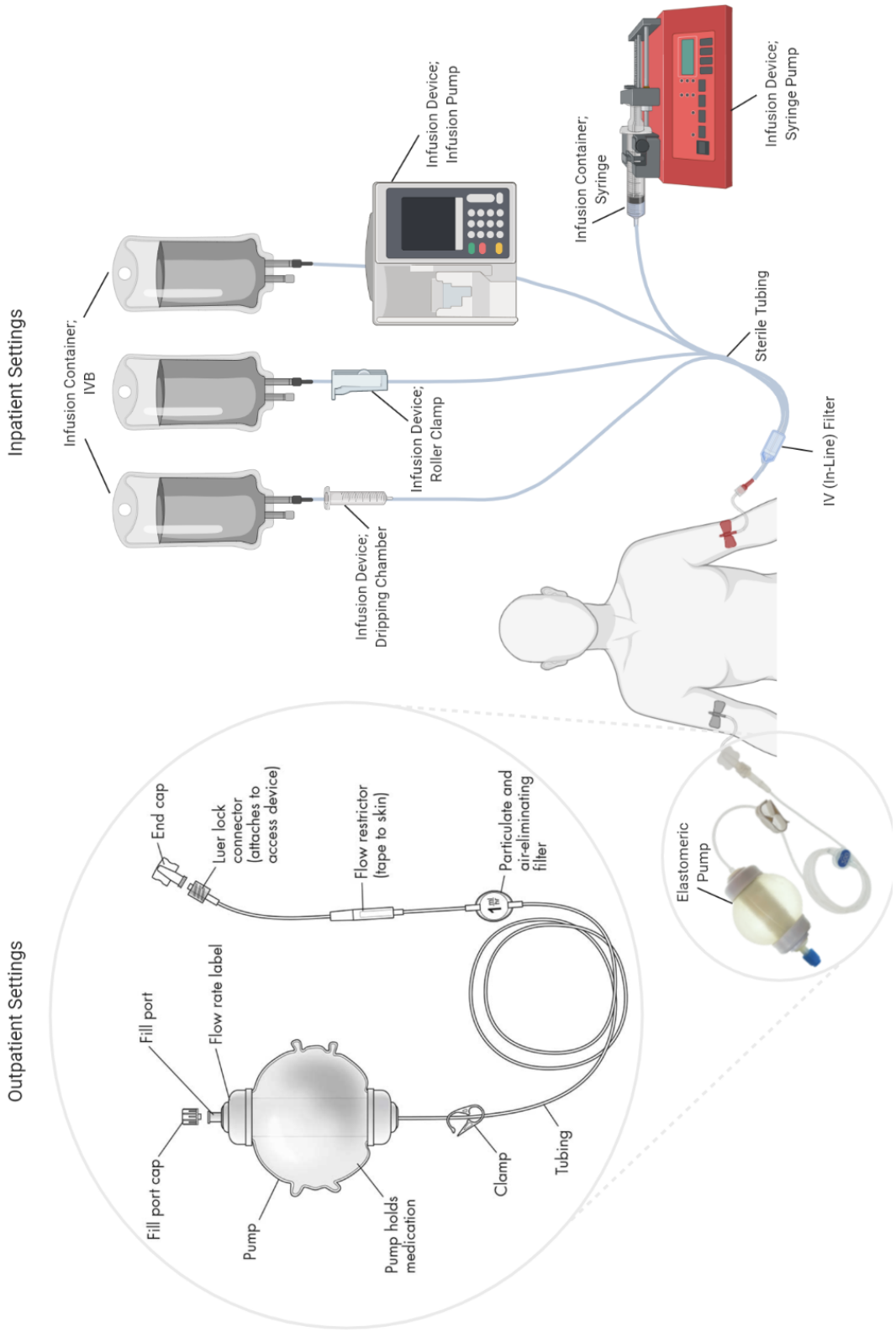


Figure 22 Infusion containers and devices used in inpatient (IVB and syringes) and outpatient (EPs) settings for parenteral antibiotic administration.

1.8.1.2 Intravenous Bags

IVBs are fully collapsible containers that are easily stored, transported and disposed and reduce the risk of airborne contamination by 10 times compared with rigid and semi-rigid containers. The IV bag containing the medication is hung above the height of the patient and the solution is allowed to flow through sterile tubing attached to a cannula (inserted into a vein) under gravity. The rate of administration cannot be controlled without additional equipment. Therefore, a clamp, drip chamber or infusion pump is often employed to allow precise control over the flow rate and total amount of medication delivered (**Figure 22**) (130).

1.8.1.3 Elastomeric Pumps

In outpatient settings, elastomeric pumps are most used to deliver medication to the patient. Elastomeric pumps use pressure to infuse medications such as antibiotics, chemotherapeutics, analgesics and local anaesthetics. These pumps have reservoir capacities ranging from 60 – 500 mL and flow rates ranging from 0.5 - 500 mL/hr with infusion times varying from 30 minutes to 14 days. These one-use, disposable devices do not require electricity, are not driven by gravity and can be conveniently relocated when necessary. They are relatively comfortable to wear due to their low weight, small size, and silent operation. The pressure within the pump is exerted by an elastomeric (silicone or polyisoprene) layer that stretches when the pump is filled. The elastic constriction pushes the medication through tubing to the flow restrictor that controls the accuracy of the flow rate and eventually out into the connection with the patient (central access device) (**Figure 22**) (130,132). It is noteworthy that in the majority of OPAT settings, EPs are utilized, however, in some cases electronic pumps are used.

Many classes of antibiotics are used in these devices for both hospitalised patients and patients treated in outpatient settings including beta-lactams and glycopeptides. The most extensively utilised antibiotics are the beta-lactam class.

1.9 Introduction to Beta-Lactam Antibiotics

Beta-lactam antibiotics (BLAs) are antibacterial agents that contain a beta-lactam (BL) ring (**Figure 23**) in their molecular structure. The BL ring is responsible for the BLAs ability to kill bacteria, thus is referred to as the killing site. BLAs are the most extensively used antibiotics in clinical practice due to their relatively high effectiveness, low cost, ease of delivery and minimal side effects. BLs represent the largest family of antimicrobial agents and comprise approximately 50% of worldwide antibiotic sales. This class of antibiotics includes penicillins, cephalosporins, monobactams and carbapenems. Penicillins (mainly effective against Gram-positive bacteria) are the treatment of choice for numerous infections, while cephalosporins which are effective against both Gram-positive and Gram-negative bacteria are widely used in surgical prophylaxis and to treat severe community acquired infections. Carbapenems are also effective against both Gram-positive and Gram-negative bacteria but are utilized in mixed nosocomial and MDR bacterial infections.

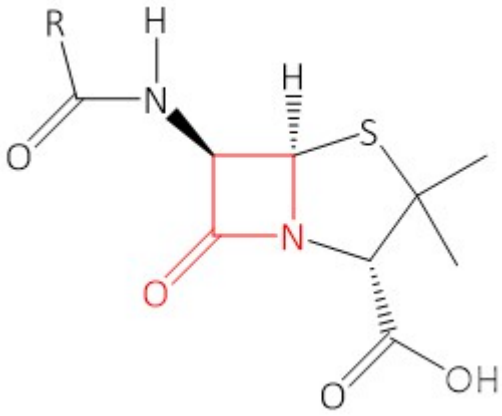


Figure 23 Structure of penicillin. The beta-lactam ring is shown in red.

1.9.1 Characteristics of Beta-Lactam Antibiotics

Collectively, BLAs display broad-spectrum activity against a wide range of clinically significant Gram-negative, Gram-positive, aerobic and anaerobic bacteria (133). All BLAs exhibit bactericidal activity which is relatively independent of plasma concentration. They act by inhibiting the PBP enzymes required to cross-link the peptidoglycan chains which make up bacterial cell walls. The resulting disruption of cell wall crosslinking leads to bacterial autolysis and cell death.

1.9.2 Beta-Lactam Antibiotic Mechanisms of Resistance

Although bacterial cell walls offer a selective target for therapy, the effectiveness of BLAs depends on their ability to reach the PBP intact and then effectively bind to the PBP. Therefore, there are two major mechanisms of bacterial resistance to BLAs:

(1) Inactivation by enzymes: Enzymatic hydrolysis of the BL ring occurs when the bacteria produce beta-lactamases that can hydrolyse the BL ring, rendering the BLA ineffective. In Gram-positive bacteria the beta-lactamase enzymes (BLEs) are generally inducible, resulting in a large amount of enzyme being produced in the presence of the BLA. In Gram-negative bacteria, the BLEs are produced constitutively, even when the BLA is not present. Gram positive bacteria release these enzymes from within the cell into the extracellular environment where it inactivates the BLA before it enters the cell. Gram-negative bacteria, however, retain the enzymes within the periplasmic space to inactivate the BLA (134).

(2) Target modification: Transpeptidases, such as PBPs, the key components involved in the construction of the peptidoglycan. The PBP active site is the target of BLAs. Some bacteria have evolved PBPs with structures to which BLAs bind with much lower affinity, limiting their ability to inhibit cell-wall synthesis (134,135).

Although BLAs have been the antibiotic agents of choice for the treatment of infections since their discovery, their efficacy is significantly threatened by bacterial BLEs. These enzymes are produced by Gram-positive and Gram-negative bacteria that catalyze the opening of the BL ring through reaction with water.

1.9.3 Beta-Lactam Antibiotic Hydrolysis

Generally, lactams (five or six membered rings) are, like amides, chemically stable. However, the four membered ring of BLs is subjected to structural strain. Thus, it is rapidly hydrolysed in aqueous solutions (136). Hydrolysis of a BLA by a beta-lactamase enzyme (BLE) requires a water molecule that carries out nucleophilic attack on the BL moiety to hydrolyse the amide

bond and open the ring structure (**Figure 24**). When the BL ring is hydrolysed, the BLAs ability to kill bacteria is interrupted (137).

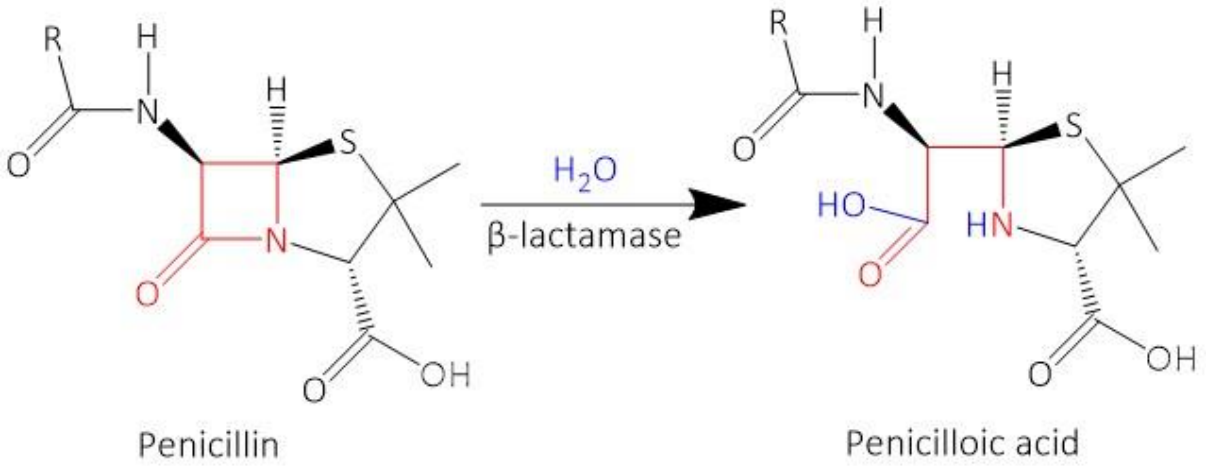


Figure 24 Showing hydrolysis of the BL ring. Hydrolysis of a BLA always involves a critical water molecule that, upon activation, carries out nucleophilic attack that opens its ring structure, rendering it ineffective.

1.9.4 Beta-Lactamase Inhibitors

To overcome BLE-mediated resistance, two strategies were proposed to preserve BLA utility: (1) discover novel BLAs that can evade bacterial enzymatic inactivation caused by BLEs and (2) inhibit the BLEs so BLA can reach the PBP unhindered. With the discovery of new antibiotics diminishing, the use of inhibitors has gained popularity. Beta-lactamase inhibitors (BLI) are co-administered with BLAs to prevent AR (138).

BLIs are drugs that exhibit relatively weak anti-bacterial activity. However, they augment the activity of BLAs against beta-lactamase producing organisms. Thus, BLIs are always combined with BLAs in clinical use (138). The combination of a BLI and a BLA offers the advantage of expanding the spectrum of activity of the BLA by binding to PBPs in bacteria which would ordinarily be protected by BLEs (139). These inhibitors greatly enhance the efficacy of BLAs in the treatment of serious antibiotic resistant infections. BLIs have been widely adopted because of their favorable safety profile and ability to limit the spread of bacterial resistance.

1.9.4.1 Beta-Lactamase Inhibitors Modes of Action

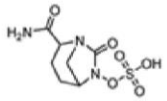
BLIs have two primary mechanisms of action. In the first of these the BLIs act as substrates that bind to the BLE with high affinity, forming a steric interaction. The second mechanism involves the BLI becoming a 'suicide inhibitor' which permanently inactivates the BLE

through chemical reactions at the active site preventing hydrolysis of the BL ring. Such agents cause irreversible inhibition of BLEs (138).

The most widely used BLIs are avibactam, relebactam, clavulanic acid, sulbactam and tazobactam (Figure 25). Avibactam and relebactam work by the first mechanism, while clavulanic acid, sulbactam and tazobactam work by the second mechanism. Each BLI has different dosage scheduling options, permitting HCPs to tailor a patient's medication regime to their unique needs (138).

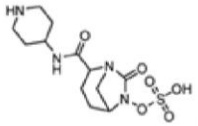
Within several years of the introduction of BLIs for clinical use, inhibitor resistant BLEs were reported. Resistance to BLA-BLI combinations currently challenges the ability of HCPs to successfully treat some common infectious diseases including those from urinary tract, respiratory and bloodstream infections. To overcome this critical challenge, novel "second generation" BLIs that demonstrate favourable inactivation properties towards resistant BLEs were developed (140).

Avibactam



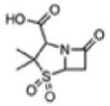
Avibactam has a half-life of approximately 2 hours, and administration is in combination with ceftazidime in a 1:4 combination. A dosing regimen of 2/0.5g ceftazidime/avibactam is used every 8 hours with a 2 hour infusion period.

Relebactam



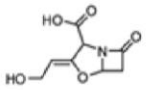
Relebactam has a half-life of approximately 1.2 hours, and administration is in combination with imipenem and cilastatinn a 1:1:0.5 combination. A dosing regimen of 0.5/0.5/0.25g imipenem/cilastatin/relebactam is used every 6 hours with a 30 min infusion period.

Sulbactam



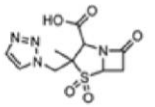
Sulbactam has a half-life of approximately 1 hour, and administration is in combination with ampicillin in a 1:2 combination. A dosing regimen of 1/0.5g ampicillin-sulbactam is used every 6 hours with a 15-30 min infusion period.

Clavulanic Acid



Clavulanic-Acid has a half-life of approximately 47 mins, and administration is in combination with amoxicillin in a 1:4 combination. A dosing regimen of 1/0.2g amoxicillin/clavulanic acid is used every 6 hours with a 15-30 min infusion period.

Tazobactam



Tazobactam has a half-life of approximately 1.2 hours, and administration is in combination with piperacillin in a 1:8 combination. A dosing regimen of 4/0.5g piperacillin/tazobactam is used every 6-8 hours with a 15-30 min infusion period.

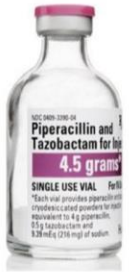


Figure 25 Current BLA and BLI combinations clinically used.

1.9.5 Beta-Lactam Dosing Regimen

Despite advances in modern medicine, BLA dosing regimens have remained largely unchanged since BLAs were discovered and are based on a 'one dose fits all' model. This approach fails to account for the wide variations in how a patient processes the drug, or the nature of their infection (141). BLA dosing schedules were empirically designed based on *in vitro* data and clinical experience. By failing to address patient variations in dose response, dosing interval, optimal duration of therapy or post-antibiotic effects, dosing regimens based on an understanding of PK and PD were therefore not established (141–144).

BLAs exhibit bactericidal activity characterized by time dependent killing which refers to the time which it takes for a pathogen to be killed by exposure to an agent. The goal of time-dependent killing is to optimise the duration of BLA exposure above the MIC of the infecting organism. The optimal time over MIC varies for different BLs. Bactericidal effects are typically observed when the antibiotic concentration exceeds the MIC for 35-40% of the dosing interval for cephalosporins, 30% for penicillins and 20% for carbapenems. However, to achieve the maximal bactericidal activity, serum concentrations must exceed the MIC for 60-70% of the dosing interval for cephalosporins, 50% for penicillins and 40% for carbapenems (145). It is therefore crucial to select a BLA and administration regimen that has a high likelihood of achieving the PK/PD targets to achieve efficacy. Various methods have been employed to maximise BLA T > MIC, including administering a higher dose, increasing dosing frequency or increasing the duration of infusion. In general, the most effective way to optimise exposure is to lengthen the administration time.

Current administration of parenteral BLs is via a bolus injection or over a 30-minute II every 6-8 hrs. Although this mode of administration has its advantages, including better utilisation of IV access and fewer compatibility concerns, it results in drug concentrations falling below the MIC between dosing intervals. However, optimisation of antibiotic dosing and administration may be a better means of improving clinical outcomes. BL dosing regimens can be optimised by employing P/CI as it can achieve a greater percentage of T > MIC in both tissue and plasma. P/CI of BLAs can be especially beneficial for the critically ill population and those infected with organisms that have a higher MIC.

The application of innovative strategies such as employing PK/PD principles to time-dependent antibiotics that: (1) are extensively utilised in practice, (2) compass a wide spectrum of activity and, (3) are associated with low toxicity, such as the BL class, could potentially improve their 'effectiveness' as well as be an efficacious way to combatting current resistance trends. Although published opinion defends the use of P/CI BLs as it maximises their time-dependent activity, dose and administration optimisation remain a significant clinical challenge. Research into optimizing how BLs are used and improving strategies on how infections are treated will help to preserve the potency of BLAs (75). The literature indicates that the main constraint regarding P/CI BLAs is their stability in aqueous solutions (146–148).

1.9.6 Beta-Lactam Antibiotic Stability

The chemical stability of a BLA should be maintained throughout the infusion time for the patient to receive the required concentration of active drug needed to achieve clinical cure while avoiding exposure to degradation products (77). This is especially important with parenteral BLAs as they could degrade post formulation. Physical instability should also be studied to avoid exposure to potential problematic particulate formation.

Improper administration of BLAs can lessen the effectiveness of the therapy as well as accelerate AR. Studies examining the chemical stability of BLAs suggest that their stability may vary to a meaningful extent when compared to manufacturers' data. This sets limitations on the potential use of BLAs by P/CI as administrations over 24-hour require frequent changes of the infusion solutions. This may be due to manufacturer sterility and stability testing not being performed according to guidelines where preparation must take place under strictly aseptic conditions.

Understanding the molecular stability of BLAs will give insight into new treatment strategies based on alternative approaches to effectively treat patients (37,77). Determining optimal treatment regimens is also critical for conserving and prolonging the effectiveness of currently available antibiotics (144).

Most commonly, the preparation of BLAs takes place on the wards by nursing staff and involves calculations, multiple manipulations, dilution after reconstitution and use of infusion pumps and is therefore classed as a moderate risk process by the National Patient

Safety Agency. The 'five rights of medication administration' (5Rs) are regarded as a standard for safe medication practices. This consists of ''the *right drug* for the *right person* in the *right dose* via the *right route* at the *right time*''. Preparation of the infusion solution just before administration can contribute to increased staff workload, delays in treatment and increased patient waiting times. Risk reduction strategies are recommended to prevent harm to patients through safer use of injectable medicines. One such strategy which may be employed is use of a pre-prepared product, for use in both inpatient and outpatient settings, which is only possible if antibiotic stability allows sufficient time for preparation and storage.

1.10 Introduction to Stability Testing

The stability of a drug substance or drug product is determined by how long it can maintain its original form without significant chemical or physical change (149). Stability is an essential attribute of drug substances and their products, due to the potential impact of exposing a patient to an unstable drug or its degradation product. Stability studies provide evidence on how the quality of a pharmaceutical formulation varies with time under the influence of a variety of environmental factors. These studies are conducted to evaluate the impact of storage conditions on the drug product and to ensure that the drug product complies with predefined quality parameters throughout its shelf-life. The importance of stability studies is shown in **Figure 26** (150).

A fundamental element of BLA stability studies involves an understanding of the chemical and physical behaviour of the active ingredients under the storage and usage conditions they are likely to encounter. Evaluating the physico-chemical stability of a pharmaceutical product requires an in depth understanding of its physical and chemical properties. Monitoring changes in a drugs chemical and physical characteristics is vital to ensure the purity, potency, and safety of the drug. For physical properties of IV infusion solutions, key changes that are monitored include any change in colour, optical density (from the presence of crystalline substances), and pH. The stability protocols for monitoring chemical changes

involve analysis of the concentration of active ingredients and checking for the presence of any degradation products.

Figure 26 The importance of stability studies. Stability testing assesses how the quality of a drug substance or drug product varies with time under the influence of environmental factors.

1.10.1 Regulatory Guidelines for Stability Testing

Regulatory guidelines have been established and circulated by international, national, and regional organisations to assist firms in the generation of stability data and to prevent the generation of inconsistent stability data. These guidelines explain the concepts, procedures, and protocols that regulatory agencies must adhere to.

The stability guidelines that are most commonly followed for BLAs include the international Conference of Harmonisation (ICH) (ICH Q1A, Q1B and Q1C) and WHO guidelines (WHO Technical Report Series, No, 953, 2009). The ICH and WHO guidelines provide a framework which industries depend upon to plan stability studies. The guidelines provide information on generating stability data for storage conditions, storage durations, and testing requirements. The WHO guidelines are modelled on the ICH guidelines, only differing in a few additional aspects including ongoing stability studies, and in-use and hold-time stability.

ICH guidelines have been adopted by many regulatory organizations including the US Food and Drug Administration, the European Medicines Agency, and the Chinese National Medicinal Products Administration.

A fundamental element of BLA stability studies involves a determination of data concerning the physicochemical behaviour of the active ingredients under the storage and usage conditions they are likely to encounter. To appropriately assess the stability of BLAs, it is vital to ascertain whether drug strength can be retained to provide a safe and efficacious drug product. Therefore, the principles adhered to when designing a stability study for BLAs reconstitution and dilution must include:

- Using a validated stability assay
- Studying stability in diluents used in practice
- Exposing infusion solutions to in-use conditions
- Determining stability in appropriate infusion containers

BLAs are deemed unstable when the active ingredient loses sufficient potency to the extent that it adversely affects the safety or efficacy of the BLA or falls outside labelled specifications as shown by stability-indicating methods. 90% of the labelled potency is

generally recognized as the minimum acceptable potency level in order to comply with the British, European and American pharmacopeias' (151).

1.10.2 Stability Requirements for OPAT

In the UK, the MHRA and NHS Standards regulate the procedures for compounding medicines in administration devices. These standards incorporate the minimum dataset required for the assessment of antibiotic stability and shelf-life and is available in the document 'Standard Protocol for Deriving and Assessment of Stability' commonly known as the Yellow-Covered Document (YCD). Prior to determining antibiotics suitability for OPAT, it is important that the antibiotic stability in solution is fully understood (152). The requirements of the NHS YCD regarding antibiotic stability are included in **Figure 27**.

Figure 27 YCD stability testing specifications. More stability data, especially in terms of narrower spectrum agents, that comply with these specifications are needed to support OPAT service expansion.

A systematic review by Jenkins *et al*., in 2017 assessed the extended stability of antibiotics in administration devices. They stated that the lack of stability data within administration

devices such as EPs is a barrier to service expansion and poses an antimicrobial stewardship dilemma. Another current challenge involves broad-spectrum antibiotics being prescribed instead of narrow-spectrum agents that could be used if stability data were available (e.g., use of once daily, broad-spectrum ceftriaxone rather than 4-6 hourly, narrow-spectrum flucloxacillin for the treatment of susceptible staphylococcal infections). The importance of using narrow-spectrum BLAs is explained in Section 1.4.3. This comprehensive review found that there were no published studies that entirely conformed to the UK national standards specific to OPAT. It was concluded that the lack of YCD-compliant studies is impeding OPAT expansion as services are compelled to use broad spectrum, once-daily agents (152,153).

The British society for antimicrobial chemotherapy (BSAC) developed a drug stability testing (DST) programme with the aim to offer data on the efficacy and stability of agents and devices used in OPAT infection management practice as required by the YCD (154). Currently there is a substantial lack of data which meet the YCD criteria available in open access. The BSAC DST programme seeks generation and open access publication of gold standard YCD (positive and negative) data on the stability of broad and narrow spectrum antibiotics in elastomeric devices. The BSAC OPAT initiative is to advise clinicians, pharmacists and nursing staff on service considerations and provide availability of robust DST, compounding, procurement, storage, and administration data (153,155).

OPAT aligns well with MHRA and NHS priorities. However, a few practical challenges limit the generalised use of continuous infusions with elastomeric pumps. An example, one that is poorly defined in the literature includes the stability of some antibiotics in real life conditions. For example, the excessive temperature increase of antibiotic solutions in 'near body' devices (such as EPs) may accelerate drug degradation which in turn could yield degradation products (120). In Chapter 3 and Chapter 4 of this thesis, the effect of temperature on antibiotic concentration is investigated in both IVBs and EPs to determine BLA stability under real life conditions.

Usually, when conducting stability studies on existing drug products, real-time stability tests are performed for the same duration as the recommended shelf-life. However, to determine whether BLAs can be administered via P/CI, in this project retained sample stability studies will be conducted to periodically inspect the BLA until the drug concentration decreases by

10% of the initial concentration (5% for OPAT). Retained sample stability studies are carried out for marketed products for which additional stability data are required. The method for obtaining stability data of these retained sample studies requires a known storage condition that mimics practice and sampling frequency at a constant interval (153,156).

The stability of a BLA over time should be determined by subjecting the API to various real-life storage conditions, thereby establishing conditions which minimise any decrease in API concentration. Typically, to establish the stability of BLAs and BLIs, a liquid chromatography method (coupled with a UV detector) that can separate the API from any impurities or degradation product/s is required. A stability indicating method (SIM) must also be developed to monitor the concentration and purity as well as identify any degradation products.

1.11 Introduction to High-Performance Liquid Chromatography

1.11.1 Chromatography

Chromatography is a process for separating components of a mixture. This technique has emerged as the most important and versatile analytical method. Chromatography was first discovered in 1906 when a Russian botanist, Mikhail Tswett, separated plant pigments through a glass column packed with calcium carbonate. Since this discovery, chromatography has developed into an invaluable tool for the separation and identification of compounds (157,158).

1.11.2 High Performance Liquid Chromatography

To date, high performance liquid chromatography (HPLC) remains the predominant chromatographic technique and is extensively used throughout the pharmaceutical industry. A HPLC system comprises several components including a solvent (mobile phase) reservoir, pump, injector, HPLC column, detector, and PC with chromatography software for data acquisition (**Figure 28**). High-pressure tubing and fittings are used to interconnect the HPLC system components (157,158).

The solvent reservoir, usually fitted with a gas diffuser, stores the mobile phase (usually a system contains a minimum of two and maximum of four reservoirs). The high-pressure

pump generates a specified flow of the mobile phase through the HPLC system, typically of several millilitres per minute (mLs/min). An injector (manual or auto sampler) introduces the sample into the continually flowing mobile phase, carrying the sample to the HPLC column (stationary phase). The mobile phase is involved in the movement of analytes flowing through the system. The analytes are retarded by non-covalent interactions with the stationary phase that is bound and packed within the column. Separation takes place due to the differing time it takes each component to travel through the stationary phase when carried through by the mobile phase (**Figure 29**) (157,158).

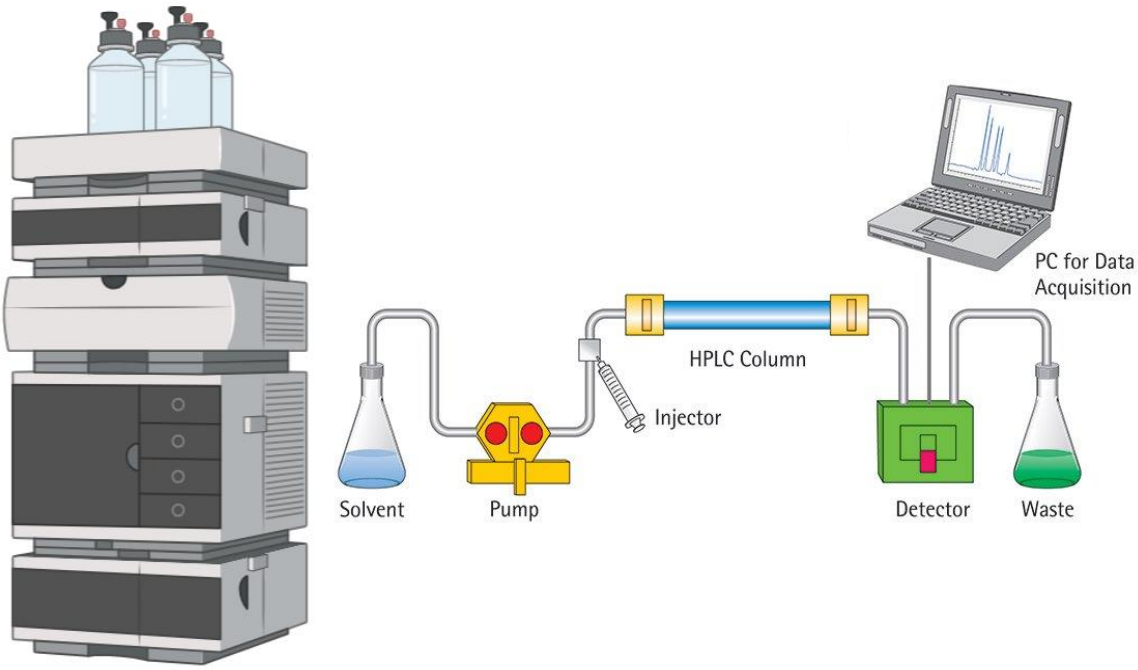


Figure 28 HPLC instrument and diagram of main instrumental components. This technique is used to separate, quantify and identify every component that is in a mixture.

A detector is needed to visualise the separated compounds post elution from the high-pressure column. The type of detector used to acquire a response is determined by the analyte. However, UV detectors are the most common as most organic compounds absorb in the UV range of the electromagnetic spectrum. The detector is connected to the computer where electrical signals of each compound are recorded at times based on their time of elution off the column to generate a chromatogram. The mobile phase then carries the compounds out of the detector to waste (or can be collected) (**Figure 28**). The chromatogram can then be analysed based on peak area to quantify the sample's components (157,158).

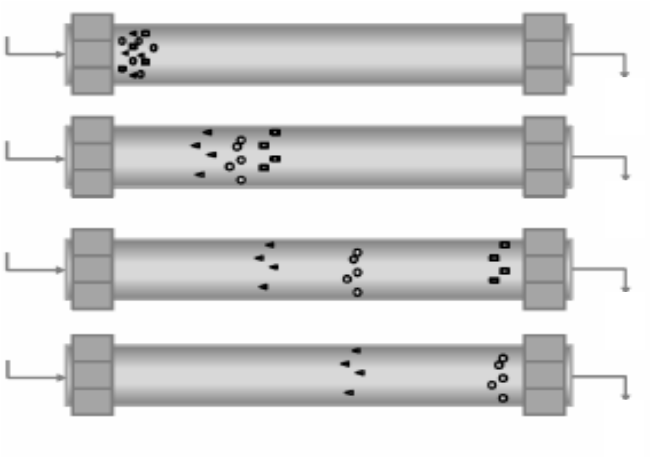


Figure 29 Separation of three compounds on a HPLC column. Each of the compounds within a mixture will interact with the stationary phase differently, eluting at different retention times according to their polarity.

1.11.3 HPLC Stability Indicating Method

HPLC is used for both quantitative and qualitative analysis of pharmaceuticals and is the preferred technique for determining BLA stability. To ensure the safety, efficacy, and quality of a BLA as well as to establish its stability and determine its shelf-life and optimal storage conditions, a HPLC SIM is required (157,158).

A SIM is a quantitative analytical method that ''is based on the characteristic structural, chemical, or biological properties of each active ingredient of a drug product (BLA and BLI)'' and ''will distinguish each active ingredient from its degradation product so that the BLA and BLI concentration can be accurately measured''. The USP <1225> (Validation of Compendial Procedures), FDA (Analytical Procedures and Method Validation) and, ICH (Validation of Analytical Procedures (Q2(R1))) guidelines exemplify the procedures required for developing a SIM that is specific to the API and can separate the API from its degradation products and/or excipients.

1.12 AIM

Despite the theoretical advantages of PI and CI, a global practice shift toward P/CI antibiotic administration has not taken place. This can be attributed to the preconceptions that they are more complicated and can increase staff workload (**Chapter 2**). These concerns are accompanied by uncertainty surrounding infusion solution stability due to changes in the drug stability after reconstitution and dilution (**Chapter 3** and **Chapter 4**).

This thesis addresses literature, practice and laboratory-based research regarding P/CI BLAs. The overarching aim is to optimise antibiotic therapy by determining the feasibility of differential dosing antibiotic regimens for inpatient and outpatient use.

1.12.1 OBJECTIVES

To achieve this aim, the specific objectives of this research include:

Literature-Based Research

- Conducting a brief historical review of the literature that formed the foundation of present understanding regarding differential antibiotic dosing regimens (**Chapter 2**)
- Conducting a contemporary review of the ever-growing body of literature referring to differential antibiotic dosing regimens (**Chapter 2**)
- Systematically reviewing the literature to critically compare clinical outcomes of II vs P/CI BLAs (**Chapter 2** and **Chapter 3**)

Practice-Based Research

- Establishing the challenges encountered regarding BLA therapy by assessing nurse's knowledge, perceptions, comfort, and experience in relation to P/CI (**Chapter 2**)
- Conducting a retrospective study of patients that received P/CI BLA therapy to establish BLA use in practice (**Chapter 2**)

Laboratory-Based Research

- Developing and validating a HPLC-SIM for piperacillin-tazobactam and amoxicillin-clavulanic acid in compliance with ICH guidelines (**Chapter 3** and **Chapter 4**).

- Designing and conducting a stability study to evaluate the physical and chemical stability of piperacillin-tazobactam and amoxicillin-clavulanic acid (Chapter 3 and Chapter 4).
- Determining the shelf life of piperacillin-tazobactam and amoxicillin-clavulanic acid after reconstitution and dilution (Chapter 3 and Chapter 4) when:
    - Using diluents used in practice.
    - Exposing infusion solutions to in-use conditions.
    - Determining stability in appropriate infusion containers.

CHAPTER 2

BETA-LACTAM ANTIBIOTIC USE IN PRACTICE

Publications

Fawaz S, Barton S, Whitney L, Swinden J, Nabhani-Gebara S. Stability of meropenem after reconstitution for administration by prolonged infusion. Hospital pharmacy. 2019 Jun;54(3):190-6.

Fawaz S, Barton S, Whitney L, Nabhani-Gebara S. Differential antibiotic dosing in critical care: survey on nurses' knowledge, perceptions and experience. JAC-Antimicrobial Resistance. 2020 Dec;2(4).

2.1 Beta-Lactam Use in Practice

Clinically, beta-lactam antibiotics (BLAs) are the most widely prescribed antibacterial agents in the infectious disease armamentarium as they are efficacious and commonly well tolerated. Since the discovery of benzylpenicillin in the 1920s, many novel penicillin derivatives and beta-lactam (BL) classes have been discovered either to broaden the spectrum of activity or to address the occurrence of resistance that have arisen in specific bacterial populations (133).

With the present absence of novel antibiotics to treat the ever-evolving emergence of multi-drug resistant (MDR) bacteria, innovative strategies that improve the ''effectiveness'' of currently available BLAs are essential. Employing principles such as optimising the pharmacokinetics/pharmacodynamics (PKs/PDs) of currently utilised agents is one of the few strategies left to effectively treat common infections. Pharmacokinetically and pharmacodynamically, the BLA class encompasses numerous compounds hence, variability in PK parameters (such as volume of distribution, half-life, and drug clearance) and PD parameters (minimal inhibitory concentration (MIC) breakpoints of infecting pathogen) certainly exist. Hence, PK and PD measurements are linked to form the PK/PD index (Section 1.3.3.4) that best correlates with antibiotic activity (159).

As previously highlighted in **Chapter 1**, the index that correlates with optimal outcomes involves extending the BLAs infusion duration to increase the time (T) where antibiotic concentrations remain above the MIC (T > MIC). Thus, the goal of therapy is to maximise T > MIC as a percentage of the dosing interval (160). Currently, with the approved intermittent infusion (II) regimen there is a wide variability for attaining PK/PD targets across different patient populations and susceptible pathogens. Retrospective clinical data indicate that patients benefit from higher concentration as well as longer BLA exposures than those expressed in *vitro* and in *vivo* clinical experiments (161,162).

As a strategy, the PD concept of prolonging a BLA's administration for the entire dosing interval (via CI) or extending its infusion time for 40-70% of the dosing interval (via PI) depending on the BL class, was previously thought to be an esoteric topic without practical applicability or clinical utility. However, in recent years, differential dosing has gained

popularity, where it is now considered essential for optimising therapy and is a core component of effective antimicrobial stewardship (AMS) and patient care (163). The PD characteristic of BLAs has fuelled interest in studies that assess and compare the PK, PD, and clinical effects of BLAs when administered via P/CI or II.

Although implementing P/CIs is encouraged for the treatment of MDR pathogens, scarce guidance exists for clinicians on the logistics of employing this strategy into the rapidly evolving changes in healthcare practice. Even though the use of P/CI is on the rise, especially in critical care, it is not well known to what extent and there is no gauge of the workforce readiness to support its widespread use. To aid in overcoming these challenges, a mixed method approach was adopted to gain a wider understanding of BLA use in practice.

The overall aim of this chapter is to provide a snapshot of BLAs use in practice and evaluate the clinical benefits and implications associated with P/CI BLAs. To provide an in-depth evaluation of current BLA utilisation, this chapter is divided into four subchapters:

- The first subchapter involves reviewing the literature that compares the clinical efficacy of BLA administration via P/CI vs II.

- The second subchapter includes systematic reviews of two BLAs to compare P/CI and II dosing regimens for clinical outcomes including clinical cure, microbiological cure, mortality, the length of hospital stays and adverse events.

- The third subchapter describes a retrospective cohort study that provides insight of how BLAs are being used in critical care wards at a tertiary care centre.

- The fourth subchapter is a survey of critical care nurses at a tertiary centre and provides an insight into the experiences and perceptions of nurses to aid in supporting the wider use of BLAs via P/CI.

2.2 Literature Review

2.2.1 Historical Review

It is important to briefly review significant nonclinical literature that formed the foundation of present understanding regarding differential BLA dosing regimens (160). Over 70 years ago, Schimdt *et al*., in 1949 and Eagle *et al*., in 1953 investigated the influence of the dosage regimen on the therapeutic activity of benzylpenicillin in various animal models. These initial studies demonstrated that continuous or regular dosing of benzylpenicillin resulted in a more rapid cure of infected animals when compared to less frequent or infrequent dosing (i.e., once or twice daily) (164,165). These findings were later supported by animal and *in vitro* studies conducted by Bakker-Woudenberg *et al*., in 1984 and Craig and Ebert in 1992, where consistent superior effects of CI benzylpenicillin were observed in either immunosuppressed or venom treated animals (166,167). However, although these findings supported potential advantages of P/CI, at this point superiority of these dosing regimens over conventional II had yet to be realised (160).

Through the 1990s, numerous clinical studies that assessed the efficacy of P/CI were conducted. Of the few randomised controlled trials (RCTs) conducted (n = 14), the majority only assessed pharmacologic endpoints (n = 12). Two RCTs reported patient outcomes. In 1979, Bodey *et al*., observed superiority in clinical outcome in the treatment of febrile episodes in cancer patients when cefamandole was administered via CI compared to II (168). In contrary, no statistically significant differences regarding clinical outcomes were observed in favour of CI in the RCT conducted in 1983 by Lagast *et al*., that compared clinical outcomes of CI and II cefoperazone in 45 patients with gram negative bacillary septicaemia (169).

Considering the differential dosing rationale and the proof of concept given data accumulated from animal and patient studies, the lack of robust, reliable clinical evidence delayed the incorporation of P/CI into traditional clinical practice. However, the increase of MDR bacteria resulted in substantial renewed interest in BLAs' PDs.

2.2.2 Contemporary Review

Within the last two decades there has been an ever-growing body of literature including non-clinical experiments, clinical studies, clinical trials, systematic reviews, and meta-

analyses supporting the correlation of PD parameters and antibiotic efficacy. Numerous studies carried out to compare traditional II with P/CI BLAs have demonstrated significant differences between the two dosing regimens (90,170–173).

Although PK/PD considerations are complicated and require subgrouping of patients by many factors, studies have found that optimal clinical outcomes of infections occur when PK/PD targets are attained, which is associated with maximal BLA activity. To predict maximal bactericidal effect of existing BLAs, clinicians and scientists have adopted administration under the guidance of PK/PD models.

2.2.2.1 Monte Carlo Simulations

A key contribution to the field, is the use of data maximisation strategies, such as computerised simulation that use probability models, to lessen the need for costly, complicated clinical trials. The best-known and most widely used strategy is the Monte Carlo simulation (MCS) (160). MCSs estimate antibiotic exposure thresholds associated with optimal bactericidal activity by combining PK and microbiological data to predict the likelihood an antibiotic regimen will achieve a therapeutic target (174).

MCSs incorporate the variability in PKs among a sample population when predicting antibiotic exposure thresholds and calculates the probability for obtaining a target antibiotic exposure for a range of MICs an organism can have to a particular antibiotic agent. For example, if a group of patients are to receive an antibiotic, it is expected that there will be variability between patients regarding the drug concentration profiles, peak drug concentrations and drug clearance.

MCSs strongly favour P/CI administration of BLAs, especially for patients with augmented renal function or when treating bacteria whose MIC is close to or higher than the administered BLA breakpoint. However, to implement these interventions into practice, clinical proof of PK/PD target achievement translating to overall clinical benefits needs to be observed. Although MCS are not sufficient to replace clinical trials, the simulations present the greatest likelihood of treatment success and are often used to guide clinical practice, when clinical data is not available or impractical (175,176).

2.2.2.2 Comparative Clinical Trials

Many trials have been performed to establish superiority of BLA dosing regimens (80,86,180–189,90,190–193,162,171–173,177–179). Numerous studies that represent various patient populations and susceptible pathogens have found that P/CIs were associated with improved clinical outcomes such as higher clinical cure/improvement and lower mortality rates. Whereas some studies found no significant difference between the dosing regimens (Table 4).

Table 4 Clinical trials comparing the clinical outcomes and efficacy of P/CI vs II BLA dosing

Study (Author, Year)	Patient population (No. of Patients/Disease)	Number of Patients		Microbiological Finding	Antibiotic	Antibiotic Dosing		Clinical Cure (%)		Mortality (%)		Major Findings
		P*/CI	II			P*/CI	II	P*/CI	II	P*/CI	II	
Hanes *et al.*, 2000 (86)	32/Nosocomial Pneumonia	17	15	G(-)B	Ceftazidime	60mg/kg/day	2g/q8h	56	71	NR	NR	No difference found between dosing regimens; thus, both are adequate treatment methods.
Nicolau *et al.*, 2001 (177)	35/Nosocomial Pneumonia	17	18	G(+)B & G(-)B	Ceftazidime	3g/day	2g/q8h	94	81	0	0	No difference found between dosing regimens. Microbiological cure (CI = 76% vs II = 80%)
Grant *et al.*, 2002 (178)	98/Patients with Mixed Infections	47	51	G(+)B & G(-)B	Piperacillin-Tazobactam	8-12g/day	3-4g/q6-8h	94	82	2.1	9.8	CI provided equivalent clinical and microbiologic cure to II. CI is a cost-effective alternative to II.
Lubasch *et al.*, 2003 (194)	81/COPD Exacerbation	41	40	G(-)B	Ceftazidime	4g/day	2g/q8h	90.2	90	NR	NR	CI was found to be clinically and bacteriologically as effective as II.
Rafati *et al.*, 2006 (172)	40/Septic Critically Ill Patients	20	20	G(-)B	Piperacillin	8g/day	3g/q6h	75	70	25	30	Clinical efficacy of CI is superior and reduces severity of illness resulting in clinical cure.

Lau *et al.*, 2006 (179)	262/Abdominal Infection Patients	81	86	G(+)B & G(-)B	Piperacillin-Tazobactam	12g/day	3g/q6h	86	88	0.8	2.3	No difference between dosing regimens. CI are a reasonable alternate mode of administration.
Lorente *et al.*, 2006 (80)	89/Patients with VAP	42	47	G(-)B	Meropenem	4g/day	1g/q6h	90	60	NR	NR	CI meropenem resulted in a significantly higher clinical cure rate than traditional II.
Lorente *et al.*, 2007 (90)	121/Patients with VAP	56	65	G(-)B	Ceftazidime	4g/day	2g/q12h	89	52	NR	NR	Ceftazidime administered via CI had greater clinical efficacy than II.
Roberts *et al.*, 2007 (180)	57/Septic Critically Ill Patients	29	28	G(+)B & G(-)B	Ceftriaxone	2g/day	2g/q24h	45	18	NR	NR	CI resulted in significantly greater clinical and bacteriological cure rates compared with II.
Sakka *et al.*, 2007 (181)	20/Nosocomial Pneumonia	10	10	G(+)B & G(-)B	Imipenem-Cilastatin	2g/day	1g/q8h	NR	NR	5	10	No significant difference between the two dosing regimens in terms of mortality.
Van Zanten *et al.*, 2007 (182)	93/COPD Exacerbation	47	46	G(+)B & G(-)B	Cefotaxime	2g/day	1g/q8h	93	93	NR	NR	CI was found to be equally effective compared with standard II.

Lodise *et al.*, 2007 (162)	194/Variable *P.aeruginosa*	*102	92	*P.aeruginosa*	Piperacillin-Tazobactam	*3.375g/q8h 4-h PI	3.375g/q6h 30 min II	*NR	NR	*12.2	13	No difference in baseline clinical characteristics were noted between the two dosing regimens.
Itabashi *et al.*, 2007 (183)	42/Severe Pneumonia	*18	24	G(+)B & G(-)B	Meropenem	*500mg/q12h 4-h PI	500mg/q12h 1-h II	*NR	NR	*5.6	37.5	Prolongation of meropenem infusion time is beneficial in terms of clinical efficacy.
Lorente *et al.*, 2009 (173)	83/Patients with VAP	37	46	G(-)B	Piperacillin-Tazobactam	16g/day	4g/q6h	89.2	56.2	21	30.4	Higher clinical efficacy achieved by CI. Higher dose reached target conc for pathogens.
Patel *et al.*, 2009 (184)	129/Mixed Infections	*70	59	G(-)B	Piperacillin-Tazobactam	*3.375g/q8h 4-h PI	3.375g/q6h 30 min II	*NR	NR	*5.7	8.5	PI yielded similar clinical outcomes compared to conventional II.
Wang 2009 (185)	30/Patients with HAP	*15	15	*Acinetobacter baumannii*	Meropenem	*500mg/q6h 3-h PI	1gmg/q8h 1-h II	*100	100	0	0	PI meropenem is a cost-effective approach and is equally clinically effective to II.
Chytra *et al.*, 2012 (186)	240/Critically Ill Patients	120	120	G(+)B & G(-)B	Meropenem	4g/day	2g/q8h	83	75	NR	NR	CI achieved significantly greater microbiological cure rates (CI = 90.6% vs II = 78.4%)
Lee *et al.*, 2012 (187)	148/ICU Patients	*68	80	G(-)B	Piperacillin-Tazobactam	*3.375g/q8h 4-h PI	2.25g/q6h 30 min II	*81	62	*19.1	37.5	Results suggest improved 30-day mortality in ICU patients treated via PI vs CI.

Laterre *et al.*, 2012 (188)	28/Critically Ill Patients	14	14	G(-)B	Temocillin	6g/day	2g/q8h	93	79	NR	NR	No significant difference in clinical outcomes however CI achieved higher clinical cure rates.
Lu *et al.*, 2013 (189)	50/Patients with HAP	*25	25	G(-)B	Piperacillin-Tazobactam	4.5g/q6h 3-h PI	4.5g/q6h 30 min II	*88	80	*NR	NR	PI of piperacillin-tazobactam for G(-)B bacteria provide stable plasma concentration.
Jamal *et al.*, 2015 (171)	16/ ICU Patients	8	8	G(-)B	Piperacillin-Tazobactam	9g/day	2.25g/q6h 30 min II	75	75	0	0	CI is advantageous as it allows achievement of rapid and consistent concentrations.
Cortina *et al.*, 2016 (190)	78/ Variable *P.aeruginosa*	40	38	*P.aeruginosa*	Piperacillin-Tazobactam	9g/day	4.5g/q8h 30 min II	50	47.4	0	2.6	No difference in efficacy between CI & II. Data indicates better performance of II than CI.
Bao *et al.*, 2017 (191)	50/Patients with HAP	*25	25	G(-)B	Piperacillin-Tazobactam	4.5g/q6h 3-h PI	4.5g/q6h 30 min II	88	80	0	0	Dosing regimen had no impact on adequacy of treatment and PI is as effective as II.
Fan *et al.*, 2017 (192)	367/ ICU Patients	*182	185	G(-)B	Piperacillin-Tazobactam	4.5g/q8-12h 4-h PI	4.5g/q8-12h 30 min II	NR	NR	11.5	15.6	No significant difference between dosing regimens in terms of mortality rate.

Zhao *et al*., 2017 (193)	50/Patients with Severe Sepsis	25	25	G(-)B	Meropenem	3g/day	1g/q8h	64	56	28	32	Cis were associated with superior bacteriological efficacy and shorter treatment duration.

CI = continuous infusion, COPD = chronic obstructive pulmonary disease, G(-)B = Gram negative bacteria, G(+)B = Gram positive bacteria, HAP = hospital acquired pneumonia, ICU = intensive care unit, II = intermittent infusion, NR = not recorded, PI = prolonged infusion and , VAP = ventilator acquired pneumonia. An ‘’*’’ indicates that the study investigated antibiotic administration via a prolonged infusion.

There is a strong scientific basis and an undisputed agreement among the majority of trials that P/CI is clinically superior compared to II. P/CI has shown optimised antibiotic PD profiles which have resulted in: BLA concentration maintenance above the MIC of the infecting pathogen for a longer period, reduction in total daily dose of drug required, reduced time to eradicating infection and a reduction in the formation of resistant bacteria (37,77).

The accumulating clinical evidence is not yet conclusive but suggests that P/CI as a strategy may be beneficial to certain patient populations with pathophysiological changes and altered PK parameters (e.g., critically ill patients). To counteract treatment failures and reduce antibiotic susceptibility in these patient populations, it is suggested that antibiotic dose optimisation should be employed. Unfortunately, published trials do not provide clear guidance regarding whether a conventional II or interventional P/CI schedule is more beneficial in terms of clinical outcomes.

Although many comparative studies favour the use of CI, rigorous analysis of clinical evidence found limitations and flaws associated with most of the conducted trials. Significant precluding elements include non-optimal study designs, highly heterogeneous patient populations, differing severity of illnesses, inconsistent PK/PD analysis and, inconsistent BLA dosages in different arms (Figure 30) (80,86,180–189,90,190–193,162,171–173,177–179).

Another limitation is the lack of significance in the published trials which is possibly due to the small sample sizes and heterogeneous patient populations used to assess the effect of CI vs II. Also, most studies are single centre in design. Only two studies were conducted as a multicentre study (179,194). The need for studies that are multicentre in nature should be accentuated as the enrolment of patients from different regions and countries will provide a stronger basis for subsequent generalisation to an extent of a possible global practice shift (195).

Most of the studies (n = 22) conducted stated that there is a need for large scale, prospective, multinational clinical studies to ascertain whether the potential benefits of CI BLAs indeed translate into optimised clinical outcomes compared to II. Large- scale, adequately designed RCTs investigating CI vs II BLAs are warranted to provide clear guidance on which dosing schedule is more beneficial for all patients. Such studies should account for

factors including enrolling homogeneous patient populations, giving equal daily antibiotic doses to both the CI and II group and performing concurrent PK/PD analysis (195)

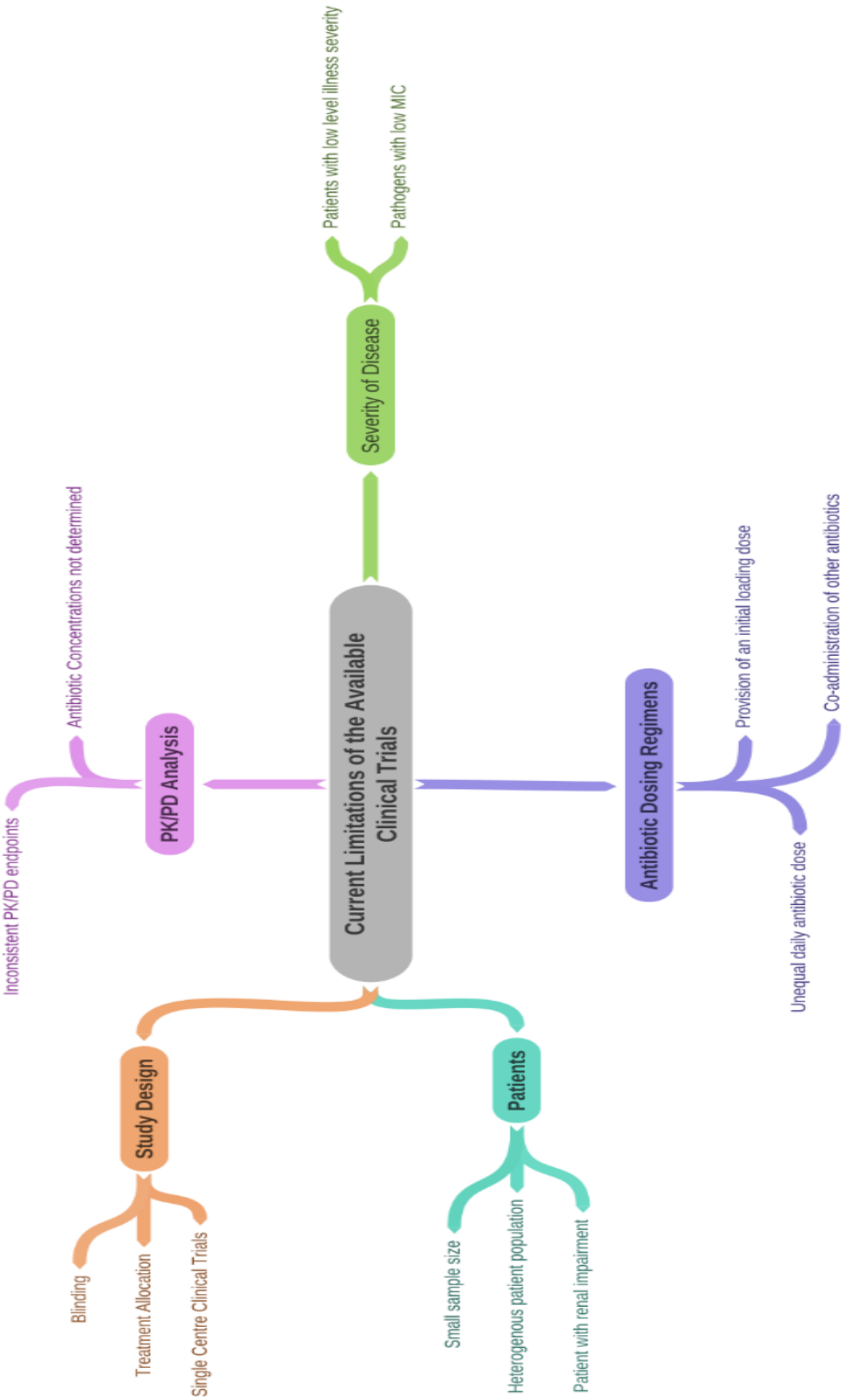


Figure 30 Mind map summarising current limitations and flaws associated with the available clinical trials [192].

A few studies have managed to overcome the above-mentioned challenges and limitation. Trials including the Beta-Lactam InfusioN Group (BLING) and Beta-Lactam Infusion in Severe Sepsis (BLISS) were specifically designed to overcome the criticisms of prior studies. Collectively, these represent the least heterogeneous and highest quality evidence available to date, demonstrating better attainment of PK/PD targets as well as higher clinical cure rates in the P/CI arm (196).

The BLING trial, a prospective, multicentre, RCT was conducted to establish whether CI BLAs offers more advantages to patients compared with II. A total of 60 patients were enrolled. PK findings demonstrated that CI achieved higher antibiotic plasma concentration (CI = 82% vs II = 29%, p = 0.001), hence supporting the concept of PK/PD superiority correlated with CI. Other results obtained showed CI superiority in terms of higher clinical cure (CI = 70% vs II = 43%, p = 0.037) and improved survival to hospital discharge (CI = 90% vs II = 80%, p = 0.47) (197).

Based on the findings from the BLING trial, a second study, BLING II, was designed. BLING II is a phase II, multicentre, RCT, designed with more rigorous and stringent methods, to determine the PK parameter of attaining plasma antibiotic concentrations above the MIC of infecting pathogen between CI and II. A total of 432 patients were enrolled. Results obtained from this trial showed that there was no difference in clinical outcomes of BLA administration between CI and II in all endpoints evaluated. This phase II trial concluded that ''Given the simplicity of an II, this should be the preferred method for BLA administration'' (195,198).

Contrasting results obtained from the two BLING trials led to the design of a phase III trial, BLING III. Currently, BLING III is ongoing and will recruit 7000 patients treated with one of two BLAs, piperacillin-tazobactam and meropenem, from 70 ICUs around the world (199).

The BLISS trial, a prospective, two centre RCT of CI vs II BLAs was conducted to determine if CI is associated with better clinical and PK/PD outcomes compared to II. A total of 140 participants were enrolled (70 allocated to CI and 70 allocated to II). Results demonstrated that CI resulted in higher clinical cure (CI = 56% vs II = 34%, p = 0.011), less days on ventilator (CI = 14 days vs II = 22 days, p < 0.043) as well as better PK/PD target attainment compared to II (T > MIC: CI = 100% vs II = 70%, p < 0.001) (200). The findings of this study suggest that

CI of BLAs is beneficial especially for critically ill patients infected with less susceptible pathogens (201).

2.2.2.3 Overview of Comparative Systematic Reviews and Meta-Analyses

Systematic reviews (SRs) and meta-analyses (MAs) comparing P/CI and II are of high value when assessing the efficacy of BLA dosing strategies considering that many available primary studies lack significance and homogeneity (80,86,180–189,90,190–193,162,171–173,177–179). There are multiple SRs and MAs comparing different BLA dosing regimens. Table 5 displays an overview of the main SRs and MAs comparing P/CI and II.

2.2.2.3.1 Clinical Cure

Data obtained from these SRs and MAs favoured the use of P/CI as they offer improved clinical outcomes (202,203). Most of the SRs and MAs showed that overall superior clinical cure rates were achieved in the P/CI arm.

Subgroup analysis conducted in five SRs showed higher rates of clinical cure in critically ill patients (170,203–206), three of these studies observed improvements in clinical cure in non-critically ill patients receiving P/CI (203–205).

Five reviews did not observe a clinical cure benefit (206–210). It is noteworthy that four of these reviews were published in 2013 or earlier (206,208–210) and two reviews combined randomised and non-randomised data (206,207).

2.2.2.3.2 Microbiological Cure

Only five SR and MAs reported microbiological cure. These studies demonstrated a statistically significant benefit in patients receiving P/CI (202,205,207,211,212).

2.2.2.3.3 Mortality

The majority of SRs and MAs also demonstrate lower mortality in the P/CI arm, however five reviews conveyed no difference between the two dosing regimens as the confidence interval suggested similar outcomes or results in favour of II (202,204,206,209,210).

Table 5 Overview of systematic reviews and meta-analyses

Review (author, year), [Reference]	Patient Population	Infusion (P/CI)	Antibiotic/s	Included Studies	Outcome of Interest									Risk of Bias
					Mortality	Clinical Cure	Clinical Failure	MBC	Length of Stay	Adverse Events	Cost	EoRes	PK/PD Outcome	
Roberts *et al.*, 2009 (210)	HP	CI	BLAs	RCT	✓	✓	×	×	×	×	×	×	×	High
Tamma *et al.*, 2011 (209)	NS	PI	BLAs	RCT	✓	✓	×	×	×	✓	×	×	×	Low
Korbila *et al.*, 2013 (206)	NS	PI	CEPH	NRT, RT	✓	✓	×	×	×	✓	×	✓	×	High
Hassan *et al.*, 2013 (208)	NS	PI	BLAs	RCT	×	✓	×	×	×	×	×	×	×	High
Falagas *et al.*, 2013 (213)	NS	PI	CRBs, PIP-TAZ	ALL	✓	✓	×	×	✓	×	×	✓	×	High
Chant *et al.*, 2012 (214)	Critically Ill	PI	BLAs	NRT, RT	✓	×	✓	×	✓	×	×	×	×	Low
Teo *et al.*, 2014 (170)	HP	PI	PIP-TAZ	RCT, RS, PS	✓	✓	×	×	×	✓	×	×	×	High
Yang *et al.*, 2015 (207)	NS	PI	PIP-TAZ	RCT, RS, PS	✓	✓	×	✓	×	✓	×	×	×	Low
Yang *et al.*, 2016 (215)	NS	PI	PIP-TAZ	RCT, RS, PS	✓	✓	×	×	✓	×	✓	×	✓	Low
Roberts *et al.*, 2016 (203)	Septic	CI	BLAs	RCT	✓	✓	×	×	×	×	×	×	×	Low
Lal *et al.*, 2016 (202)	Pneumonia	PI	BLAs	RCT, NRT, RS	✓	✓	×	✓	×	✓	×	×	×	Low
Lee *et al.*, 2018 (204)	Critically Ill	CI	BLAs	RCT	✓	✓	×	×	×	×	×	×	✓	High
Yu *et al.*, 2018 (211)	Critically Ill	PI	MERM	RCT, OS	✓	✓	×	×	✓	✓	×	×	×	Low
Vardakas *et al.*, 2018 (216)	Septic	PI	BLAs	RCT	✓	✓	×	×	×	✓	×	✓	×	Low

Rhodes *et al*., 2018 (205)	Critically Ill	PI	PIP-TAZ	RCT, OS, RS, QS	✓	✓	✗	✓	✗	✗	✗	✗	✗	Low
Chen *et al*., 2020 (217)	NS	CI	MERM	RCT, PS	✓	✓	✗	✓	✗	✓	✗	✗	✗	Low
Aboulatta *et al*., 2020 (218)	Critically Ill	P/CI	BLAs	RCT, OS	✓	✓	✗	✗	✓	✗	✗	✗	✗	Low
Fawaz *et al*., 2020 (212)	Critically Ill	P/CI	PIP-TAZ	ALL	✓	✓	✗	✓	✓	✓	✓	✓	✗	Low

BLA = beta-lactam antibiotic, CRB = carbapenems, CEPH = cephalosporins, HP = hospitalised patients, MBC = microbiological cure, MERM = meropenem, NRT = non-randomised trials, NS = not specified, OS = observational studies, PIP-TAZ = piperacillin-tazobactam, PS = prospective studies, RCT = randomised control trials, RS = retrospective studies, RT = randomised trials.

Although most SRs and MAs conducted show favourable outcomes in terms of BLA administration via P/CI, considerable variability exists in the inclusion/exclusion criterions across studies, in terms of population, spectrum of infection (site, organism and severity), BLA, infusion protocol and outcomes. The variations among studies as well as differences in conclusions between studies make it difficult to elucidate a true effectiveness of P/CI BLAs.

Overall, this brief overview of the literature demonstrates a wealth of studies, both in terms of SRs and MAs as well as primary studies (Table 4 and Table 5). Despite this, this review supports the need for better conducted, definitive trials and SRs given variability in scope of the available studies. Well-designed RCTs that employ rigorous methods and specifically evaluate the proposed benefits of P/CI compared with II are necessary (Table 6).

Table 6 Recommendations for future studies

Studies	Recommendations for Future Studies
RCTs	To overcome reduced comparability among the studies available in the literature, recommendations for future RCTs to address include: • Clinical heterogeneity by which confounding factors including patient sample size, patient population and the severity of patient illness are accounted for. • Multicentre research to enrol a larger number of participants and improve the validity and generalisability of the findings. The results from these studies are likely to be more applicable to a variety of settings. • More rigorous study designs regarding the use of concurrent control groups and random assignment of treatments are crucial for valid conclusions about treatment effects.
SRs	To overcome the given variability in scope of published SRs and MAs, recommendations for future studies include: • Rigorous deconstruction of the research question at the onset of the reviewing process in terms of population, intervention, comparator, and outcome to ensure that the SR and MA remains tightly focused. This enhances the likelihood of generating clearer more objective answers to the research questions. • Clearly stating study characteristics and the quality of included studies to enable for evidence-informed cross-study comparisons will inform as well as encourage the reader to critically engage with studies and prioritise empirical evidence over pre-conceived knowledge (219).

2.3 Systematic Reviews on The Revival of Older Antibiotics via Differential Dosing Regimens to Fight Antibiotic Resistance

Antibiotic resistance (AR) significantly reduces the effectiveness of treating infectious diseases resulting in a dramatic increase in the rates of morbidity and mortality, thus has been deemed one of the greatest threats to human health globally (220). AR has been linked to antibiotic overuse and misuse through selection pressure (37). With the rapid approach towards a post-antibiotic era combined with a scarcity of new antibiotic agents, there is a growing need to optimise the use of previously and currently used antibiotics to treat infections, mandating that clinicians and researchers strive to maximise the utility of antibiotic therapy (210,220).

It has been demonstrated that the prevalence of antibiotic resistance traits can be reversed through decreased antibiotic consumption however this is dependent on the individual, the bacterial strain, and the mechanisms of resistance (37). Studies have demonstrated that if the selection pressure that is applied by the presence of an antibiotic is removed, the bacterial population can potentially revert to a population of bacteria that responds to antibiotics (221). In this context, one major step in optimising antibiotic therapy involves reconsidering and reintroducing active and available, previously used - 'forgotten' or 'less frequently prescribed' - antibiotics and enhance their use, thus, revaluating their efficacy and safety to optimise their therapy (220).

The latter is a growing area for reducing the development of antibiotic resistance, and it involves differential dosing regimens such as prolonged or continuous infusions of time-dependant antibiotics (37,76–80).

Current emphasis on clinical efficacy within healthcare settings involves ensuring that the practice of HCPs is based on knowledge derived from research rather than personal experience. SRs can provide an invaluable resource to encourage evidence-based practice among clinicians. These studies search, appraise, compare and compile information regarding frequent and infrequent outcomes of all evidence to provide a complete interpretation of research results. Two SRs were conducted to compare the clinical outcomes of differential dosing of ampicillin and temocillin. SRs are especially important for

previously used BLAs that perhaps may have re-gained susceptibility to pathogens due to the elimination of resistant plasmids (222).

2.3.1 Clinical Outcomes of Continuous Infusion Ampicillin, A Narrative and Systematic Review.

2.3.1.1 Introduction

Ampicillin, an extended spectrum penicillin, exhibits broad-spectrum activity against both gram-positive and gram-negative bacteria. In practice, ampicillin is administered via II where an adult dosage of 12g/day is administered in 6 equally divided doses over a 3-minute infusion. After II of ampicillin, peak serum levels are reached however serum and tissue concentrations rapidly decrease due to the short half-life of ampicillin. Ampicillin has no significant post-antibiotic effect, therefore, when concentrations drop lower than the MIC (T < MIC), bacterial growth resumes immediately, facilitating the development of resistance, especially when serum concentrations fall below the MIC threshold for longer than half of the dosing interval.

Ampicillin is mainly prescribed for the treatment of respiratory tract, urinary tract, and skin and tissue infections (223). Previously, it was widely used in clinical practice, thus, many organisms have acquired resistance to it (223). Although resistance against ampicillin has emerged predominantly among gram negative rods, it is still one of the first-line treatments for the few infections by ampicillin susceptible organisms (224). Destruction of ampicillin through the expression of pathogens BLEs has led to the use of ampicillin in combination with other antibiotics, such as aminoglycosides, or a BLI, such as sulbactam to improve its efficacy, extend its spectrum of activity and reduce the development of AR (223).

In recent past, the use of ampicillin has decreased for the treatment of infections that were previously sensitive to it, including UTI and enteric fever, due to bacterial resistance. The mechanism of resistance to ampicillin is carried by bacterial cells plasmids. With decreased antibiotic use, bacterial plasmids are lost in the bacterial population through natural selection resulting in antibiotics regaining sensitivity. Thus, the decrease in ampicillin use in practice has perhaps led to the re-emergence of susceptibility of pathogens to ampicillin due to the elimination of resistant plasmids.

A re-emergence of sensitivity, undisputed safety as well as availability at low cost favours the use of ampicillin over other antibiotics and offers an opportunity to evaluate alternative dosing regimens for the treatment of infections. One such strategy involves administration

via CI to maximise PK and PD properties and maintain ampicillin concentration above the MIC to improve microbiological and clinical outcomes.

At the time of ampicillin approval, decades ago, PK and PD principles were largely unknown, thus, recommendations for optimal usage were not identified. The optimisation of ampicillin therapy is a relatively unexplored area where further research is needed. Limited evidence is currently available on the clinical efficacy and safety of ampicillin by CI relative to conventional II. The aim of this section is to systematically review existing literature to establish the clinical benefits of CI ampicillin and appraise the strengths and the weaknesses of current evidence.

2.3.1.2 Methods

2.3.1.2.1 Literature Search

A systematic review of the literature was conducted; references for this review were acknowledged through searches on PubMed between 1998 to present in compliance with PRISMA guidelines. Retrieval of additional articles using supplementary approaches through other sources such as hand searching of journals, Google Scholar and checking reference lists of articles to identify additional text were applied. A full review of published studies was implemented addressing clinical outcome of CI ampicillin. The last search was run on the 16th of February 2021.

Search strategy used to retrieve studies relating to ampicillin is: (("beta-lactams" OR "beta lactam") AND ("anti-bacterial agents" OR ("anti-bacterial" AND "agents") OR "anti-bacterial agents" OR "antibiotics" OR "antibiotic") OR "beta-lactam antibiotics" OR "beta-lactam antibiotic" OR ampicillin OR ampicillin-sulbactam OR sulbactam-ampicillin OR Acimpil) AND ("Drug Administration Schedule" OR "Infusions, Intravenous" OR "continuous infusion" OR "extended infusion" OR "intermittent therapy" OR ((continuous OR bolus OR extended OR intermittent) AND (administration OR infusion OR dosing)) AND ("inpatient" OR "hospitalized" OR "hospitalised" OR "hospitalization" OR "hospitalisation" OR "Bacterial Infections").

2.3.1.2.2 Study Selection

Study eligibility criteria include the types of: a) studies, b) participants, c) interventions and d) outcome measures; these measures are presented in Table 7. Report eligibility criteria include publications written in English language, study status is published and inclusion of old and new data. Exclusion criteria include rejecting studies on: Pharmacoeconomics, non-human subjects, non-adult subjects and non-English studies. Systematic reviews and meta-analysis were also excluded.

Table 7 Showing eligibility criteria for study selection process.

	Eligibility Criteria
a) Studies	Prospective, randomised, controlled trials/studies comparing/evaluating clinical efficacy or clinical outcome of ampicillin administration via P/CI and/or II, written in English language, were included.
b) Participants	Hospitalised adult participants aged 18 and over, suffering from a bacterial infection and requiring treatment using ampicillin. Non-adult, non-human and non-hospitalised patient studies were excluded.
c) Interventions	Studies comparing the beneficial and harmful/limiting effects of P/CI and/or II. Infusions of all types (P/CI and/or II), dose and regimen were adequate for the review. Pharmacoeconomics studies were excluded.
d) Outcome measures	All studies were eligible if specifically related to clinical outcome/efficacy of ampicillin dosing regimens. All outcomes were included to reduce risk of bias because of selective reporting.

P/CI= prolonged/continuous infusion; II= intermittent infusion

Articles were initially analysed by titles and abstracts for relevance and presence of inclusion criterion. Articles that were perceived as irrelevant were excluded; the full text of selected abstracts were acquired for further eligibility analysis. Screening of full texts obtained was conducted considering the clear inclusion and exclusion criterion. All relevant studies that described the clinical outcome of CI of ampicillin in English language literature were evaluated. Only studies that described clinical outcome were selected and tabulated.

2.3.1.2.3 Data analysis

A data extraction form was developed for this overview (based on Cochrane data extraction template). The data was extracted from included studies by one reviewer (Sarah Fawaz) and

the second and third reviewer checked and verified the relevance of the extracted information (Shereen Nabhani-Gebara and Stephen Barton). Variances in opinions were resolved by discussion between the three reviewers.

2.3.1.2.4 Risk of Bias and Study Quality Assessment

To determine the validity of eligible references two reviewers independently screened abstracts of articles that were found relevant based on their titles to identify if theoretically they met the inclusion criteria. The full texts of citations that passed the initial screening were retrieved and the pair of reviewers independently assessed each against the eligibility criteria. The reviewers compared results and disagreements about whether the inclusion criteria were met and were resolved through discussion with a third reviewer. 'Blinding' was not assessed as the interventions under study could not be blinded.

The methodological quality of included RCT's was assessed with the Jadad Scale (225) that evaluated the trial's randomisation, double blinding and reports of withdrawals and dropouts. An overall score of 0–5 points was assigned, where an overall score of three and above was regarded as adequate trial quality (212).

The Newcastle-Ottawa Scale is a quality assessment tool for selection, comparability and outcome assessment used to assess the quality of included observational studies (retrospective and prospective) (226). Studies scoring more than six or more stars are considered as being 'good quality' (212). Studies with a score from 4-6 are considered as 'high risk of bias', and those with score of 0-3 are considered 'very high risk of bias'.

2.3.1.3 Results

The search of PubMed and science direct provided 124 citations. Of these, 98 studies were excluded following the review of abstract as they did not meet the pre-defined inclusion criteria. Twenty-two articles were discarded after full article review due to the following reasons: non-human (n = 11), on children (n = 6) and non-English (n = 5). Five additional studies that met the inclusion criteria were acknowledged through checking the reference list of relevant studies. Six more studies were eliminated due to the focus being on pharmacoeconomic (n = 4) as well as kinetics and dose calculations (n = 2). Three studies

met the described inclusion criteria and were included in the systematic review. The article selection process is illustrated in **Figure 31**.

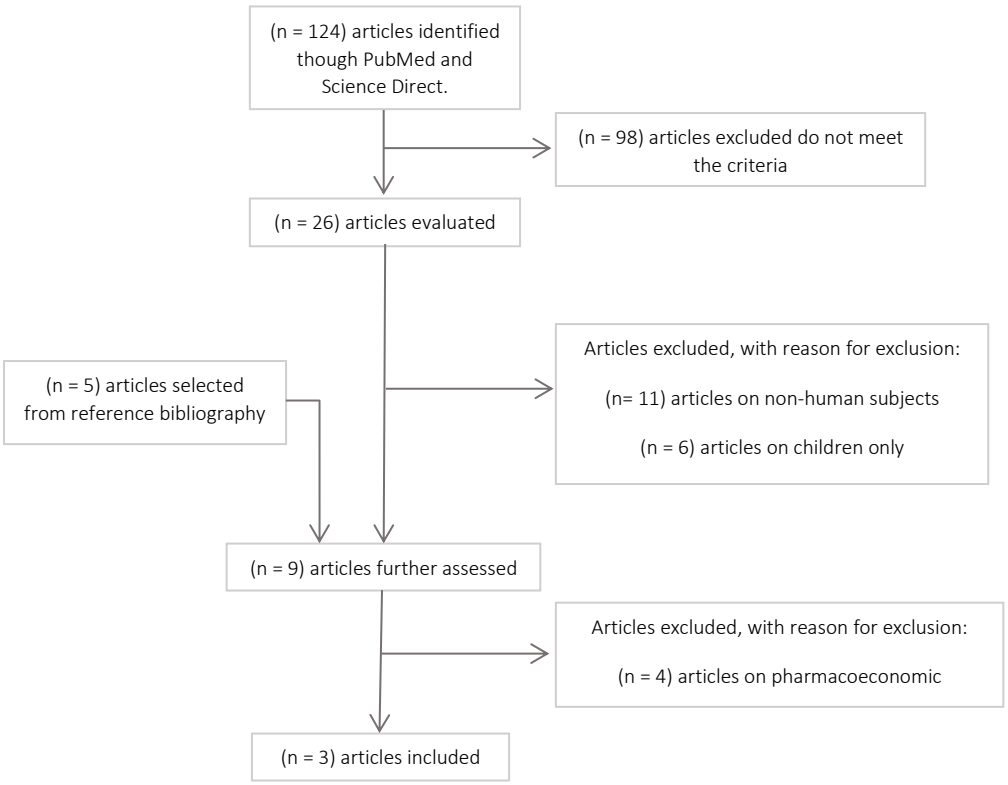


Figure 31 Identification, screening, and selection of articles for systematic review. Flow diagram illustrating the selection process for studies chosen for Ampicillin.

Demographic characteristics, drug regimen comparison and treatment results were extracted from included studies. **Table 8** summarises the included studies.

Table 8 Characteristics of studies comparing outcomes for continuous versus intermittent infusions of ampicillin

Study/ Country	Study Design/ Patient Population	Dosage	Clinical Cure (%)		Mortality (%)		Outcomes
			P/CI	II	P/CI	II	
Martin et al., 1998/ France (227)	Randomised control trail/ 16 CS Patient	CI (n=8) – 2g LD + 2g every 4h as a CI II (n=8) – 2g every 2h over 3 min II	100	100	0	0	CI and II were equally effective in maintaining conc in abdomen tissue. CI is recommended since it is easier to handle.
Ogawa *et al.*, 2013/ Japan (228)	Case report/ 1 Patient suffering from IE	Strain was highly resistant to gentamicin and sensitive to streptomycin. CI- 12g DD over a 24h infusion	100	NA	0	NA	CI is considered effective for CC when appropriate dose is set. However, when dosage is low time above the MIC become 0%.

Ogawa *et al*., 2014/ Japan (224)	Retrospective study/ 5 Hospitalized inpatients	Dosage regimen varied in each case. 8-12g DD over 24h CI. Syringes/IV bags changed every 6h	100	NA	0	NA	Serum and tissue conc above MIC of causative pathogen. Further study needed to address optimal dosing regimen.

CI= continuous infusion; II= intermittent infusion; MIC= minimal inhibition concentration; LD= loading dose; IV=intravenous; DD= daily dose; CS=colorectal surgery; IE= infective endocarditis; CC= clinical cure; NR= not reported.

2.3.1.3.1 Characteristics of the Included Studies

Three published articles examined ampicillin modes of administration. The primary outcome of the included studies was to assess the clinical efficacy of CI.

A randomised control trial conducted by Martin *et al.,* compared serum and tissue concentrations of ampicillin when administered via CI and II intraoperatively in 16 colorectal surgery patients. Enrolled patients had no history of allergies to BLAs and had normal hepatic and renal function. Patients were randomly assigned to one of two groups, CI or II (Table x). Blood samples were collected from patient prior to initiating BLA therapy, ten minutes after initiation (peak level), and at the end of treatment (trough level). Ampicillin concentrations were determined by HPLC. The authors observed no significant difference in serum and tissue concentrations for both dosing regimens as high levels of ampicillin-sulbactam were achieved in both CI and II groups. It was also determined that there was no significant modification in ampicillin to sulbactam concentration ratios by the method of administration. They concluded that both modes of therapy were equally effective in maintaining concentration in abdomen tissue (227).

An article by Ogawa *et al*., in 2013, reported a case of resistant infective endocarditis in a 73-year-old patient. Initially, the patient received empiric piperacillin at the dose of 2g twice daily, which was changed to flomoxef at the dose of 1g twice daily after piperacillin did not show any improvement. Vancomycin at the dose of 0.5g twice daily was initiated when flomoxef failed to show any effect. Blood test results detected *Enterococcus faecalis* and combined antibiotic therapy (vancomycin 1g twice daily and gentamicin 50g thrice daily) was initiated. Susceptibility test results obtained showed that the pathogen was resistant to routinely prescribed agents and antibiotic therapy was deescalated. The patient was switched to CI ampicillin monotherapy (**Table 8**), and it was found to be an effective therapeutic choice for treating endocarditis without the use of adjunctive aminoglycosides

(common treatment). CI was found to be an effective alternative administration method to traditional II, however, ampicillin concentrations need to be maintained above the MIC of the infecting organism. It was concluded that CI ampicillin is considered an acceptable method and is effective when an adequate dosage is set. Further study is needed on a larger group of patients to clarify the relationship between ampicillin dosages and serum concentration (228).

In 2014, Ogawa *et al.,* conducted a retrospective study that involved reviewing cases of 5 patients who were treated with CI ampicillin. Medical records of the hospitalised adult patients treated with CI and those who had one or more serum ampicillin concentration determinations were reviewed to evaluate the efficacy and safety of the dosing regimen. Although the dose varied in each case, ampicillin serum concentrations were maintained above the MIC for the causative pathogen in all patients and no significant complications were observed. The study confirmed CI is a safe and effective alternative to II and that further studies are needed to address optimal dosing regimen (224).

2.3.1.3.2 Study Quality Assessment

The methodological quality of the studies was assessed using the Jadad Scale and Newcastle-Ottawa Scale (Table 9 and Table 10). Although blinding was not described, randomisation was clearly described in the RCT by Martin *et al.*, and the clinical study was regarded to exhibit adequate trial quality with the score of 3/5 on the Jadad Scale (Table 9). Studies conducted by Ogawa *et al.*, in 2013 and 2014 were considered to have a 'high risk of bias' with scores of 5/9 on the Newcastle-Ottawa Scale (Table 10).

Table 9 Quality assessment of RCT included based on the Jadad Scale.

Quality assessment of RCT's	Martin *et al*., 1998 (227)
[(1)] Described as randomised	1
[(2)] Described as double blind	0
[(3)] Description of withdrawals	1
[(4)] Randomisation method described	1
[(5)] Double blinding method described	0
Score (-/5)	3/5

Randomisation:

Up to two points are given: [(1)] described as randomised (yes = 1) (no = 0) and [(4)] randomisation method described (yes = 1) (no = 0)

Double blinding:

Up to two points are given: [(2)] described as double blind (yes = 1) (no = 0) and [(5)] double blinding method described (yes = 1) (no = 0)

Reports of withdrawals and dropouts:

Up to one point is given: [(3)] Description of withdrawals (yes = 1) (no = 0)

RCT's = randomised control trials

Table 10 Quality assessment of observational studies based on Newcastle-Ottawa Scale

Study	Selection				Comparability	Outcome			Score
	A	B	C	D	E	F	G	H	
Ogawa 2013 (228) [(C)]	*	-	-	*	--	*	*	*	5*
Ogawa 2014 (224) [(R)]	*	-	-	*	--	*	*	*	5*

Selection:

*A: representation of the exposed cohort (yes = *) (no= -)*

*B: selection of non-exposed cohort (yes = *) (no= -)*

*C: ascertainment of exposure (yes = *) (no= -)*

*D: demonstration that outcome of interest was not present at start of study (yes = *) (no= -)*

Comparability:

*E: comparability of cohorts on the basis of the design or analysis [controls for: age, sex and marital status (yes = *) (no= -) and for other factors (yes = *) (no= -)]*

Outcome:

*F: assessment of outcome (yes = *) (no= -)*

*G: was follow up long enough for outcome to occur (yes = *) (no= -)*

*H: adequacy of follow up of cohorts (yes = *) (no= -)*

[(R)] = retrospective cohort study, [(C)] = case report

2.3.1.3.3 Clinical Cure

All three included studies reported clinical cure rates (224,227,228). 100% of patients that received CI ampicillin therapy in all studies were clinically cured from their infections.

2.3.1.3.4 Mortality

None of the included studies reported patient mortality rates as all patients included were successfully clinically cured (224,227,228).

2.3.1.3.5 Microbiological Cure

The 2013 case study by Ogawa *et al.,* reported that the patient achieved microbiological cure (228).

2.3.1.3.6 Adverse Events

Information relating to adverse events was only reported by Ogawa 2013; they reported that no side effects to ampicillin therapy were observed during the course of the treatment (228).

2.3.1.3.7 Length of Hospital Stay

None of the included studies reported length of hospital stay.

2.3.1.4 Discussion

The decreased use of ampicillin in recent years has resulted in ampicillin regaining efficacy against pathogens that had previously gained resistance to it, thus paving a way for renewed clinical use of ampicillin. However, indiscriminate use of ampicillin via inappropriate dosing regimens will impede its regained efficacy and facilitate the development of resistance. Therefore, a multidisciplinary approach is needed, where new solutions and strategies to re-develop the use of older antibiotics using modern standards and communicating these findings – bench to bedside – are required to address the major global threat of AR.

This is the first systematic review that assesses the clinical benefits of differential dosing parenteral ampicillin, where the primary outcome in the included studies assessed the clinical efficacy of CI ampicillin. Results obtained from this study show that CI ampicillin

resulted in excellent clinical and microbiological cure rates, no cases of mortality and no occurrences of adverse events.

The outcomes of the current study correlate and expand upon reviews previously published on the clinical efficacy of CI beta-lactams (210,213,229). A recent systematic review comparing CI and II piperacillin-tazobactam observed similar beneficial effects of administration via CI (212). Falagas *et al*., 2013 (213) and Vardakas *et al*., 2018 (216) found that there was a significant reduction in mortality rates among patients receiving P/CI. Roberts *et al*., 2016 (203) observed higher clinical rates and reduced mortality in P/CI patients and Lal *et al*., 2016 (202) found P/CI to reduce clinical failure rates (212).

Despite the positive findings from this study, there is a paucity of PK/PD information relating to older antibiotics that could potentially support dosing recommendations to optimise efficacy, minimise side effects, and address the emergence of resistance. More in-depth open access data on clinical studies relating to ampicillin are necessary to overcome issues with respect to stability and interventions in terms of dosing and schedule and dissemination of knowledge to healthcare professionals, academics, governments, and the public is vital.

Several observations encountered while reviewing this data led to reduced comparability among studies. First, clinical heterogeneity was present as selected studies studying clinical outcomes of CI ampicillin have confounding factors including patient sample size, study settings, and study design. Second, information regarding monotherapy and combination antibiotic therapy were not reported in the included studies. A limitation of this review is that a medical librarian was not involved in this study.

2.3.1.5 Conclusion

In conclusion, with the limited data available, the included studies demonstrated that CI ampicillin is associated with improved clinical outcomes to conventional dosing. There is an urgent need for approaches that stimulate coordinated reconsideration processes for previously used antibiotics, particularly regarding exposure-response relationships, to justify adequate dosing regimens. Updated knowledge from both academia and clinicians that involves re-analysis of old pharmacokinetic data is warranted for the revival of older agents to bear the current global challenge of AR.

2.3.2 Differential Dosing of Revived Temocillin in the Fight Against Antibiotic Resistance; A Systematic Review Comparing Clinical Outcomes of Intermittent and Continuous Infusion.

2.3.2.1 Introduction

Temocillin is a narrow spectrum BLA that was previously active against Gram-negative bacteria and is used to treat septicaemia, urinary tract, biliary tract, and respiratory tract infections. Temocillin was first introduced in 1981, however remained widely neglected due to the availability of other antibiotics that were also active against Gram-positive organisms and anaerobes (230). It has recently been reintroduced as an alternative therapy for problematic Gram-negative resistant pathogens with the increasing concern of emerging resistance (231,232).

After the discovery of temocillin in the 1980s, its use was soon after abandoned due to a lack of activity against Gram-positive pathogens. Although temocillin possesses a narrow spectrum of activity, it demonstrates remarkable stability to beta-lactamase hydrolysis which is currently recognised as an important bacteriological advantage as its use can spare the use of broad-spectrum agents (230,233). The scarce use of temocillin indicates low resistance rates therefore its reuse represents a promising strategy to fight AR (234).

Like all penicillin's, temocillin exhibits time-dependant microbiological activity, related to the time at which drug concentrations exceeds the MIC (T > MIC) for ~50% of the dosing interval (232). Currently in practice, temocillin is administered via II, 2g every 12 hours, where peak serum level is promptly attained, however, serum and tissue concentrations rapidly decrease due to temocillin's short half-life (8 hours) resulting in the concentration falling below the MIC. The most effective way to optimise exposure, particularly against resistant Gram-negative bacteria, is to prolong the infusion to maximise bactericidal exposure time. Thus, maximising the periods at which temocillin concentrations are maintained above the MIC by administering as a P/CI (188,235).

Currently in-practice first and second-line antibiotic treatments are limited or unavailable, leading to the uses of agents that are more toxic to the patient (36). Temocillin's spectrum of activity has previously been under estimated and seen as a disadvantage, however, this characteristic has turned to an advantage when used in targeted therapy, especially in the

current era of ever-increasing AR (231). Temocillin has an excellent safety/tolerability profile and chemically is relatively stable, rendering it potentially suitable for administration by P/CI (230). The aim of this study is to systematically review existing literature to compare the clinical outcomes of CI and II temocillin and appraise the strengths and the weaknesses of current evidence.

2.3.2.2 Methods

2.3.2.2.1 Literature Search

Studies included in this review were retrieved from PubMed (between 2008 to present) in compliance with PRISMA guidelines. Additional articles were acknowledged through other sources such as hand searching of journals, google scholar and checking reference lists of articles. The last search of the literature was conducted on the 16th of February 2021.

Search strategy used to retrieve studies relating to temocillin is: (("beta-lactams" OR "beta lactam") AND ("anti-bacterial agents" OR ("anti-bacterial" AND "agents") OR "anti-bacterial agents" OR "antibiotics" OR "antibiotic") OR "beta-lactam antibiotics" OR "beta-lactam antibiotic" OR temocillin OR Nagaban OR Timentin) AND ("Drug Administration Schedule" OR "Infusions, Intravenous" OR "continuous infusion" OR "extended infusion" OR "intermittent therapy" OR ((continuous OR bolus OR extended OR intermittent) AND (administration OR infusion OR dosing)) AND ("inpatient" OR "hospitalized" OR "hospitalised" OR "hospitalization" OR "hospitalisation" OR "Bacterial Infections" OR "sepsis" OR (("intensive" OR "critical" OR "acute") AND ("care" OR "unit" OR "illness")).

2.3.2.2.2 Study Selection

Study eligibility criteria include the types of a) studies, b) participants, c) interventions and d) outcome measures; these measures are presented in Table 11. Report eligibility criteria include publications written in English language, study status is published and inclusion of old and new data. Exclusion criteria include rejecting studies on: Pharmacoeconomics, non-human subjects, non-adult subjects and non-English studies. Systematic reviews and meta-analysis were also excluded.

Table 11 Showing eligibility criteria for study selection process.

	Eligibility Criteria
a) Studies	Prospective, randomised, controlled trials/studies comparing/evaluating clinical efficacy or clinical outcome of temocillin administration via P/CI and/or II were included.
b) Participants	Hospitalised adult participants aged 18 and over suffering from a bacterial infection and requiring treatment by the use temocillin. Non-adult, non-human and non-hospitalised patient studies were excluded.
c) Interventions	Studies comparing the beneficial and harmful/limiting effects of P/CI and/or II. Infusions of all types (P/CI and/or II), dose and regimen were adequate for the review. Pharmacoeconomics studies were excluded.
d) Outcome measures	All studies were eligible if specifically related to clinical outcome/efficacy of dosing regimens. All outcomes were included to reduce risk of bias because of selective reporting.

P/CI= prolonged/continuous infusion; II= intermittent infusion

Initially, articles were analysed by titles and abstracts for relevance in compliance with inclusion criterion. Articles perceived 'irrelevant' were excluded. The full texts of the selected abstracts were acquired for further eligibility analysis. Screening of full texts obtained was conducted considering the clear inclusion and exclusion criterion. All relevant studies that described the clinical outcome of CI of temocillin in English language literature were evaluated. Only studies that described clinical outcome were selected and tabulated.

2.3.2.2.3 Data analysis

A data extraction form was developed for this overview (based on Cochrane data extraction template). The data was extracted from included studies by one reviewer (S.F) and the second and third reviewer checked and verified the relevance of the extracted information (S.N-G and S.B). Variances in opinions were resolved by discussion between the three reviewers.

2.3.2.2.4 Risk of Bias and Study Quality Assessment

To determine the validity of eligible references two reviewers independently screened abstracts of articles that were found relevant based on their titles to identify if theoretically they met the inclusion criteria. The full texts of citations that passed the initial screening

were retrieved and the pair of reviewers independently assessed each against the eligibility criteria. The reviewers compared results and disagreements about whether the inclusion criteria were met and were resolved through discussion with a third reviewer. 'Blinding' was not assessed as the interventions under study could not be blinded.

The Newcastle-Ottawa Scale is a quality assessment tool for selection, comparability and outcome assessment used to assess the quality of included observational studies (retrospective and prospective) (226). Studies scoring more than six or more stars are considered as being 'good quality'. (212). Studies with a score from 4-6 are considered as 'high risk of bias', and those with score of 0-3 are considered 'very high risk of bias'.

2.3.2.3 Results

Two studies for Temocillin met the inclusion criteria and were included in this systematic review. The search of PubMed provided a total of 58 studies. Of these, 51 articles were discarded as after reviewing the abstracts it appeared that they noticeably did not meet the criteria. Two additional studies that met the inclusion criteria were identified through checking references of relevant studies. Five articles were discarded as after reviewing they were: on non-adult patients (n = 3) and non-human subjects (n = 2). The full texts of the remaining four citations were examined in more detail. Two of the four remaining studies were eliminated as the inclusion criteria were not met (studies on Pharmacoeconomics's and non-IV route of administration). Two studies met the described inclusion criteria and were included in the systematic review. Article selection process is illustrated in Figure 32.

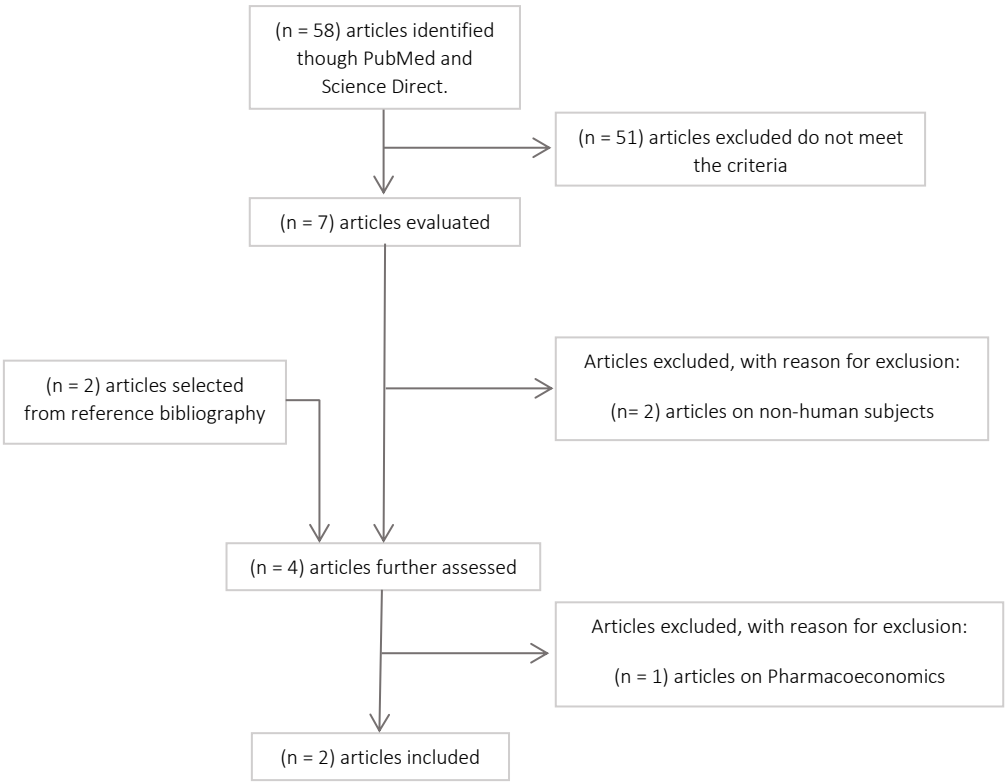


Figure 32 Identification, screening, and selection of articles for systematic review. Flow diagram illustrating the selection process for studies chosen for temocillin

Demographic characteristics, drug regimen comparison and treatment results were extracted from included studies. **Table 12** summarises the included studies.

Table 12 Characteristics of studies comparing outcomes for CI vs II of temocillin.

Study/ Country	Study Design/ Patient Population	Dosage	Clinical Cure (%)		Mortality (%)		Outcomes
			P/CI	II	P/CI	II	
De Jongh et al., 2008/ Netherlands (235)	Randomised prospective study/ 12 IC NP patients	CI (n=6) – 2g LD + 4g DD over 24h CI II (n=6) – 2g every 12hs over 30 min II	NR	NR	NR	NR	CI maintained drug conc above the MIC and simplified process. Further studies needed to provide info on clinical outcome.
Laterre *et al.*, 2015 Belgium	Prospective study/ 28 ICU patients	CI (n=14) – 2g LD +6g DD over 24h CI II (n=14)- 2g every 8hs over 30 min II	93	79	14	36	High clinical cure rates were obtained and a trend towards superiority was observed for patients in the CI vs the II group.

CI= continuous infusion; II= intermittent infusion; MIC= minimal inhibition concentration; LD= loading dose; DD= daily dose; ICU= intensive care unit; IC= intensive care; NP = nosocomial pneumonia, NR = not recorded

2.3.2.3.1 Characteristics of the Included Studies

Two published studies compared the two modes of administration: CI and II of temocillin.

A two-part study by De Jongh *et al.*, assessed both the clinical outcomes of CI temocillin compared to II (twice daily) and the stability of temocillin to determine the feasibility of CI administration. The clinical aspect of the study enrolled 12 patients (CI = 6 patients and II = 6 patients) that were similar in demographic and disease related characteristics. The clinical outcome of all patients was favourable with no temocillin-related adverse effects however, found that CI maintained drug concentration above the MIC and simplified the infusion process. The stability aspect of the study found that temocillin solutions maintained 98% of initial concentration for 24-hour when incubated at temperatures up to 37^{o}C. These results are an improvement over the published stability data in the European Pharmacopoeia. They concluded that further laboratory and clinical studies are needed to obtain information on antibiotic stability to determine feasibility of CI BLA administration and the utility of CI for clinical treatment to determine the efficacy of this dosing modality (**Table 12**) (235).

A study conducted in 2015 by Laterre *et al.*, researched the PK parameters of temocillin when administered by CI vs II. 28 patients were enrolled (CI = 14 patients and II = 14 patients) in this study. All patients were treated for either lower respiratory tract, intra-abdominal, blood stream or urinary tract infections and the MICs of isolated pathogens were determined. No adverse events occurred due to temocillin administration in both groups however, the clinical cure rate was higher in the CI arm (CI = 93% vs II = 79%) and mortality rate was also lower. In terms of PK, II adequately reaches the necessary serum antibiotic concentration to achieve %T>MIC. For patients with high PK variations or to cover strains against which temocillin would have higher MICs, CI is useful and practical alternative as it is associated with a higher %T>MIC without apparent toxicity or administration issues (**Table 12**) (188).

2.3.2.3.2 Study Quality Assessment

The methodological quality of the studies was assessed using the Newcastle-Ottawa Scale. Both included studies were 'good quality' with the scores of 7/9 on the Newcastle-Ottawa Scale (**Table 13**).

Table 13 Quality assessment of observational studies based on Newcastle-Ottawa Scale.

Study	Selection				Comparability	Outcome			Score
	A	B	C	D	E	F	G	H	
De Jongh 2008 (228) [P]	*	*	*	*	--	*	*	*	7*
Laterre 2015 (224) [P]	*	*	*	*	--	*	*	*	7*

Selection:

*A: representation of the exposed cohort (yes = *) (no= -)*

*B: selection of non-exposed cohort (yes = *) (no= -)*

*C: ascertainment of exposure (yes = *) (no= -)*

*D: demonstration that outcome of interest was not present at start of study (yes = *) (no= -)*

Comparability:

*E: comparability of cohorts on the basis of the design or analysis [controls for: age, sex and marital status (yes = *) (no= -) and for other factors (yes = *) (no= -)]*

Outcome:

*F: assessment of outcome (yes = *) (no= -)*

*G: was follow up long enough for outcome to occur (yes = *) (no= -)*

*H: adequacy of follow up of cohorts (yes = *) (no= -)*

[P] = prospective cohort study

2.3.2.3.3 Clinical Cure

Although the difference is not statistically significant, Laterre *et al*., reported superior clinical cure rates in patients receiving CI (CI = 93% and II = 79%) (188).

2.3.2.3.4 Mortality

Mortality was not reported in De Jongh *et al*'s., study (235). Laterre *et al.*, reported mortality rates for the patient population in each infusion group (CI = 14% and II = 36%), however, stated that ''No death was related to the primary infection treated with temocillin'' (188).

2.3.2.3.5 Microbiological Cure

None of the included studies reported findings on microbiological cure.

2.3.2.3.6 Adverse Events

Both studies reported that no temocillin-related adverse events were observed during treatment in any of the included patients (188,235).

2.3.2.3.7 Length of Hospital Stay

Both included studies did not report length of hospital stay, though, both reported duration of antibiotic therapy. Laterre *et al*., although not significant, the average duration of therapy was found to be lower in the II patients (6±2 days) compared with patients in the CI group (7±5 days) (188). The study by De Jongh *et al*., also observed no significant difference between the two study arms in terms of the duration of therapy (CI = 8.5 (6-12) and II = 8.8 (6-13) (235).

2.3.2.4 Discussion

This is the first SR that assesses and describes clinical outcomes of differential dosing parenteral temocillin. The present study suggests the CI is associated with improved clinical cure and mortality rates as well as no occurrences of adverse events.

The results from this study correlate with findings from previous reviews on CI and II BLAs. A recent SR and MA conducted by Aboulatta *et al*., in 2020, evaluated the effects of CI vs II BLAs and observed that P/CI resulted in a significantly lower mortality rate than for II (218). Findings from a SR and MA by Fawaz *et al*., 2020, comparing clinical outcomes of P/CI and II piperacillin-tazobactam demonstrated P/CI significantly improved clinical cure rates and reduced mortality and length of hospital stay (212). Also, in 2017, Lee *et al*., found that CI was associated with significantly improved clinical cure rates (204).

Although the interest of administering revived antibiotics by CI has been repeatedly advocated, the optimisation of temocillin is a relatively unexplored area where further research and support from both laboratory and clinical studies are needed to determine its feasibility. Temocillin was never developed using current standard, structured drug assessment and regulatory approval processes. Consequently, resurgent temocillin is being prescribed using limited knowledge generated at the time of its discovery. A better understanding of temocillin PK/PD is needed, including exposure-effect and exposure-emergence of resistance relationships to enable dose-finding approaches and optimising dosing regimens.

Some observations encountered while reviewing this data led to reduced comparability among studies. First, clinical heterogeneity was present as selected studies studying clinical outcomes of CI vs II temocillin have confounding factors including patient sample size, study

settings, and study design. Second, information regarding monotherapy and combination antibiotic therapy were not reported in the included studies. A limitation of this review is that a medical librarian was not involved in this study.

2.3.2.5 Conclusion

In conclusion, from the limited data available, the included studies demonstrated that CI temocillin is associated with improved clinical outcomes compared to conventional dosing. There is an urgent need for approaches that stimulate coordinated reconsideration processes for previously used antibiotics, particularly regarding exposure-response relationships, to justify adequate dosing regimens. Updated knowledge from both academia and clinicians that involves re-analysis of old pharmacokinetic data is warranted for the revival of older agents to bear the current global challenge of AR.

2.4 Retrospective Practice Review of Prolonged Infusion BLAs in Critical Care at Tertiary Centre

2.4.1 Introduction

Optimising BLA exposure in critically ill patients demonstrates a greater challenge due to highly variable, unpredictable and commonly sub-optimal BLA serum concentrations, potentially causing therapeutic failure and selection of resistant pathogens (236).

Current empirical dosing schedules have been derived from studies in healthy volunteers with normal physiology and are therefore not representative of different real-world infected patient populations (160). Dosing strategies that have been validated in patient populations that are non-critically ill fail to consider the substantial changes in organ function that occur with critical illness (237,238).

Antibiotic dosing concentrations will vary greatly within ICU patients with normal kidney function or renal failure as the pharmacokinetic target attainment is dependent on kidney function (236). Increased volume of distribution and augmented renal clearance of antibiotics is increasingly reported in critically ill patients which leads to lower initial and faster decreasing BLA serum levels.

Given the enhanced renal elimination reported in critically ill patients, antimicrobial dosing requires extensive consideration due to important clinical consequences as accurate and timely drug exposure is essential for clinical success (238).

Piperacillin-tazobactam and meropenem are widely used for empiric therapy in clinical practice and are often used in the treatment of MDR infections as they have proven efficacy in a wide range of bacterial infections. As other BLAs, the primary determinant of piperacillin-tazobactam and meropenem efficacy is the time at which free drug concentrations are maintained above the MIC (239). As previously investigated in **Section 2.1**, PK/PD studies have shown that their administration via P/CI significantly increases the likelihood of maintaining serum levels above the MIC when compared to conventional II (80,171,172,186,193,210).

It is uncertain if dose optimisation of antibiotic therapy, guided by PK and PD principles achieves desired clinical outcomes, however, in recent years, PI is increasingly used in ICU.

Therefore, the aim of this retrospective cohort study was to evaluate the efficacy of PI piperacillin-tazobactam and meropenem in ICU patients at a tertiary centre.

The main objectives of this study were to:

- Investigate the prescribing patterns (dose, indications, administration etc) of piperacillin-tazobactam and meropenem in critical care
- Investigate the clinical profile of patients receiving these antibiotics

2.4.2 Methods

2.4.2.1 Research setting, design, and study subjects

This retrospective cohort study was a single centre study investigating the use of P/CI piperacillin-tazobactam and meropenem at St Georges Hospital (SGH). Approval to conduct this 'audit of practice' was obtained from 'The Clinical Audit Committee' (audit registration number: CADB002442).

Eligibility criteria for patient inclusion are (1) patient is over the age of 18, (2) patient was admitted onto critical care ward and, (3) patient had received piperacillin-tazobactam or meropenem antibiotic therapy.

The clinical notes of patients admitted to SGH were reviewed and information regarding P/CI use in practice was extracted for evaluation. This study was conducted using an investigator developed data extraction sheet.

2.4.2.2 Data extraction instrument

The instrument was comprised of four sections: (1) Patient Details, (2) Susceptible Bacteria, (3) Agent Administered and (4) Outcomes (**Appendix 1**). The following sections included:

The first section of the data extraction form, **Patient Details**, requires the retrieval of information relating to the patients age, gender, and body weight. This section is essential and will provide a snapshot of demographic information regarding the included participants.

The microbiology section, **Susceptible Bacteria**, entails accumulating information relating to the patient's infection. Patients' records were examined to recover data relating to the source of infection, indication, whether cultures were taken, whether the pathogen was isolated and the diagnosis.

The '**Agent Administered**' section requires information regarding: the agent used (name and whether it was administered alone or in combination with other antibiotics), administration (dose, volume, frequency, infusion time) and duration of treatment.

The last section on instrument, '**Patient Outcomes**', involves retrieving data from standard clinical tests and observations associated with inflammation. Inflammatory marker levels, pre and post antibiotic treatment, were noted. Markers investigated include serum creatinine (SrCr) [normal SrCr range; male = 65.4 - 119.3 µM/L and female = 52.2 - 91.9 µM/L], C-reactive protein (CRP) [normal CRP = up to 10 mg/L for male and female] and white blood cell count (WBC) [normal WBC range = 4.5 - 11.5 × 10^9 count/L for male and female].

2.4.2.3 Ethical Considerations and Negotiation of Access

The main ethical issues were patients' anonymity and confidentiality. The names and addresses of patients were unrecorded making the collected data anonymous. Only information required for answering the research question/s were retrieved. Collected information was utilised for the intended purpose of the study and collected patient data were kept confidential.

2.4.2.4 Data extraction procedure

Prior to beginning data collection, the instrument was approved by consultant pharmacist at SGH. Patient data were retrieved from medical records and noted on to the predesigned data extraction instrument.

Data was extracted using the structured devised instrument by Sarah Fawaz who was familiarised with the electronic database at SGH. Data sources used were inpatient case files, pharmacy records and discharge letters; these medical records provide a detailed account of the patients, symptoms, diagnosis, prescribed medication/s and evolution of the disease.

2.4.2.5 Data collection and analysis of data

Data collection took place between 12 February and 31 March 2018. Data were computed and analysed using SPSS version 24.0 and Microsoft Excel 2012. This study uses descriptive statistics to evaluate the data collected.

2.4.2.6 Statistical analysis

Descriptive analysis of all extracted variables was carried out and data were statistically expressed as absolute or percentage frequencies and means ± standard deviation (SD) as appropriate.

2.4.3 Results

A total of 128 adult patients admitted to ICUs at SGH were identified through medical records as having received study medications, piperacillin-tazobactam and meropenem.

2.4.3.1 Patient Details

Information regarding identified patients' gender, age in years and weight in kilograms (kg) were retrieved from the patient's admission data (**Table 14**).

Table 14 Enrolled patient demographic characteristics (n = 128).

Gender	No. of Patients (%)
Male	83 (64.8)
Female	45 (35.2)
Age (years)	Mean ± SD (range)
All Patients	63 ± 14 (21 – 88)
Male	62.2 ± 15.2 (21 – 88)
Female	64.4 ± 11.4 (27 – 84)
Weight (kg)	Mean ± SD (range)
All Patients	78 ± 18.4 (50 – 140)
Male	81.9 ± 18.7 (50 – 140)
Female	71.1 ± 12.2 (53 – 119)

Kg = kilograms, SD = standard deviation

Of the 128 patients undergoing antibiotic treatment, 83 (64.8%) patients were male, and 45 (35.2%) patients were female. Overall, the average patient age was 63 years (male = 62.2 years and female = 64.4 years), and the overall average patient weight was 78 kg (male = 81.9 kg and female = 71.1 kg) (**Figure 33**).

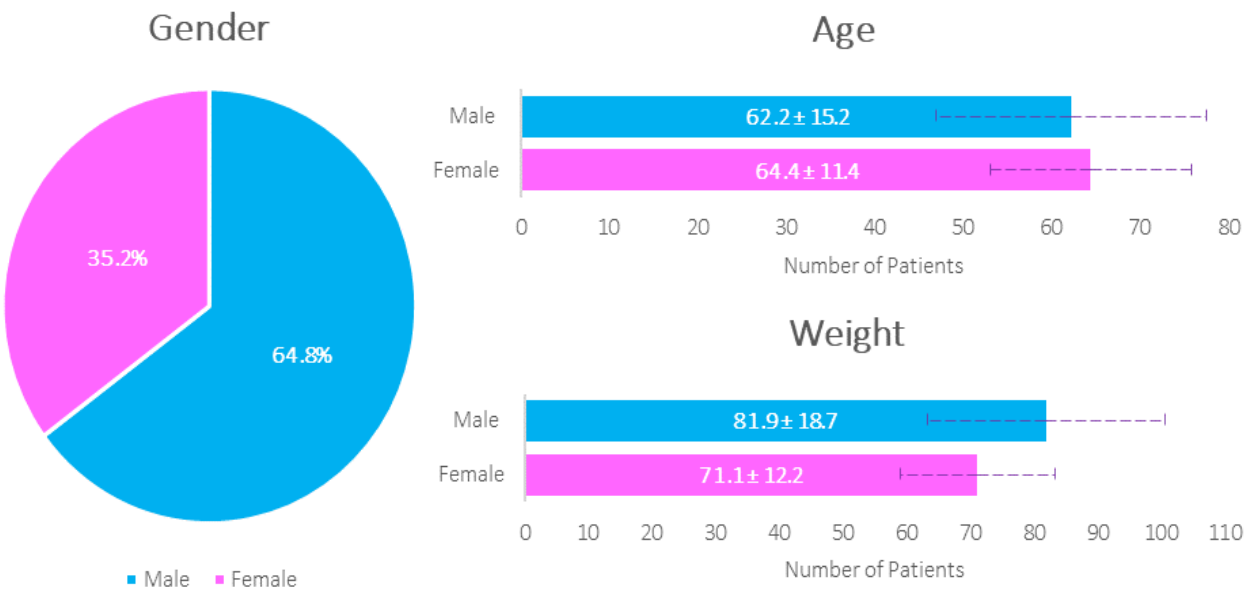


Figure 33 Representation of patients' demographic characteristics

2.4.3.2 Susceptible Bacteria

Table 15 summarises clinical characteristics regarding the study population.

Table 15 Source of infection, indication, isolated pathogen and diagnosis.

Source of Infection	No. of Patients (%)
Community Acquired	47 (36.7)
Hospital Acquired	81 (63.3)
Indication	**No. of Patients (%)**
Empirical	107 (83.6)
Definitive	21 (16.4)
Positive Cultures	**No. of Patients (%)**
Yes	32 (25)
No	96 (75)
Diagnosis	**No. of Patients (%)**
Respiratory	93 (72.7)
Sepsis	56 (43.8)
HAP	22 (17.2)
CAP	3 (2.3)
VAP	7 (5.5)
Chest Infection	4 (3.1)

	Unknown	1 (0.8)
Skin and Soft Tissue		5 (3.9)
	Soft Tissue Abscess	2 (1.6)
	Surgical Wound Infection	2 (1.6)
	Line Infection	1 (0.8)
Abdominal		2 (1.6)
	Biliary Sepsis	1 (0.8)
	Liver Laceration	1 (0.8)
Other		28 (21.9)
	Sepsis	14 (10.9)
	Meningitis	2 (1.6)
	Osteomyelitis	2 (1.6)
	Other	10 (7.8)

Isolated Pathogen	No. of Samples (%)
Escherichia coli - G(-)B	9 (25)
Pseudomonas aeruginosa - G(-)B	13 (36.1)
Enterobacter cloacae - G(-)B	3 (8.3)
Klebsiella pneumonia - G(-)B	2 (5.6)
Staphylococcus aureus - G(+)B	3 (8.3)
Other	4 (11.1)
Unknown	2 (5.6)

CAP = community acquired pneumonia, G(-)B = Gram-negative bacteria, G(+)B = Gram-positive bacteria, HAP = hospital acquired pneumonia, VAP = ventilator acquired pneumonia

The majority of patients, 63.3%, had an infection that was hospital acquired and the remaining 36.7% had an infection that was community acquired. 32 of 128 patients (25%) had positive cultures corresponding to 36 isolated pathogens (**Figure 34**).

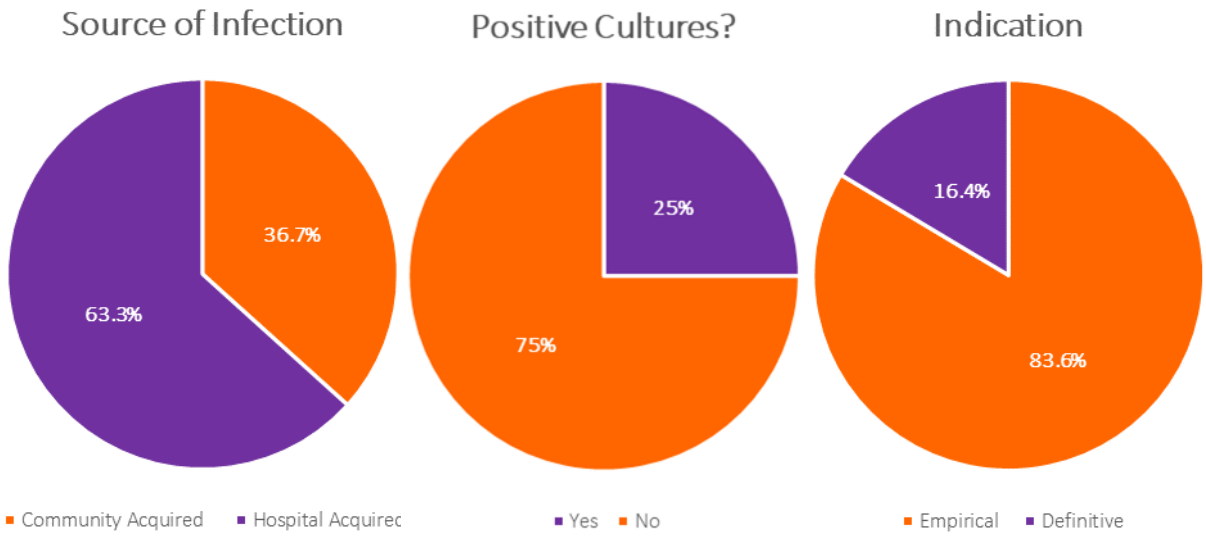


Figure 34 Source of infection, cultures, and indication representation

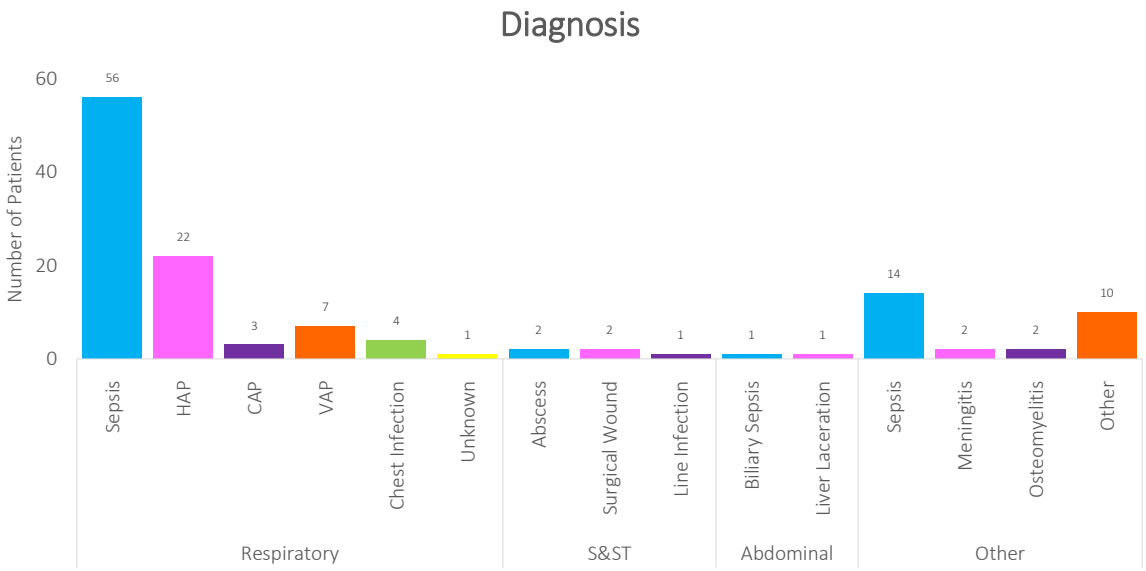


Figure 35 Showing identified patients' diagnosis. CAP = Community Acquired Pneumonia, HAP = Hospital Acquired Pneumonia, S&ST = Skin and Soft Tissue, VAP = Ventilator Acquired Pneumonia

72.7% of patients were diagnosed with a respiritory infection, 3.9% were diagnosed with a skin and soft tissue infection, 1.6% had an abdominal infection and the remaining 21.9% had an infection, such as meningitis and osteomyelitis, that fell into the 'other' category on data

extraction form (**Figure 35**). Within the population diagnosed with a respiritory infection, the majority of patients, 56 cases, suffered from sepsis.

Of the 36 pathogens isolated from 32 patients, Gram negative bacteria were predominant with *Pseudomonas Aeruginosa* being the most common isolate overall (33.3% of all isolates). Besides *Escherichia Coli* was also commonly found (25% of all isolates). Three patients had Gram positive *Staphylococcus Aureus* (8.3% of all isolates). 92 patients (71.9%) did not have an isolated pathogen and for 6 patients the isolated pathogen was not recorded (16.6% of all isolates) (**Figure 36**).

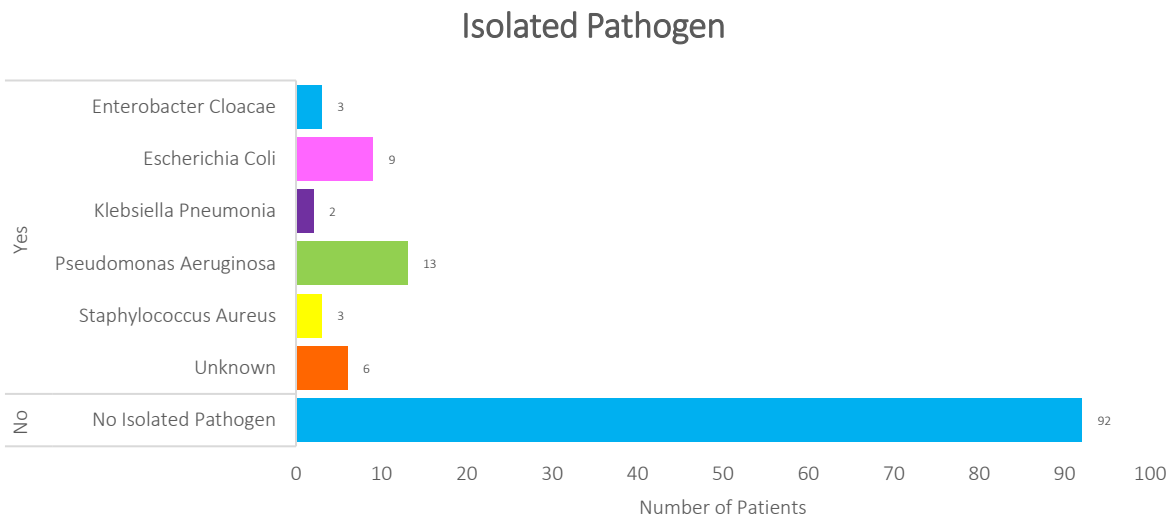


Figure 36 Bar chart representation showing the isolated pathogens.

2.4.3.3 Agent Administered

Prior antibiotic use was recorded in 59 out of 128 patients with co-amoxiclav being the most frequently used first-line antibiotic (30 of 59 patients received co-amoxiclav intermittent infusions). A total of 95 patients (74.2%) received piperacillin-tazobactam via a 4-hour PI. Of these patients 79 received (61.7%) piperacillin-tazobactam as monotherapy, whereas 15 patients (11.7%) received combined therapy. A total of 33 (25.2%) patients received meropenem via PI therapy. 27 patients (21.1%) received meropenem as a monotherapy and 6 patients (4.7%) received combined therapy (**Table 16**) (**Figure 37**).

Table 16 Antibiotic agent administered

Antibiotic	No. of Patients (%)
Piperacillin-tazobactam	95 (74.2)
Piperacillin-tazobactam 4.5g (q8h)	94 (73.4)
Monotherapy PI	79 (61.7)
Combined Therapy PI	15 (11.7)
Gentamicin	6 (4.7)
Vancomycin	2 (1.6)
Clarithromycin	1 (0.8)
Fluconazole	1 (0.8)
Clindamycin	1 (0.8)
Doxycycline	1 (0.8)
Acyclovir	1 (0.8)
Amikacin	1 (0.8)
Co-Amoxiclav	1 (0.8)
Piperacillin-tazobactam 1.5g (q6h)	1 (0.8)
Gentamicin	1 (0.8)
Meropenem	33 (25.2)
Meropenem 0.5g	2 (1.6)
Monotherapy (q8h) PI	1 (0.8)
Monotherapy (q12h) PI	1 (0.8)
Meropenem 1g (q8h)	25 (19.5)
Monotherapy PI	19 (14.8)
Combined Therapy PI	6 (4.7)
Vancomycin	1 (0.8)
Erythromycin	1 (0.8)
Gentamicin	1 (0.8)
Amikacin	1 (0.8)
Clarithromycin	1 (0.8)
Flucloxacillin	1 (0.8)
Meropenem 2g (q8h) PI	6 (4.7)

PI = prolonged infusion, q8h = every 8 hours, q12h = every 12 hours.

Figure 37 Summarises the prescribing patterns of agents administered. CT = combined therapy, MT = monotherapy, PI = prolonged infusion, PT = piperacillin-tazobactam, M = meropenem

2.4.3.4 Patient Outcomes

Table 17 summarises the information obtained on patients' outcomes. Clinical outcomes of interest were clinical cure, mortality and duration of antibiotic treatment.

Table 17 Showing enrolled patient outcomes.

Patient Outcomes	
Duration of Antibiotic Treatment (Days)	Mean ± SD (range)
Piperacillin-tazobactam	6.8 ± 3.4 (1 – 20)
Meropenem	16.9 ± 35.8 (1 – 180)
Both Antibiotics	9.4 ± 18.7 (1 – 180)
Male	7.4 ± 4.5 (1 – 28)
Female	13.2 ± 30.9 (1 – 180)
Patient Labs	Mean (range)
SrCr (Prior Antibiotics)	105.2 (15 – 834)
Male	105.6 (15 – 834)
Female	104.1 (34 – 313)
CRP (Prior Antibiotics)	144 (1.5 – 476)
Male	145.8 (1.5 – 476)
Female	140.2 (29 – 374)
WBC (Prior Antibiotics)	18.3 (1 – 420.3)
Male	21.9 (4.1 – 420.3)
Female	11.1 (0.1 – 21)
SrCr (Post Antibiotics)	82.9 (15 – 369)
Male	82.4 (15 – 369)
Female	84.2 (24 – 275)
CRP (Post Antibiotics)	101.3 (1 – 474)
Male	104.4 (1.1 – 474)
Female	93.9 (1 – 374)
WBC (Post Antibiotics)	11 (2 – 123.7)
Male	11.5 (2 – 123.7)
Female	9.9 (3.1 – 24.8)
Clinical Cure	No. of Patients (%)
Yes	117 (91.4)

Piperacillin-tazobactam	89
Meropenem	28
No	7 (5.5)
Patient started on alternative antibiotic	5 (3.9)
Developed HAP	1 (0.8)
Not Recorded	1 (0.8)
Mortality	4 (3.1)
Piperacillin-tazobactam	3 (2.3)
Meropenem	1 (0.8)

CRP = c-reactive Protein, HAP = hospital acquired pneumonia, SD = standard deviation, SrCr = serum creatinine, WBC = white blood cell.

Overall, the duration of antibiotic treatment was 9.4 days. Treatment duration was longer for female patients (13.2 days) when compared to male patients (7.4 days) (p = 0.095). It was also clear that the duration of treatment was significantly shorter for patients receiving piperacillin-tazobactam (6.8 days) than those receiving meropenem (16.9 days) (p = 0.007) (Figure 38).

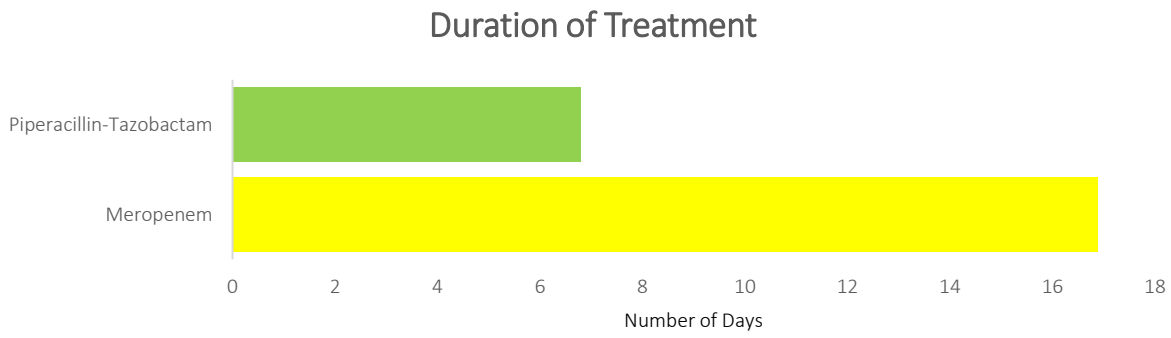


Figure 38 Average duration of piperacillin tazobactam and meropenem treatment

Figure 39 and Table 17 displays the average prior and post antibiotic treatment WBC, SrCr and CRP for male and female patients identified for inclusion in this study.

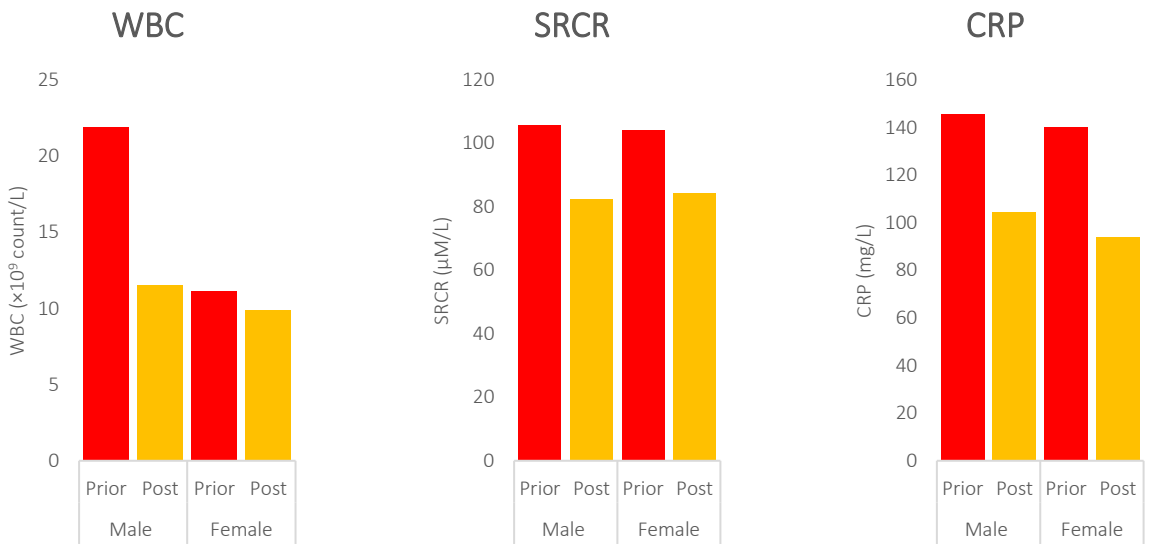


Figure 40 Average prior and post antibiotic treatment WBC (normal range = 4.5-11.5 x 10^9 count/L for males and females), SrCr (normal range – male = 65.4 – 119.3 µM/L and female = 52.2 – 91.9 µM/L) and CRP (normal level = 10 mg/L for males and females)

SrCr levels prior to antibiotic treatment (mean = 99.4 µM/L, SD = 63.5) were significantly higher than SrCr levels post antibiotic treatment (mean = 83.4 µM/L, SD = 70.1), t (83) = 3.015, p = 0.003. Prior to antibiotic treatment, CRP levels (mean = 143.6 mg/L, SD = 93.4) were significantly higher than levels after antibiotic treatment (mean = 97.4 mg/L, SD = 73.9), t (82) = 4.054, p < 0.001. Although not statistically significant, WBC prior to antibiotic treatment (mean = 18.6 x 10^9 count/L, SD = 41.2) was higher than post antibiotic treatment (mean = 11.1 x 10^9 count/L, SD = 12.2), t (100) = 1.751, p = 0.083. Overall, Clinical cure was

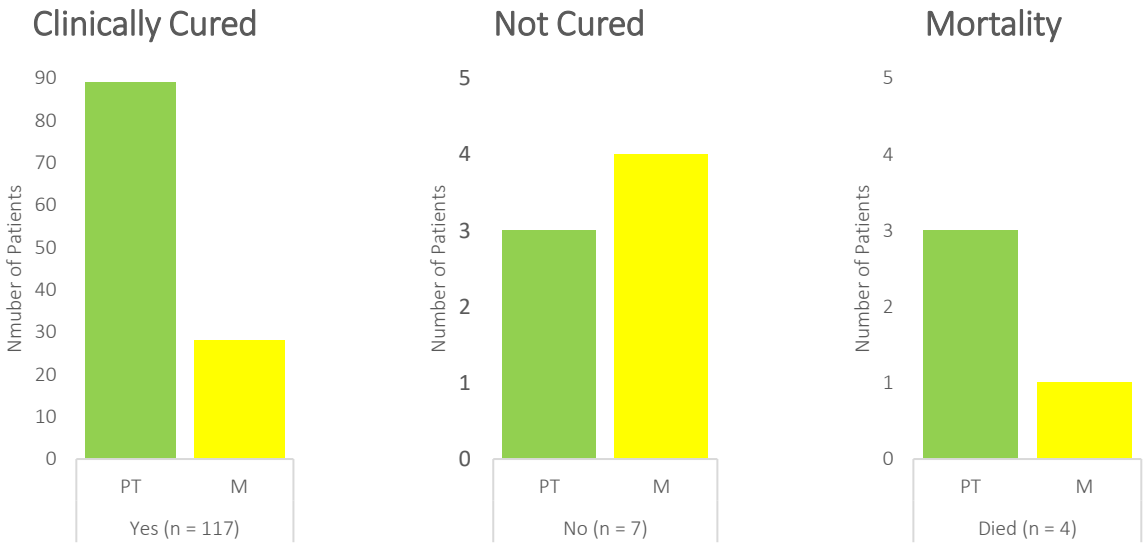


Figure 39 Clinical outcomes of PI piperacillin-tazobactam (PT) and meropenem (M).

achieved in 117 patients (91.4%) at the time of data collection: 89 patients receiving piperacillin-tazobactam (93.7% of all piperacillin-tazobactam patients) and 28 patients (84.8% of all patients receiving meropenem). Clinical cure was not achieved in 7 patients (5.5%) and there were 4 deaths (3.1%) reported: 3 patients receiving piperacillin-tazobactam (75% of all deaths) and 1 patient receiving meropenem (25% of all deaths) (**Figure 40**).

2.4.4 Discussion

This study investigated the use of BLAs in practice for the treatment of bacterial infections in ICU patients. Due to piperacillin-tazobactam and meropenems large antibacterial spectrum and low toxicity, they are among the first line therapy for critically ill patients (240).

This is the largest study to investigate the use of these BLAs in practice looking at patient profiles, prescribing patterns, dosing, and administration. Previous studies comparing the dosing regimens have demonstrated that PIs have at least similar or in many studies better clinical outcomes than IIs. Although not comparative in nature, the findings of this retrospective study suggest that PIs are an effective dosing strategy thus, support their use in the critically ill patient population.

Several observations were encountered from reviewing the retrieved data. Firstly, the majority, 117 patients (91.4%) were clinically cured. Secondly, there was a relatively low mortality rate, 4 patients (3.1%). Thirdly, SrCr levels prior to antibiotic treatment were significantly higher than SrCr levels post antibiotic treatment. Fourthly, prior to antibiotic treatment, CRP levels were significantly higher than levels after antibiotic treatment. Lastly, WBC prior to antibiotic treatment was higher than post antibiotic treatment.

2.4.4.1 Antibiotic Prescription

In ICUs, antibiotics are mostly prescribed prior to or without knowing the pathogens and their susceptibilities to antibiotics (241). Prompt broad-spectrum antibacterial therapy is delivered to patients with the onset of fever, a common symptom of sepsis. The results obtained in this study are in alignment with this, as only 21 patients (16.4%) were definitively treated whereas the majority, 107 patient (83.6%) were treated empirically.

The conventional procedure for the identification of the causative pathogen that usually takes several days (24 - 72 hours) has established the need to ‘empirically’ treat patients

while waiting for the definitive microbiological report. Noteworthy, in many cases, patients are initiated with empiric antibiotic therapy prior to collection of clinical samples thus previous antibiotic exposure may render culture results unreliable.

The small percentage of positive cultures potentially leads to inappropriate use of broad-spectrum therapy. Although, this study observed no correlation between indication and length of hospital stay, it is notable that 75% of the mortality cases did not have an isolated pathogen, thus were treated empirically. Several studies investigating the significance of appropriate antibiotic therapy found that mortality was significantly higher in patients receiving inappropriate empirical treatment (242–245). Also, a systematic review evaluating the relationship between appropriate antibiotic therapy and mortality in ICU patients found that there was a correlation between inappropriate empiric therapy and higher mortality (246).

A conservative approach to aid in overcoming inappropriate prescription of broad-spectrum BLAs involves developing diagnostic point-of-care tools such as novel molecular assays that rapidly identify biomarkers associated with pathogens such as bacteria or viruses in clinical samples. The clinical value of such tools includes identifying the infecting pathogen. This limits unnecessary antibiotic use when bacterial infections are ruled out thus encouraging HPC to seek alternative diagnoses and guides empiric therapy before the availability of culture results (241).

2.4.4.2 Antibiotic Administration

As previously highlighted in **Chapter 1**, BLA are relatively unstable due to their inherent hydrolysis reaction after reconstitution and dilution. Studies have determined piperacillin-tazobactam is stable for 24 hours at 20-25°C and 48 hours at 2-8°C (further discussed in **Chapter 3**) making administration via a PI feasible. However, studies to date have shown that carbapenems, in particular meropenem, are fairly unstable in solution (77,247,248). A recent study by **Fawaz *et al.***, suggests that meropenem is stable for 7-hours at room temperature and that stability is significantly dependant on the temperature the infusion solutions are exposed to (77).

Appropriate, timely BLA therapy given at an adequate dose is of paramount importance in ICU. All, but one patient in the study population receiving meropenem therapy were given

0.5 – 2g, q6 - 12h over a 4-hour infusion (**Table 16**). One patient received meropenem over an 8-hour infusion for 180 days. Thus, suggesting a high possibility the patient received sub-therapeutic meropenem doses resulting in antibiotic concentration falling below the MIC of the infecting pathogen, halting bactericidal activity.

Another reflection that indicates that BLAs are not being used to their optimal level is the infusion time of piperacillin-tazobactam. Patients receiving PI piperacillin-tazobactam were given 4.5g, q8h over a 4-hour infusion (**Table 16**). Published studies and stability data in **Chapter 3** demonstrate feasibility of administering piperacillin-tazobactam solutions via a 24-hour CI.

Findings of this study should be interpreted with consideration of certain limitations. Firstly, with the retrospective nature of this study, data is limited to the depth and accuracy of the documented medical records. Secondly, this study addressed a heterogenous population that was limited to ICUs in a single centre. Thirdly, data on bacteria MIC was not available for analysis, thus, not permitting the identification of patients that did not attain piperacillin-tazobactam and meropenem PD targets or patients with toxic antibiotic concentrations. Fourthly, the number of patients receiving meropenem was relatively small compared to those receiving piperacillin-tazobactam making it difficult to compare or draw conclusions on the efficacy of PI meropenem.

2.4.5 Conclusion

This is a real-world study examining the practice of PI BLAs in ICU. This study provides insight into how BLAs are used in terms of dose, dosing regimen and duration of treatment. Despite the above limitations, this study provides information that supports the use of PI in critically ill patients. Further well-designed studies are warranted to corroborate these findings and evaluate the impact of prolonging piperacillin-tazobactam and meropenem infusions to potentially encourage broader implementation of this dosing modality.

2.5 Differential Antibiotic Dosing in Critical Care: Survey on Nurses' Knowledge, Perceptions and Experience

2.5.1 Introduction

Nurses play a key role in supporting efforts to reduce antibiotic resistance within the AMS programme. They are first responders, central communicators, and coordinators of care for antibiotic therapy. Nurses are integral providers of comfort that monitor the patients status, safety and response to treatment (249,250). As nurses are the first point of contact for patients and all the stakeholders in antibiotic use, they promote the prevention and the subsequent need for antibiotics. This central role, whether in the hospital, home, or community, puts nurses in a unique and vital position for optimising antibiotic use (**Figure 41**). Thus, nurses can be educators, advocates as well as ambassadors for widespread change regarding antibiotic therapy (251).

Depending on the circumstances and the scope of practice, nurses undertake advanced roles, e.g. nurses can be instrumental in leading antimicrobial improvement initiatives like P/CI antibiotic therapy in ICU settings (252). ICU nurses play a crucial role in the rational use of IV antibiotics, preventing the emergence and spread of antibiotic resistant bacteria through AMS and infection control programmes. They are involved in preparing, administering and prescribing IV antibiotics as well as monitoring their effects on patients (252). There are multiple activities or tasks integral to successful AMS where nursing coincides with roles of stakeholders (**Figure 41**), however, these roles have not formally been recognised in guidelines for implementation and operation by nurses (**Table 18**) (250).

Nurses undertake activities that directly contribute to optimal antibiotic use in practice (**Table 18**), yet formal participation and recognition in AMS programmes are lacking. The lack of routine education and training creates barriers to nurse's engagement within the AMS (i.e., lack of awareness and knowledge gaps within their role). Facilitators to nurse's engagement in ASM programmes include a framework that clearly states their roles and responsibilities and highlights the impact of their contributions on patient outcomes (253).

Figure 41 Nurse workflow communication; showing the central position of the nurse with the patient and all stakeholders in antibiotic use.

The appropriate use and administration of IV antibiotics in ICU's could reduce mortality and morbidity as well as impede the development of difficult to treat antibiotic resistant organisms (254). Contemporary nursing literature suggests that the administration of IV medication has the potential for greatest harm and that antibiotics have been implicated in 22% of nurse medication errors in ICU. Lapses related to antibiotic therapy mainly occur when the incorrect antibiotic or when the incorrect dose of the correct agent is prescribed, and when inaccurate preparation and administration of the correct antibiotic transpires. These medication errors result in treatment failures due to several reasons including sub-therapeutic antibiotic levels at the site of infection (255). The absence of clear guidelines regarding P/CI administration of antibiotics within nursing IV therapy standards or from nursing associations, can lead to sub therapeutic dosing which may lead to AMR (255).

Table 18 Overlap of activities undertaken by nursing staff that coincide with other stakeholders in antibiotic use [239]–[241].

Patient Admissions	Nursing	Doctors	Administration	Pharmacy	Microbiology	Infectious Diseases	Infection Control	Case Management
Triage and appropriate isolation	●						●	
Accurate allergy history	●	●		●		●		
Early and appropriate cultures	●	●				●		
Timely antibiotic initiation	●	●	●			●		
Medication reconciliation	●	●		●				
Clinical Progress Monitoring								
Progress monitor and report	●	●				●		●
Preliminary micro results and antibiotic adjustment	●	●		●	●	●		
Antibiotic dosing and de-escalation	●	●		●		●		
Patient Safety and Quality Monitoring								
Adverse events	●	●		●		●		
Change in patient condition	●	●				●		
Final culture report and antibiotic adjustment	●	●		●	●	●	●	●
Antibiotic resistance identification	●	●			●	●	●	●
Clinical Progress/ Patient Education/ Discharge								
IV to PO antibiotic, outpatient antibiotic therapy	●	●		●		●		
Patient education	●	●				●	●	
Length of stay	●	●	●			●		
Outpatient management, long term care, readmission	●		●			●	●	

The lack of studies in the literature investigating nurses' clinical practice in the AMS and particularly in antibiotic administration suggests that this has been overlooked. Statistics on nurses' own perspectives regarding antibiotic knowledge contributes significantly to educational preparation and quality in healthcare. It is important that nurses practising in ICU settings take an active role in ensuring their knowledge of developments and advancement in antimicrobial stewardship remains up to date. Therefore, the purpose of this study was to assess ICU nurses' level of knowledge on antibiotic use in critical care settings, perceptions on antibiotic preparation and administration as well as to assess their comfort and experience concerning P/CI antibiotic therapy. The main objective of this study was to gain a better understanding of nurse's knowledge and perceptions regrading P/CI antibiotic therapy to provide an evidence base to support future needs in terms of education and training.

2.5.2 Methods

2.5.2.1 Research Design and Study Participants

This was a cross-sectional study investigating the knowledge, perceptions and workload of nurses working within ICU. This study was conducted using an investigator-developed, self-administered survey instrument.

2.5.2.2 Setting and Participants

The study was conducted at St Georges Hospital ICU wards: neuro, cardiac and general. All day-shift critical care nurses, both full and part time, from three ICU wards were invited to participate.

2.5.2.3 Survey Instrument

The survey had twenty-one questions, five open-ended and sixteen close-ended, Likert scale questions. Closed-ended questions allowed for comparison between respondent's responses whereas utilising open-ended questions gave participants the opportunity to frame their answers in their own words. The instrument was divided into five sections: (1) Demographics, (2) Knowledge, (3) Perceptions, (4) Comfort and, (5) Experience (Appendix 2). The following instruments utilized include:

The Demographics section included two questions that pertained to nursing years of ICU experience and nurse band grading. `

The Knowledge section included three questions that related to nurse's knowledge- two of which used a five-point Likert scale to assess knowledge of ICU antibiotic administration and one open-ended question on nurse's opinions of why P/CIs are used.

The Perceptions section included eight questions associated with nurse perceptions on the preparation and administration of P/CI. Nurse's opinions on the impact different dosing regimens had in terms of workload, time consumption and ease of preparation and delivery, were considered, to gain an insight of how these factors influence, guide and support their practice.

The Comfort section included three questions on nurse comfort discussing antibiotic treatment and interpreting microbiology results using a five-point Likert scale.

The Experience section included five questions pertaining to nurse experience- one of which used a five-point Likert Scale and four open-ended questions to gain an insight into the advantages and disadvantages of P/CI as well as investigate nurse opinions of what changes could be made to improve the preparation and administration of P/CI.

2.5.2.4 Ethical considerations and Negotiation of Access

Audience-appropriate language was utilised to write survey questions and the participants were informed of the nature and purpose of the research. Collected information was utilised for the intended purpose of the study. The main ethical issues were participants' anonymity and confidentiality. The names, addresses and dates of birth of participants were unrecorded, making collected data anonymous. The survey data was kept confidential and participants were assured their right to withdraw at any time (256–258).

2.5.2.5 Sample Size Determination

Implementation of this study was to yield useful information about nurse's perceptions on antibiotic therapy in ICU settings. To fulfil the research objectives proposed, a cross-sectional survey design was utilised. A total population of 75 nurses working within three ICUs at St Georges Hospital (SGH) were open to voluntary participation in the survey.

A sample size calculation was utilised to ensure attainment of a representative sample size to draw meaningful conclusions that are statistically significant. A sample size of at least 43

participants would be necessary to draw meaningful conclusions that are statistically significant.

2.5.2.6 Survey Procedure

The investigator-developed survey described in Section 2.5.2.3 was distributed to all nurses (n=75) that work during the day in three ICU units at SGH. Prior to distribution, the survey questions and participant information sheet, explaining the purpose and confidentiality of the survey, got approval from the head nurse at SGH.

2.5.2.7 Data Collection and Analysis of Data

Data collection took place between 12th February and 26th February 2018. Data were computed and processed using Statistical Package for the Social Sciences (SPSS) software, version 24.0 and Microsoft Excel 2012. This study used descriptive and inferential (parametric and non-parametric) statistics to analyse the data.

2.5.2.8 Statistical Analysis

Descriptive analysis of all survey variables was carried out by using absolute and percentage frequencies. Inferential statistics involved conducting parametric and non-parametric statistical analysis. The associations and correlations between ranked variables were determined using Cramer V (V) (measure of association between two nominal values) and Kendall's tau-b (τb) non-parametric coefficient statistics. The association and correlation between ranked and ordinal data were determined by employing the Gamma (ɣ) and Kendall tau-b statistics. Non-parametric test 'Spearman's rank correlation' (r_s) was utilised to determine the monotonic relationship between ordinal variables.

2.5.2.9 Association and Correlation Parameters

Cramer's V levels of association between 0.0-0.1 represent weak association, 0.1-0.3 indicate moderate association and 0.3+ represent a strong association.

Kendall tau-b (τb) correlation coefficients between 0.10 and 0.29 represent a small association, coefficients between 0.30 and 0.49 represent a medium association, and coefficients of 0.50 and above represent a large association or relationship.

A gamma value (ɣ): between 0.00-0.30 represent a weak association; between 0.30-0.60 indicate a moderate relationship; greater than 0.6 represents a strong association, between variables.

Pearson (r (50)) and Spearman Rho (r_s) size of correlation is interpreted as: 0-0.3 indicates negligible correlation, 0.3-0.5 interprets low correlation, 0.5-0.7 representing moderate correlation and 0.7-1.0 indicates high correlation.

2.5.3 Results

A total of 52 critical care nurses participated in the survey (response rate: 69.3%). An overview of nurse's responses to close-ended questions is available in the published article or in **Appendix 3**.

2.5.3.1 Demographics

The majority of participating nurses (71.2%) had three or more years' experience working in ICU's and in band 5 (76.9%). Every year nurses move up their band by one increment, experience, further training, and clinical knowledge aid in the achievement of each stage. There are eight increments in 'band 5' and nine increments in 'band 6'. 15.4% (8/52) of nurses were in band 7 (deputy ward manager or ward manager) and 7.7% (4/52) nurses were in band 8. This indicated a very experienced group of participants (**Figure 42** and **Figure 43**).

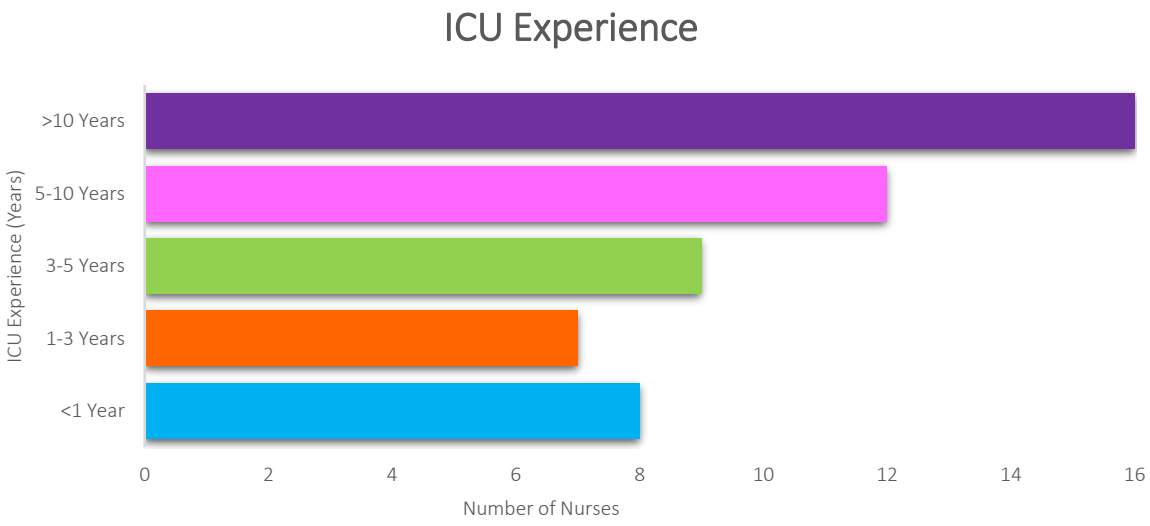


Figure 42 ICU experience of nurses

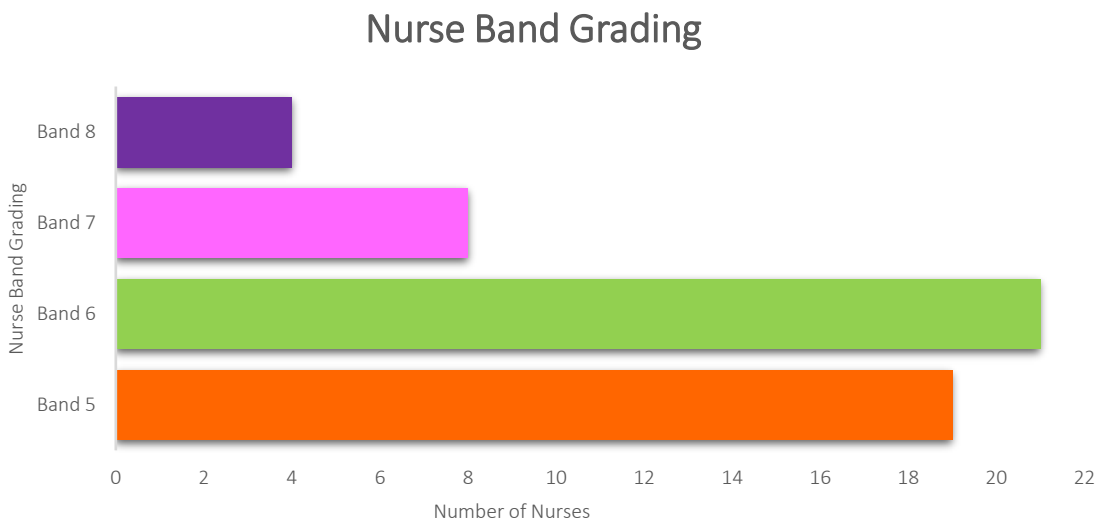


Figure 43 Band grading of ICU nurses

Demographic data and survey questions correlations and associations were performed using parametric (Pearson product-moment correlation) and non-parametric (Cramer V, Kendal Tau B and Spearman's rank correlation) statistics depending on the distribution and the skewness of the data (**Table 19**).

Table 19 Showing distribution and skewness of retrieved data.

Statement	Distribution	Skewness
My general knowledge about antibiotics in the ICU is…	N-D	0.048
My general knowledge about administering antibiotics via prolonged/continuous infusion is…	N-D	-0.606
Prolonged/continuous infusions of antibiotics aids in achieving higher clinical cure rate compared with conventional intermittent infusions…	N-S	-1.636
The preparation of antibiotics for prolonged/continuous infusions results in an increased workload on nurses compared with conventional intermittent infusions…	P-S	1.072
The preparation of antibiotics via prolonged/continuous infusions is more time consuming compared with conventional intermittent infusions…	P-S	1.698
Prolonged/continuous infusions are easier to prepare compared with conventional intermittent infusions…	P-S	1.193
The administration of antibiotics by prolonged/continuous infusions results in an increased workload on nurses compared with conventional intermittent infusions…	P-S	1.373
The administration of antibiotics via prolonged/continuous infusions is more time consuming compared with conventional intermittent infusions…	N-D	0.618
Prolonged/continuous infusions are easier to administer compared with conventional intermittent infusions	P-S	1.615
I am comfortable discussing antibiotic therapy with other healthcare professionals	N-D	0.000

I am comfortable discussing laboratory results related to infections with other healthcare professionals	N-D	-0.547
I am comfortable interpreting microbiology results	N-D	-0.711
I routinely conduct visual inspection for of the antibiotics being administered as prolonged/continuous infusions for precipitation throughout the infusion time	N-D	-0.981

N-D= normal distribution; N-S= negatively skewed; P-S= positively skewed

Correlations and association between ranked variables (years of ICU experience and band grading) indicated a strong, positive relationship (V = 0.578 and τb = 0.719; correlation significant to the 0.01 level).

2.5.3.2 Knowledge

The majority of nurses considered their self-perceived knowledge on antibiotic use in ICU to be very good or good (77%) and similarly on antibiotics administration via P/CI (80.8%). However, 23% and 19.2% weighed their knowledge as acceptable or poor in terms antibiotic use in ICU and antibiotic dosing regimens, respectively (Figure 44).

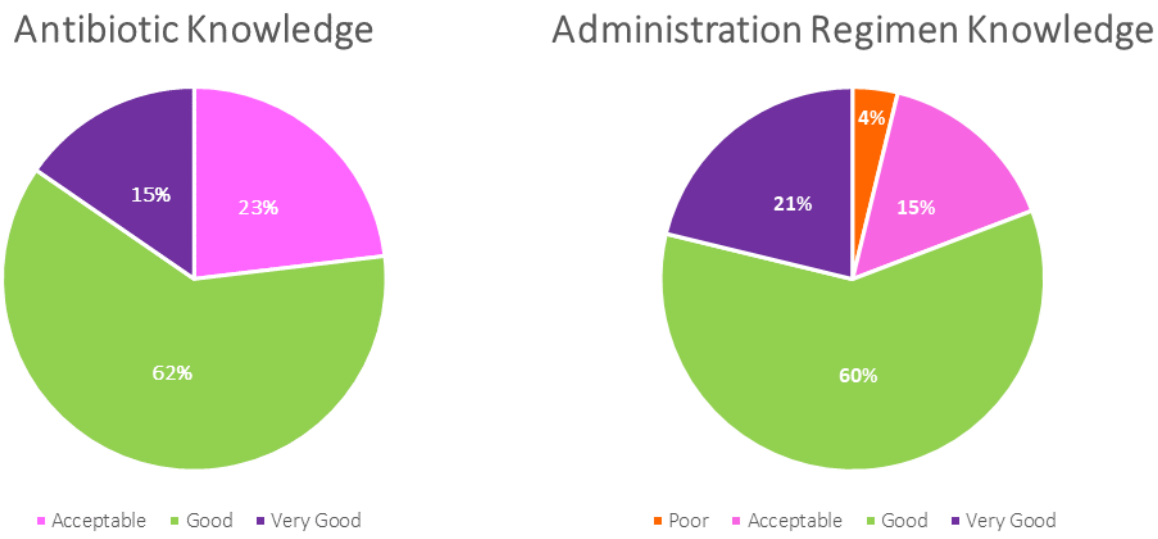


Figure 44 Nurse's knowledge on antibiotics in ICU and antibiotic administration regimens.

Nurses stated that P/CIs are used to: improve efficacy of antibiotics (33%), maintain antibiotic levels above the MIC (32%) and aid in preventing antimicrobial resistance (31%). A few participants (4%) mentioned that administering via P/CIs would reduce the need for regular dosing (Table 20) (Figure 45).

Table 20 Nurse responses to open-ended questions.

Statement	Responses		
	Response	Frequency	%
Knowledge			
Why do you think prolonged/continuous infusions are used?	Maintain antibiotic level above MIC	17	32
	Prevents resistance	16	31
	Improves efficacy	17	33
	No need for regular dosing	2	4
Experience			
What do you think are the advantages of prolonged/continuous infusions compared with intermittent infusions?	Better clinical outcome	28	43
	Less resistance	9	14
	Reduced workload	8	13
	No need for regular dosing	16	25
	Cost effective	3	5
What do you think are the disadvantages of prolonged/continuous infusions compared with intermittent infusions?	Patient discomfort	3	5
	Infusion interruptions	3	5
	No routine monitoring	4	7
	Fluid overload	5	9
	IV-line access	12	22
	No disadvantages	14	25
	Errors	15	27
What changes can be made for administration of prolonged/continuous infusions to improve the process?	Pre-made antibiotics	28	53.8
	Regular monitoring	8	15.4
	Specific protocol/ easy to use manual	6	11.5
	No changes	10	19.2
What changes can be made for preparation of prolonged/continuous infusions to improve the process?	More drugs via P/CI	20	38.5
	Pre-prepared antibiotics	21	40.4
	No changes	6	11.5
	Micro IV-line access	5	9.6

Figure 45 Nurse response to 'what do you think P/Cis are used for?' with statements

The association and correlation between 'ICU experience' and self-perceived 'knowledge' displayed a very weak, non-significant association and correlation between: (1) ICU experience and administration knowledge (γ = -0.085 and τb = -0.059) and (2) band grading and administration knowledge (γ = 0.044 and τb = 0.029).

2.5.3.3 Perceptions

Nurses perceived P/CIs advantageous over conventional intermittent infusions. Participants responded that P/CI antibiotics aid in achieving higher clinical cure rates (88%). From the 52 participants, 92.3% believed that antibiotic preparation for P/CI: does not increase workload nor is it more time consuming when compared to conventional II. The majority of participants also found that antibiotic administration via P/CI: does not increase workload (82.7%) nor is more time consuming (69.2%). However, participants did not find the preparation and administration of P/CI antibiotics easier than intermittent infusions. All but four nurses believed that P/CI antibiotics are not more prone to medical errors. Of the four nurses, three put medical errors down to calculation error and one believed these errors

were due to multiple manipulations (Figure 46 and Figure 47). It is important to note that these were more experienced nurses with higher band grades.

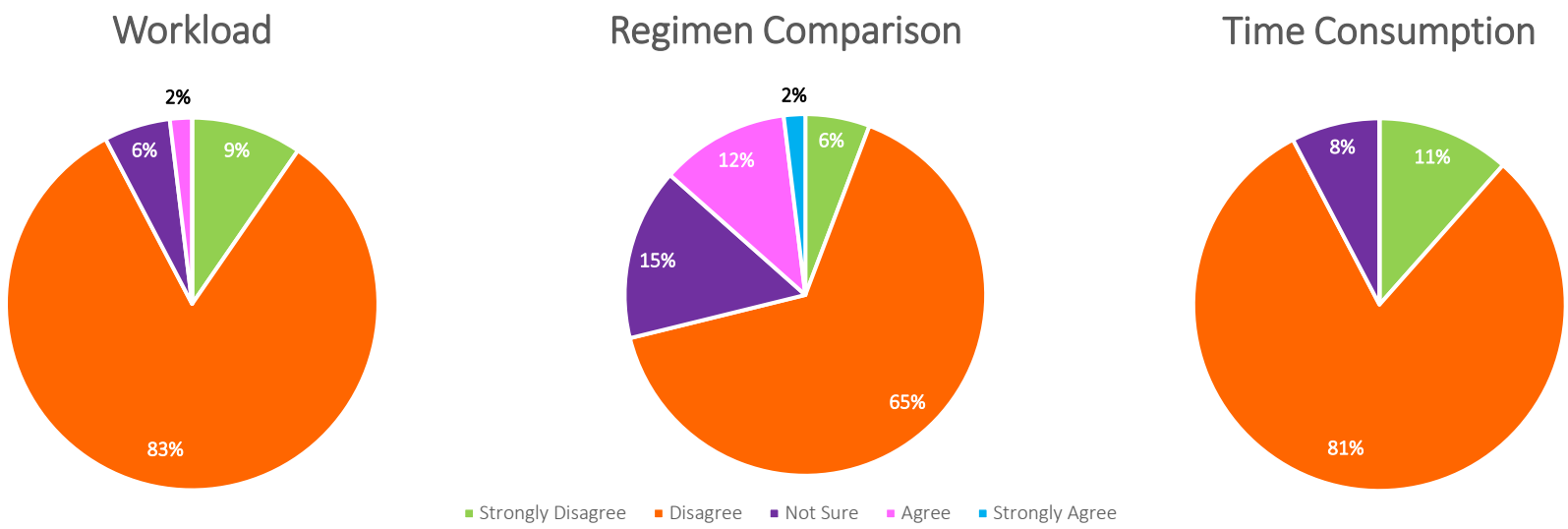


Figure 47 Pie charts demonstrating nurses' perceptions on the preparation of P/CI antibiotics in comparison to conventional II in terms of workload, ease, and time consumption.

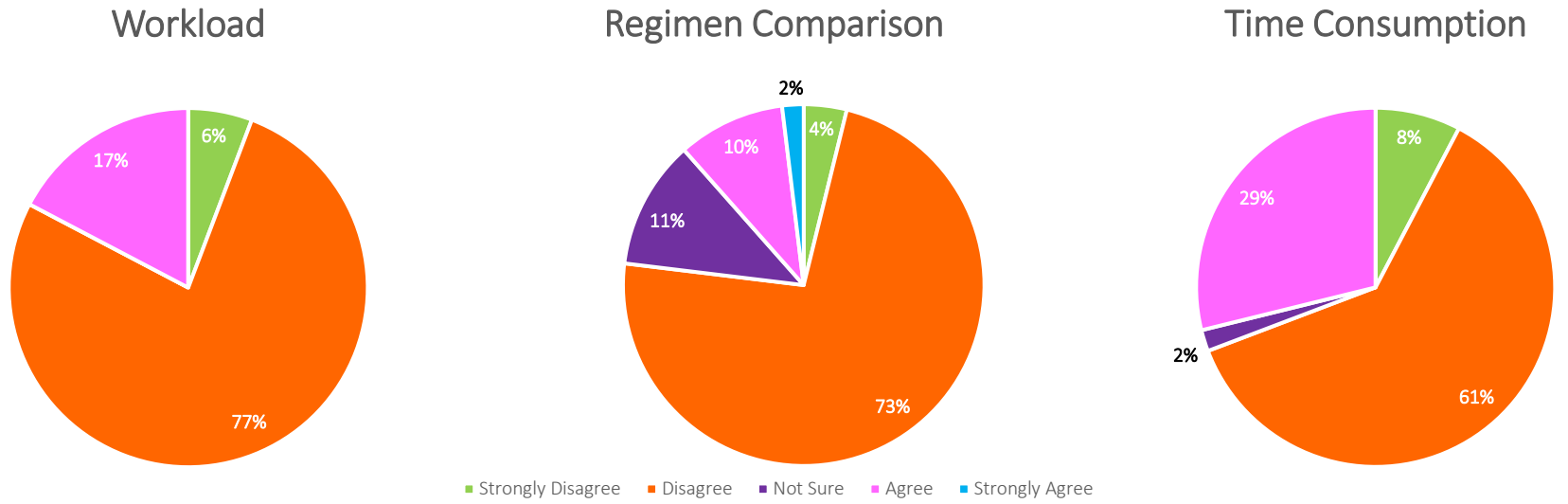


Figure 47 Pie charts demonstrating nurses' perceptions on the administration of P/CI antibiotics in comparison to conventional II in terms of workload, ease, and time consumption.

Nurses that stated their knowledge on prolonged/continuous administration of antibiotics was 'good' or 'very good' also believed that P/CIs aid in achieving higher clinical cure rates compared with conventional intermittent infusions (r_s = 0.453; p = <0.01). There is a strong positive association between 'nurse knowledge on antibiotic modes of administration' and 'the achievement of higher clinical cure rates when administration is via P/CIs' (ɣ = 0.679; P = <0.01).

Overall nurses did not feel that P/CI increased their workload. A strong positive association between participant responses to statements 7 and 8 (ɣ = 0.981; P = <0.01) (τb = 0.727; P = <0.01) was observed, where: nurses that thought that antibiotic preparation for P/CI did not increase workload also thought that preparation for this dosing regimen did not take more time. Nurses that also found that the administration of antimicrobials via P/CI did not involve additional workload observed that this dosing regimen was not more time consuming (ɣ = 0.907; P = <0.01) (τb = 0.583; P = <0.01)

Participants that specified the 'preparation' of antibiotics for P/CI did not increase workload also stated that 'administration' via this dosing regimen did not increase workload (ɣ = 0.925; P = <0.01). Nurse that stated P/CI 'preparation' was not more time consuming also thought that the 'administration' utilising this dosing regimen did not consume more time when compared with intermittent infusion (ɣ = 0.661; P = 0.01).

2.5.3.4 Comfort

Most nurses considered themselves comfortable: (1) discussing antibiotic therapy, (80.7%), (2) discussing laboratory results related to infection (86.5%) and (3) interpreting microbiology results (76.9%). However, a significant number of nurses were 'neutral', 19.2%, 11.5% and 15.4% respectively (**Figure 48**).

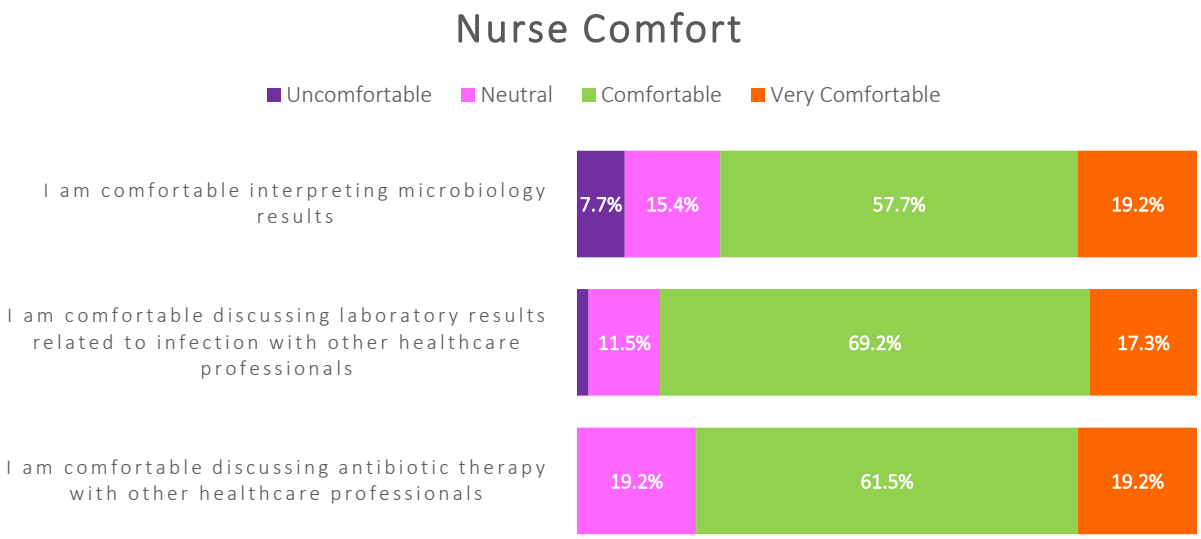


Figure 48 Stacked bar chart demonstrating nurse comfort levels in terms of antibiotic therapy.

Nurses that were comfortable discussing antibiotic therapy were also comfortable discussing laboratory data with other healthcare professionals (r (50) = 0.5.13; P = <0.01) (ɣ = 0.778; P = <0.01) and interpreting microbiology results (r (50) = 0.426; P = <0.01) (ɣ = 0.638; P = <0.01). Participants that were comfortable discussing patient laboratory results were also comfortable interpreting microbiology results (r (50) = 0.442; P = 0.01) (ɣ = 0.715; P = <0.01).

Also, nurses that believed P/CI of antibiotics aided in achieving higher clinical cure rates were more comfortable: (1) discussing antibiotic therapy with healthcare professionals (r_s = 0.460; p = <0.01) (ɣ = 0.675; P = <0.01), (2) discussing laboratory results related to infection with other healthcare professionals (r_s = 0.549; p = <0.01) (ɣ = 0.869; P = <0.01) and, (3) interpreting microbiology results (r_s = 0.778; p = <0.01) (ɣ = 0.561; P = <0.01).

The relationship between 'knowledge' of administering antibiotics via P/CI and 'comfort' in terms of discussing antibiotic therapy with other healthcare professionals was determined. Nurses that perceived their knowledge as 'very good' and 'good' felt more comfortable discussing antibiotic therapy (r (50) = 0.387; P = <0.01) (ɣ = 0.664; P = <0.01). Participants that perceived themselves knowledgeable about antibiotic therapy in ICU were also comfortable interpreting microbiology results (r (50) = 0.451; P = <0.01). There is a strong, positive association between knowledge and comfort (ɣ = 0.703; P = <0.01).

A Pearson product-moment correlation and a Gamma statistic test to determine the relationship between nurse's general antibiotic knowledge in ICU and comfort levels in terms of discussing laboratory results related to infection found a positive correlation (r (50) = 0.314; P = <0.05) and association (ɣ = 0.548; P = <0.01).

2.5.3.5 Experience

84.6% of participants 'strongly agree' (32.7%) or 'agree' (51.9%) that visual inspection of the antibiotic's physical compatibility during the infusion time of a P/CI should be conducted. Of the remaining participants, 7.7% (4/52) were 'not sure' and 7.7% (4/52) disagreed (**Figure 49**). Responses to open ended questions are displayed in **Table 20, Figure 50, Figure 51, Figure 52** and **Figure 53**.

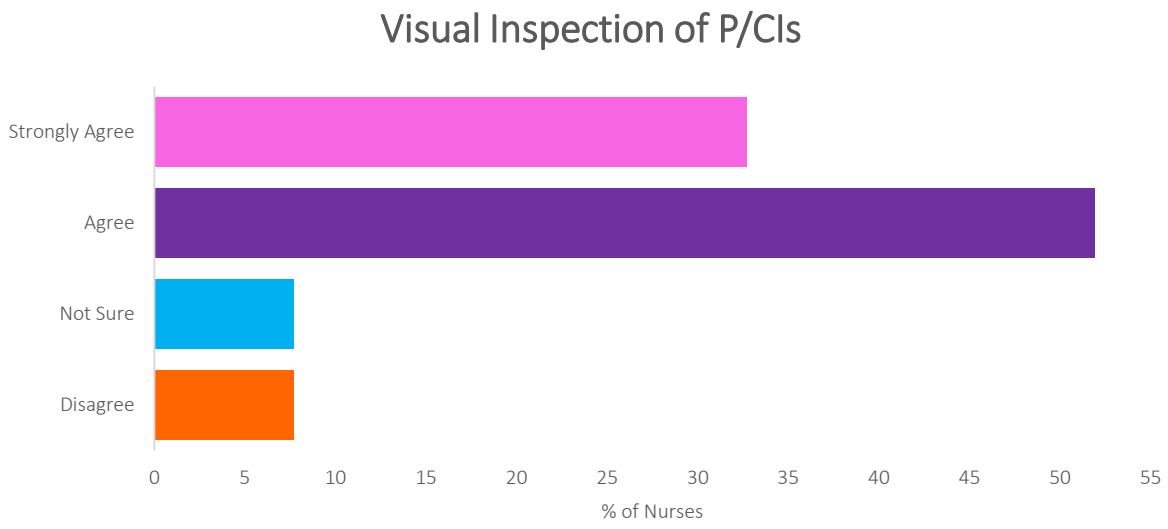


Figure 49 Nurse's responses to conducting visual inspection to assess the physical compatibility of IV antibiotics.

Interestingly, years of ICU practise (r (50) = 0.054; P = 0.7) (ɣ = 0.197; P = <0.5), band grading (r (50) = 0.246; P = <0.01) (ɣ = 0.246; P = <0.2), and comfort (r (50) = 0.374; P = <0.01) (ɣ = 0.338; P = 0.06) were not predictive of nurse's experience with P/CI. However, a positive correlation and association between 'knowledge' and 'experience' was found. Routine visual inspection of antibiotic being administered via P/CI was carried out by nurses who perceived they were knowledgeable in terms of (1) antibiotic in ICU's (r (50) = 0.356; P = 0.01) (ɣ = 0.457; P = <0.05) and (2) administering antibiotic via P/CI (r (50) = 0.357; P = 0.01) (ɣ = 0.544; P = <0.01).

The majority of participant responses (44%) included that P/CI is advantageous as it is associated with better clinical outcomes. A quarter of the responses were that P/CI antibiotic benificial to patients as there is no need for regular dosing whereas 14% and 13% of respondents answered that P/CI antibiotics correlate with less resistance and reduced workload, respectively. 5% of nurses replies were that P/CI is more cost effective than traditional II (Table 20) (Figure 50).

Figure 50 Nurse response to 'what do you think are the advantages of P/Cis compared with IIs?' with statements

Over half (56%) of responses to disadvantages of P/CI antibiotics were related to calculation errors (29%) or requiring dedicated IV-line access (27%). Other disadvantages were patient discomfort (4%), routine monitoring (8%) and applicability in fluid restricted patients (8%). A quarter of the nurses believed that there were no disadvantages associated with P/CI antibiotics (Table 20) and (Figure 51).

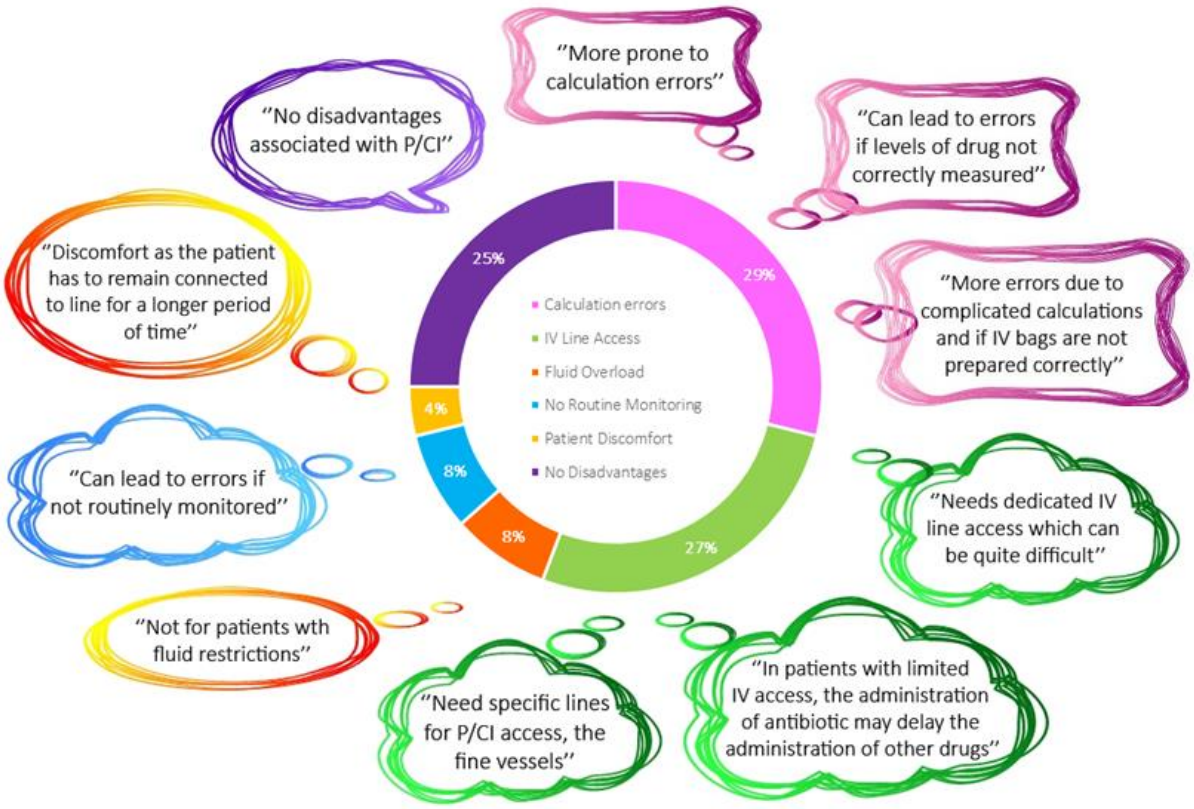


Figure 51 Nurse response to 'what do you think are the disadvantages of P/Cis compared with IIs?' with statements.

Nurses stated that changes required to improve the preparation process of P/CI antibiotic administration include the need for: preprepared antibiotic (54%), regular monitoring (15%) and specific protocol (12%). 19% of participants indicated that no changes are needed.

Figure 52 Nurse response to 'what changes can be made for preparation of P/CI to improve the process?' with statements.

Nurses stated that changes required to improve the administration process of P/CI antibiotic administration include the need for: preprepared antibiotic (40%), more antibiotics for P/CI administration (38%) and readily available micro IV-line access (10%). 12% of participants indicated that no changes are needed (Figure 53).

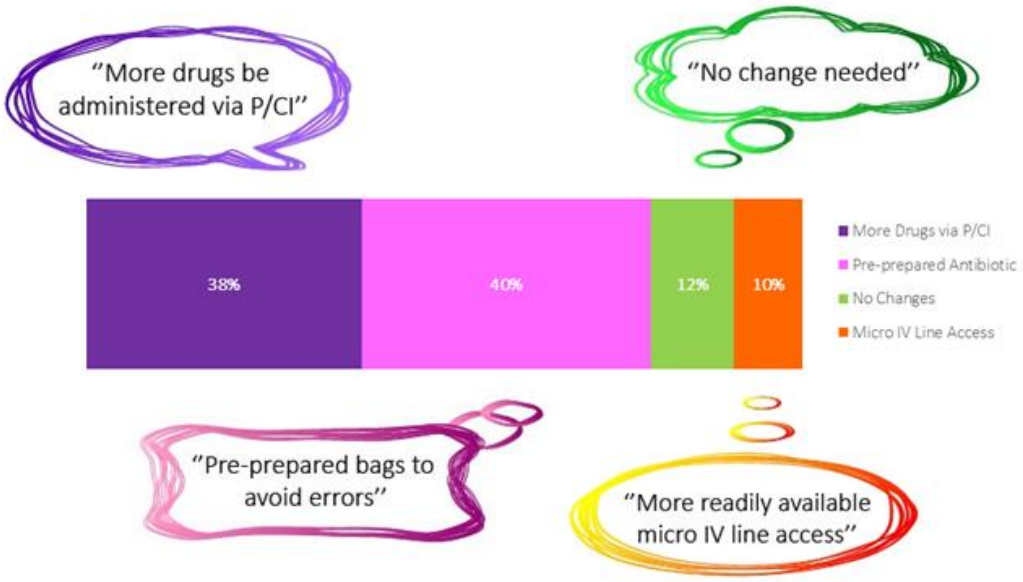


Figure 51 Nurse response to 'what changes can be made for administration of P/CI to improve the process?' with statements.

2.5.4 Discussion

This is the first study to assess ICU nurse's knowledge, perceptions, comfort and experience on antibiotic preparation, administration and use in critical care settings. The literature suggests that the use of P/CI is successful, safe and is aiding in optimising currently available antibiotics. Although its use is not widespread, it is on the rise. The appropriate use and administration of antibiotics in critical care settings contributes to the reduction in mortality and morbidity as well as impede the development of difficult to treat antibiotic resistant organisms (254).

Overall, the results revealed that nurses believe they have adequate levels of knowledge and comfort relating to the use of P/CI antibiotics along with the ability to communicate effectively on the topic. Statistical analysis showed that the more qualified nurses did not correlate with better experience with P/CI. Therefore, to support the wider implementation of P/CI treatment regimens in critical care as well as general wards, there is a need to upskill

the workforce and create specialised leadership roles for nurses within the AMS programmes to support nursing colleagues.

Knowledge accrued through professional practice and life experiences influences a nurses' ability to obtain and use knowledge (259). Studies have confirmed that experienced nurses use multiple sources of knowledge to guide their practice (254,259,260). Interestingly, results obtained in this study revealed that the years of ICU experience or banding position were not predictive of the nurse's self-perception of knowledge on antibiotic therapy. With the rapidly evolving changes in practice, there is a need for a structured approach to ensure an informed and consistent clinical practice for administration BLAs via P/CI. Therefore, continuous education and training is an absolute necessity for nurses who are required to provide high quality up to date care (261).

Studies have shown the P/CI may offer improved clinical outcomes when compared with intermittent infusion given that majority of studies published demonstrated improved clinical cure rates or significant difference between the two dosing regimens (90,170–173). Participants stated that P/CI of antimicrobials aided in achieving higher clinical cure rates when compared to traditional bolus infusions (88.5%), complying with previous clinical studies that recommend this mode of administration for patients with severe infection (in ICU) or patients infected by less sensitive pathogens (172,173,211).

Participants were able to categorise intrinsic factors (e.g. prevents antimicrobial resistance and improves antibiotic efficacy) and extrinsic factors (cost/time saving and patients length of hospital stay) known to be associated with antibiotic administration via P/CI that corroborate literature (172,262–264). Nurses stated that P/CIs reduced the need for regular dosing, hence, are beneficial for both patients and nurses as it reduces patient discomfort and, in some occasions, nurse's workload.

The preparation of P/CIs takes place on the wards mostly by nursing staff and involves calculations, multiple manipulations, dilution after reconstitution and use of infusion bags/pumps (265). The multiplicity of methods for preparing antibiotics for continuous administration creates a situation where mistakes may easily occur. Nurses affirmed that the preparation and administration of antibiotics for p P/CIs is more intricate in comparison to intermittent infusion preparation and administration; however, they did not believe it was

more time consuming or associated with increasing workload. It is evident that the use of P/CI requires multiple manipulations compared to traditional II due to the need for multiple steps, loading doses, more complex calculations and more stringent monitoring (266).

Studies indicate that drug equilibration takes longer in P/CIs than bolus administration, delaying the onset of antibacterial activity (171–173,267). Thus, circumvented by the administration of an initial loading dose. Although the loading dose ensures the rapid onset of antibacterial activity, the preparation of two doses is needed to initiate patient antibiotic therapy (268). However, participants did not identify the preparation or administration of P/CIs to be more time consuming or increase workload. This indicates a false sense of security and suggests that there is a need to provide post-graduate and continuous professional trainings.

A vital skill required is the ability to calculate antibiotic doses prescribed, however, the most recurrently cited error resulting in the wrong dose being administered stems from miscalculating doses (269). From participants that 'agreed' (4/52) that this dosing regimen is more prone to medical errors however, 75% (3/4) believed it was due to calculation errors. When calculating and preparing the correct dose for a patient, nurses need to understand different measurements used for drug dosages and be able to convert between different units of measurement (269). A series of decimally related dilutions for preparing individual antibiotic dosage that are patient specific require skills and additional effort. For example, with some ICU patients in whom severe fluid restriction may be necessary, solutions double or quadruple the strength are prepared (265).

To ensure the safe IV delivery of infusion antibiotics, nurses must be observant for potentially dangerous precipitates often caused by drug or diluent incompatibilities. Some participants (15%) do not conduct visual inspection of the physical compatibility of antibiotics administered via P/CI. Nurses should identify and avoid drug incompatibilities when preparing and administering antibiotics and monitor infusions adherently (270).

Although, most participants considered their knowledge, comfort, and experience satisfactory, there is a need for further learning beyond information gained from nursing education courses. Developing and employing a variety of strategies and mechanisms to improve and update nurses' knowledge on antibiotic dosing regimens used in ICU is crucial.

Educational support including; (1) staff presentations, (2) attendance at conferences as well as (3) in-ICU educational posters, are strategies that could be employed to raise awareness of antibiotic use (271).

It is noteworthy that this study provided an insight into the knowledge and practices of critical care nurses; however, it is important to mention that these nurses tend to be more advanced in their knowledge and expertise. Therefore, it is recommended that another study on general ward nurses is conducted to gauge their understanding, knowledge, and expertise on this topic.

Findings of this survey should be interpreted in view of certain limitations. Firstly, this was an investigator developed survey. Therefore, prior to distribution, the survey questions and participant information sheet were validated and approved by the head nurse at SGH. Secondly, this survey involved in-person dissemination, limiting the exposure of the survey to wider audiences. Thirdly, survey was completed by day shift staff, therefore, the data obtained doesn't account for the difference in experience between day and night shift nurses. Although the survey was only conducted on day staff these results provide a realistic indication of nurses' knowledge, experience, and comfort with antibiotic therapy in critical care settings. Fourthly, the survey was disseminated only in St Georges hospital. Although dissemination was conducted in a single setting, the data obtained is representative and included a wide range of nurses from three different ICU wards within the hospital.

2.5.5 Conclusion

Results indicate that ICU nurses at SGH have a good understanding surrounding the use of P/CI antibiotics. Findings from this study indicate that nurses are supportive of P/CI antibiotics. Participants considered their knowledge, comfort, and experience with antibiotic therapy high; however, key misperceptions were identified, indicating that nurses may not be aware of their knowledge deficits. Therefore, incorporating education, assessment and reinforcement on nurse competence associated with injection, infusion safety and infection control is required. Further research is needed to determine the most effective antibiotic mode of administration and continued stability studies will aid in ameliorating current dosing regimens to optimise antibiotic efficacy.

CHAPTER 3

SUITABILITY AND FEASIBILITY OF PIPERACILLIN-TAZOBACTAM FOR ADMINISTRATION VIA PROLONGED/CONTINUOUS INFUSIONS IN HOSPITAL AND OPAT SETTINGS

Publications

Fawaz S, Barton S, Nabhani-Gebara S. Comparing clinical outcomes of piperacillin-tazobactam administration and dosage strategies in critically ill adult patients: a systematic review and meta-analysis. BMC infectious diseases. 2020 Dec;20(1):1-6.

Fawaz S, Barton S, Merzouk M, Bukhari N, Nabhani-Gebara S. Suitability and feasibility of piperacillin-tazobactam for administration via prolonged/continuous infusions in hospital and OPAT settings. Drug design, development and therapy.

Conferences

Fawaz S, Barton S, Nabhani-Gebara S. Development and Validation of an RP-HPLC Method for the Simultaneous Determination of Piperacillin-Tazobactam Stability after Reconstitution. UKPharmSci International Conference 2018. Oral Presentation.

Fawaz S, Barton S, Nabhani-Gebara S. Development and Validation of an RP-HPLC Method for the Simultaneous Determination of Piperacillin-Tazobactam Stability after Reconstitution. UKPharmSci International Conference, Greenwich, 2019. Poster Presentation

3.1 Introduction to Piperacillin-Tazobactam

Piperacillin-tazobactam is a penicillin beta-lactam antibiotic (BLA) that's used to treat a wide variety of bacterial infections, including intra-abdominal infections, skin infections and pneumonia. Piperacillin has a broad spectrum of activity; it is active against most clinically important gram negative bacteria and demonstrates activity against gram positive aerobic bacteria (272,273).

3.1.1 Rationale for the use of Piperacillin in Combination with Tazobactam

Pharmaceutical formulation contains two active ingredients; (1) piperacillin, a penicillin antibiotic and, (2) tazobactam, a beta-lactamase inhibitor (BLI) that prevents bacteria from inactivating piperacillin. Piperacillin alone lacks strong activity against bacteria as the beta-lactam ring is hydrolysed by the pathogens beta-lactamase enzymes (BLEs) (273). Piperacillin is most commonly used in conjunction with BLI tazobactam as it enhances its effectiveness by inhibiting many BLEs to which it is susceptible and extends piperacillin's spectrum of activity, permitting its use for various clinical infections (274). It is available for parenteral administration only in combination, with an 8:1 ratio of piperacillin to tazobactam by weight (275).

3.1.1.1 Piperacillin Mechanism of Action

Piperacillin exerts bactericidal activity via inhibition of bacterial cell wall synthesis (peptidoglycan) by binding to penicillin binding protein (PBP) enzymes located on the inner membrane of the bacterial cell wall. PBPs are responsible for catalysing the D-alanine – D-alanine amino acid cross linkages of peptidoglycan cell wall. Piperacillin irreversibly inhibits PBPs, preventing cross-link formation, leading to weakened bacterial cell wall and ultimately cell lysis (275).

3.1.1.2 Tazobactam Mechanism of Action

Tazobactam is a penicillanic acid sulfone that exhibits negligible antibacterial activity. It inhibits the destruction of piperacillin by BLEs that catalyse the hydrolysis of the beta-lactam (BL) ring (275).

3.1.2 Dosage and Administration

Administration of piperacillin-tazobactam is exclusively intravenous as it is not absorbed in the gastrointestinal tract, deeming it orally inactive. It is routinely administered intermittently as a bolus injection over 3-5 minutes or by infusion over 20-30 minutes. Dosage is dependent on the severity of infection, ranging between 2/0.25g (piperacillin/tazobactam) every 6-12 hours for the treatment of mild infections to 4/0.5g every 6-8 hours for the treatment of patients with more severe infections (276).

3.1.3 Tolerability and Adverse Effects

Piperacillin-tazobactam is generally well tolerated. The most frequent adverse events include gastrointestinal symptoms (diarrhoea, constipation, nausea, vomiting), insomnia, fever and headaches. In rare cases patients may suffer from haemolytic anaemia, seizures and raised liver enzymes.

3.1.4 Pharmacokinetic Profile

The distribution of piperacillin and tazobactam is rapid, where peak plasma concentrations are attained immediately upon completion of IV infusion (277). Distribution into various tissue sites is generally regarded as good (except for fat tissue) due to the hydrophilic nature of the two compounds (274). The strained BL ring system of piperacillin is cleaved to produce a pharmacologically active metabolite (*N-desethyl-piperacillin*). Tazobactam also undergoes hydrolysis to produce an inactive open ring metabolite (276).

After an intermittent infusion (II) of 4.5g piperacillin-tazobactam, peak serum levels of 264.4-368mg/L and 29.1-39mg/L, respectively, are reached; by one hour the level drops to 105mg/L and falls to 15mg/L within 4 hours (276). The major route of elimination of both piperacillin and tazobactam is via renal excretion, predominantly through active tubular secretion and glomerular filtration. Approximately 70–80% of the piperacillin dose is eliminated in the urine is unmetabolized and around 80% of tazobactam dose is excreted unchanged in the urine (274). Both compounds have a mean plasma elimination half-life of approximately 0.8-1 hour (276).

Dosage reduction is recommended for patients with renal impairments as plasma concentration values are prolonged. Patients receiving peritoneal dialysis generally don't require dosing adjustments as 6% and 13% piperacillin and tazobactam, are respectively dialysed. However, during haemodialysis, 30-50% of piperacillin tazobactam is removed within 4 hours; these patients should receive an additional 2/0.25g dose post each dialysis period. Piperacillin-tazobactam PKs are not altered in patients with hepatic impairment (276).

3.1.5 PD Profile

Piperacillin-tazobactam is a time-dependant antibiotic; hence, its bactericidal activity is closely correlated to the time at which antibiotic concentrations in tissue and serum exceed the MIC threshold of the infecting organism (T > MIC). Periods at which piperacillin-tazobactam concentrations are above the MIC is a major parameter determining efficacy where optimum bactericidal activity is achieved when time above the MIC is approximately 50-60% of the dosing interval. However, piperacillin-tazobactam has no significant post-antibiotic effect, therefore, when concentrations drop lower than the MIC (T < MIC), bacterial growth resumes immediately, facilitating the development of resistance, especially when serum concentrations fall below the MIC threshold for longer than half of the dosing interval.

3.1.6 Mechanism of Resistance

There are four potential mechanisms in which bacteria can develop resistance to piperacillin-tazobactam; these include: (1) BLE production, (2) target site (PBPs) modification, (3) alterations in membrane permeability or (4) an increase in the membrane efflux (274,278). A major resistance mechanism towards piperacillin involves its inactivation via BLEs by gram negative bacteria. Tazobactam makes piperacillin effective against BLEs that would normally degrade piperacillin.

3.1.7 Continuous vs Intermittent Infusion Piperacillin-Tazobactam

Maintaining BLA concentrations above the MIC has been correlated with improved clinical outcome. Leveraging piperacillin-tazobactam's PK and PD parameters to maximise T > MIC is one strategy to enhance its efficacy while lowering costs of treatment.

In practice, piperacillin-tazobactam is typically dosed up to every 6 hours at varying doses (2.25-4.5g) depending on renal function and/or indication and is infused over 30 minutes. Extending the infusion time can reduce the dosing schedule to every 8-12 hours, with a lower total daily dose compared with traditional dosing.

Over the last few decades, several studies have investigated P/CI vs II piperacillin-tazobactam. Parameters investigated include, clinical cure rates, mortality rates, length of hospital stay, adverse effects, cost and workload on health-care practitioners. A summary of studies that compared P/CI and II piperacillin tazobactam are presented in the table below (Table 21).

Table 21 Summary of studies that compared P/CI and II piperacillin-tazobactam

Study/Year/Country	Study Design/Patient Population	Dosage	Clinical outcome
Richerson *et al.*, 1999 (279) USA	Crossover Study 12 Healthy Volunteers	CI (n=12) – 13.5g over 24hr CI II (n=11) – 4.5g every 6hrs over 30min II	CI maintains drug conc above the MIC and allows the least amount of drug to be given with the smallest amount of labour
Burgess *et al.*, 2002 (280) USA	Crossover Study 11 Healthy Volunteers	CI (n=11) – 6.75g-13.5g over 24hr CI II (n=11) – 3.375g every 6hrs over 30min II	The efficacy of piperacillin tazobactam appears to be maximised in CI as it maximises the time above the MIC.
Buck *et al.*, 2005 (281) Germany	Prospective Randomised Study 24 Hospitalised Patients	CI (n=12) – 2.25g LD + 4.5g-13.5g over 24hr CI II (n=12) - 4.5g every 8hrs via bolus injection	Resolution or improvement of clinical and laboratory signs of infection (such as fever and normalization of leucocytosis)
Patel *et al.*, 2009 (184) USA	Retrospective Cohort Study 129 Patients - Gram (-) infections	PI (n=70) – 3.375g every 8hrs over 4hr PI II (n=59) - 3.375g-4.5g every 6-8hrs over 30min II	PI yielded similar clinical outcomes compared with II among healthy patients. With PI administration there is a lower cost.

Brunetti *et al.*, 2015 (282) USA	Retrospective Cohort Study 2150 Hospitalised Patients	PI (n=632) – 2.25g-4.5g every 6hrs over 4hr PI II (n=1518) - 2.25g-4.5g every 6hrs over 30min II	PI is safe and associated with significant cost saving. However, PI was not associated with a reduction in mortality and LOHS.

A 1999 study conducted by Richerson *et al*., compared serum drug concentrations of piperacillin-tazobactam when administered as either CI or II. 12 healthy volunteers were enrolled in this crossover study. Each subject received a regimen of CI piperacillin-tazobactam and serum samples were obtained at multiple sampling intervals. After a one week wash out period, the II study commenced on the same subjects. Results obtained from this study show that CI maintained drug concentrations above the MIC between dosing intervals with no infusion related adverse events. They concluded that CI allows the least amount of drug to be given with the smallest amount of labour and supply cost, while also maintaining piperacillin-tazobactam concentrations above the antibiotics MIC for the entire dosing interval (Table 21) (279).

In 2002, Burgess *et al*., compared the PK/PD of piperacillin-tazobactam when administered as CI vs II against clinical isolates of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. This crossover study involved each subject receiving both II and CI over 24 hours. The order of administration was randomly determined and a washout period of >7 days was required between the receipt if the regimens. Blood samples for PK and PD analysis were obtained at multiple sampling intervals for both dosing regimens. Results obtained confirmed that that the efficacy of piperacillin-tazobactam is maximised in dosing regimens that maximise the time above the MIC; this can best be accomplished by CI administration. They also conclude that this mode of administration has the potential to be cost effective (Table 21) (280).

In 2005, Bucks *et al*., studied the difference in concentration-time profiles of piperacillin and tazobactam administered either by CI or II in hospitalised patients with different infections. A total of 12 patients were enrolled in this study, 12 in the CI group and 12 in the II group. Blood samples were drawn from patients at different sampling intervals depending on the regimen administered. Clinical outcomes in both the CI and II groups were comparable. In both groups 8 of 12 patients responded to antibiotic therapy. This study concluded that CI piperacillin-tazobactam provided adequate antibacterial activity over 24 hr dosing period and offers the potential for a substantial reduction in the total daily dose (Table 21) (281).

A 2009 study by Patel *et al*., compared clinical outcomes of II and PI piperacillin-tazobactam among patients with gram-negative infections, the majority of which resulted from urinary and respiratory sources. For this study, 129 patients (59 for II and 70 for PI) met the inclusion criteria. Results show that clinical outcomes did not differ between those receiving PI and II. Mortality rate and median length of hospital stay were also similar in both groups. Patel and colleagues concluded that both dosing regimens yielded similar clinical outcomes. However, there is a lower daily acquisition and administration cost with PI. Future studies evaluating the outcomes of II and PI should focus on patients with high disease severity (Table 21) (184).

More recently, in 2015, Brunetti *et al*., evaluated the clinical impact and cost-effectiveness by comparing administration of piperacillin-tazobactam via PI and II. A total of 2150 patients were included in the analysis, 1518 in the II group and 632 in the PI group. It was found that bactericidal activity is enhanced when antibiotic concentrations were maintained above the MIC for a longer period of time. They concluded that PI piperacillin-tazobactam is safe and associated with significant treatment cost saving. However, additional studies are warranted to identify the optimal dosing strategy in obese patients as there appeared to be an increased mortality rate in patients treated with PI as BMI increased (Table 21) (282).

Piperacillin-tazobactam is frequently utilised due to its relatively high effectiveness against a broad spectrum of micro-organisms, rapid penetration into inflammatory fluids as well as tissue concentrations reaching corresponding plasma concentrations.

The above data (Table 21) were obtained from healthy patients with mild or moderate infections. Critically ill patients, however, suffer from substantial changes in haemodynamic, renal and biliary excretion mechanisms and extravascular fluid overload. Consequently, it is important to recognise that treating septic patients presents a challenge for dose optimisation as PK/PD parameters vary and drug excretion (renal function) is affected. Therefore, data from healthy volunteers do not reflect the PK in critically ill patients. In view of this, a systematic review and meta-analysis comparing clinical outcomes of piperacillin-tazobactam administration and dosing strategies in critically ill patients was conducted and published.

3.2 Comparing Clinical Outcomes of Piperacillin-Tazobactam Administration and Dosage Strategies in Critically Ill Adult Patients: A Systematic Review and Meta-Analysis

3.2.1 Abstract

Recently, continuous administration of piperacillin-tazobactam has been proposed as a valuable alternative to traditional intermittent administration especially in critically ill patients. However, antibiotic dosing remains a challenge for clinicians as antibiotic dosing regimens are usually determined in non-critically ill hospitalized adult patients. The aim was to conduct a systematic review to identify and highlight studies comparing clinical outcomes of piperacillin tazobactam dosing regimens, continuous/prolonged infusion vs intermittent infusion in critically ill patients. Meta-analyses were performed to assess the overall effect of dosing regimen on clinical efficacy.

Studies were identified systematically through searches of PubMed and Science Direct, in compliance with PRISMA guidelines. Following the systematic literature review, meta-analyses were performed using Review Manager.

Twenty-three studies were included in the analysis involving 3828 critically ill adult participants in total (continuous/prolonged infusion = 2197 and intermittent infusion = 1631) from geographically diverse regions. Continuous/prolonged resulted in significantly: higher clinical cure rates (Odds Ratio 1.56, 95% Confidence Interval 1.28-1.90, P = 0 .0001), lower mortality rates (Odds Ratio 0.68, 95% Confidence Interval 0.55-0.84, P = 0 .0003), higher microbiological success rates (Odds Ratio 1.52, 95% Confidence Interval 1.10-2.11, P = 0.01) and decreasing the length of hospital stay (Mean Difference - 1.27, 95% Confidence Interval - 2.45-0.08, P = 0.04) in critically ill patients.

Results from this study show that there is a significant level of evidence that clinical outcome in critically ill patients is improved in patients receiving piperacillin-tazobactam via continuous/prolonged infusion. However, more rigorous scientific studies in critically ill patients are warranted to reach a sufficient level of evidence and promote further implementation of P/CI as a dosing strategy.

3.2.2 Background

Recently, continuous administration of piperacillin-tazobactam has been proposed as a valuable alternative to traditional intermittent administration especially in critically ill patients. However, correct antibiotic dosing remains a challenge for clinicians as antibiotic dosing regimens are usually determined in non-critically ill hospitalized adult patients. Patient that are in intensive care units (ICU) differ from other hospitalized patients in terms of pathophysiology and disease severity; these factors not only affect metabolism but also drug PK/PD behaviour. Critically ill patients also have an increased risk (5-10 times more likely) of having or developing infections and infectious complications than those in general wards (283).

Dosing strategies that have been validated in patient populations that are non-critically ill fail to consider the substantial changes in organ function that occur with critical illness (237). Augmented renal clearance of antibiotics is increasingly reported in critically ill patients. Antibiotic dosing concentrations will vary greatly within intensive care patients with normal kidney function or renal failure as the PK target attainment is dependent on kidney function (236). Given the enhanced renal elimination reported in critically ill patients, antimicrobial dosing requires extensive consideration due to important clinical consequences as accurate and timely drug exposure is essential for clinical success. The augmented renal clearance is possibly associated with the (1) immune response to infection, (2) inflammation to fluid loading and, (3) use of vasoactive medications. An increase in both cardiac output and blood flow is therefore observed, leading to enhanced glomerular filtration that results in sub-therapeutic piperacillin-tazobactam concentrations due to substantial drug elimination (229).

The optimisation of antimicrobial agents is a relatively unexplored area where further research is needed. Continuous infusions (CI) and prolonged infusions (PI) of piperacillin-tazobactam has been directly linked to improved clinical outcome displaying capabilities such as lowering the possibility of resistance and decreasing mortality (79,210,237). The aim here is to systematically review the literature comparing the clinical outcome of piperacillin tazobactam dosing regimens, continuous/prolonged infusion P/CI and II.

3.2.3 Methods

3.2.3.1 Literature Search

A systematic review of the literature was conducted (284–287); references published between 1998 and 2019 were acknowledged through searches on PubMed and Science Direct, in compliance with PRISMA guidelines. Search terms used were: (penicillin OR penicillins OR piperacillin OR tazobactam OR piperacillin-tazobactam OR piperacillin/tazobactam) AND (intermittent OR bolus OR short OR prolonged OR extended OR continuous) AND (infusion OR duration OR administration OR interval OR dosing) AND (intensive care OR ICU OR critically ill OR critical care OR septic shock OR sepsis OR severe sepsis).

However, like any database, their coverage is not complete, therefore the authors retrieved additional articles using supplementary approaches such as manual searching of journals, Google Scholar and checking reference lists of articles to identify additional text. A full review of published studies was implemented addressing and comparing clinical outcome of IV piperacillin-tazobactam dosing regimens administered to infected critically ill patients. The last search was on the 1st of August 2019 [PROSPERO registration number: CRD42019117303].

3.2.3.2 Study Selection

Initially, all articles reporting comparative outcomes of critically ill patients treated with P/CI versus II piperacillin-tazobactam were considered eligible. The eligibility criteria were separated into two components: study characteristics and report characteristics. Study eligibility criteria included the types of a) studies, b) participants, c) interventions and d) outcome measures; these measures are presented in **Table 22**. Report eligibility criteria included: publications written in English language, study status is ''published'' and inclusion of both old and new data. Exclusion criteria included: Pharmacoeconomic studies, non-human subjects, non-adult subjects, non-critically ill subjects, non-English language studies and pilot studies. Systematic reviews, meta-analysis and editorials were also excluded.

Table 22 Showing eligibility criteria for study selection process

	Eligibility Criteria
a) Studies	Prospective and retrospective trials/studies comparing/evaluating clinical efficacy or clinical outcome of piperacillin/tazobactam administered via CI vs II in critically ill patients. Pilot studies excluded
b) Participants	Critically ill adult participants aged 18 and over suffering from documented bacterial infection and requiring treatment with piperacillin-tazobactam. Non-adult, non-human and non-critically ill patient studies were excluded.
c) Interventions	Studies comparing the beneficial and harmful/limiting effects of CI and II. Infusions of all types (CI, PI and II), dose and regimen are adequate for the review. Pharmacoeconomic studies were also excluded.
d) Outcome measures	All studies were eligible if specifically related to clinical outcome/efficacy of dosing regimens. All outcomes were included to reduce risk of bias because of selective reporting.

CI= continuous infusion; II= intermittent infusion

3.2.3.3 Data Analysis

A data extraction form was developed based on Cochrane data extraction template. The information extracted from each of the included studies consisted of:

1. Characteristics of participants (didn't necessarily comprise characteristics such as age and sex, however, includes characteristics such as the disease patient is diagnosed with and the method of diagnosis) and the eligibility criteria (inclusion and exclusion measures)

2. The type of intervention – mode of administration, continuous vs intermittent dosing (including the drug, dose, duration of infusion and frequency)

3. Type of outcome measure (including clinical outcome and clinical efficacy in terms of clinical cure)

One reviewer extracted the following data from included studies (Sarah Fawaz (S.F)). The second and third reviewers verified the relevance of the extracted information (Shereen Nabhani-Gebara and Stephen Barton (S.N-G and S.B)). Variances in opinions were resolved by discussion between the three reviewers.

3.2.3.4 Risk of Bias and Study Quality Assessment

Methodological assessment of included RCTs was undertaken using the Cochrane risk of bias tool. Two reviewers individually assessed the risk of bias (S.F and S.N-G) with disagreements resolved by a third reviewer (S.B). Six domains of bias were assessed including: (1) random sequence generation, (2) allocation concealment, (3) blinding of participants and personnel, (4) incomplete outcome data, (5) selective reporting and (6) other biases. Publication bias was evaluated using funnel plots.

The methodological quality of included RCT's was assessed with the Jadad Scale (225) that evaluated the trial's randomisation, double blinding and reports of withdrawals and dropouts. An overall score of 0-5 points was assigned, where an overall score of three and above was regarded as adequate trial quality.

The Newcastle-Ottawa Scale is a quality assessment tool for selection, comparability and outcome assessment used to assess the quality of included observational studies (retrospective and prospective) (226). Studies scoring more than six stars are considered as being good quality.

No studies were excluded based on quality assessment however their quality scores were taken into account when describing results.

3.2.3.5 Statistical Analysis

Meta-analysis was performed using Review Manager for Windows Version 5.3 to compare the clinical efficacy of P/CI vs II in terms of clinical cure, mortality, microbiological cure rates, adverse events and length of hospital stay. Pooled odds ratio (OR) and 95% confidence intervals (C.I) were calculated for dichotomous data, considering all outcomes from included studies. Pooled mean difference (MD) and 95% C.I were calculated for continuous data. Statistical heterogeneity was assessed by employing χ^2 test and I^2 statistic. The presence of heterogeneity between studies was assessed by χ^2 test ($P < 0.10$ indicates significant heterogeneity) and the extent of the inconsistencies was considered using I^2 statistic ($I^2 >$ 70% indicates considerable heterogeneity). The pooled outcomes were calculated using Mantel-Haenszel fixed effect model when there was no significant heterogeneity otherwise the random effects model was chosen. 'Emergence of resistance' was narratively reviewed instead of statistical analysis considering the few sample sizes included.

3.2.4 Results

3.2.4.1 Search Results

The search of PubMed and Science Direct provided 199 citations. Of these, 154 studies were excluded following review of the abstracts, as they did not meet the inclusion criteria. Twenty articles were discarded after reviewing the full article due to the following reasons: non-human (n=2), on non-critically ill (n=10) and children (n=8) subjects. A further four studies were eliminated due to the focus being on pharmacoeconomic's and renal replacement therapy.

An additional two studies that met the inclusion criteria were acknowledged through checking references of relevant studies. Twenty-three studies met the described inclusion criteria and were included in the systematic review (162,171,192,200,240,263,267,288–292,172,293,294,173,178,179,187,189–191). The article selection process is illustrated in Figure 54 and selected studies comparing clinical outcome between CI and II of piperacillin are listed in Table 23. Characteristics of included studies comprising of demographic characteristics, P/CI and II dosage, drug regimen treatment results as well as study outcomes and suggestions were extracted from all studies and summarised (Table 23). Out of the twenty-three studies included, only an abstract (and no full article) could be obtained for four of the studies (189,288,289,292).

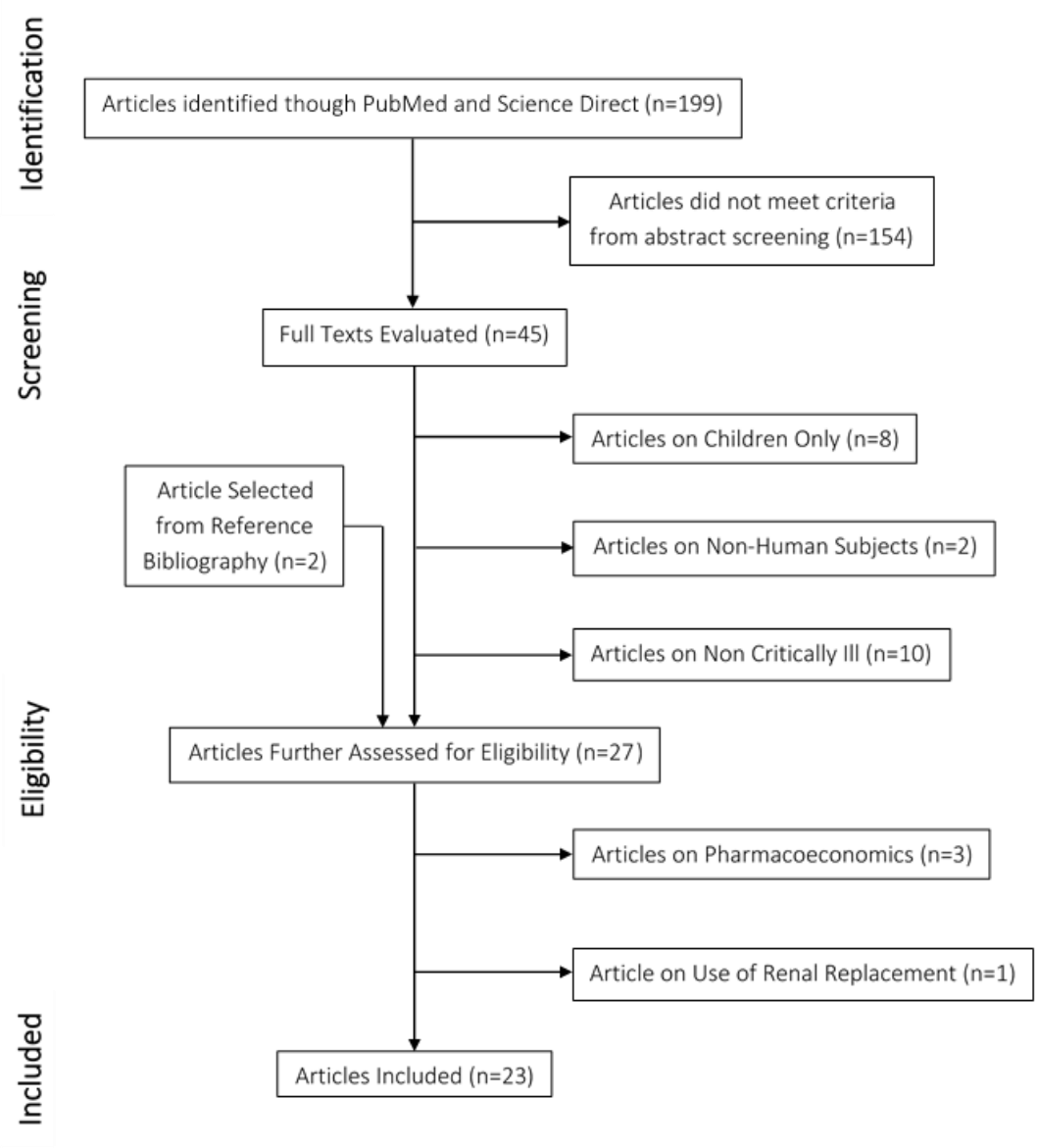


Figure 52 Flow diagram illustrating the selection process for included studies

3.2.4.2 Definitions

'Clinical cure' was defined as 'the complete resolution of clinical signs and symptoms of infection, with no new signs or symptoms associated with the original infection' (191,207).

'Microbiological cure' was defined as 'the eradication and presumed eradication of organisms at the infection site' (207).

'Adverse events' were defined as 'any unexpected medical occurrences in patients administered piperacillin-tazobactam caused by either the drug or dosing regimen being received' (207).

3.2.4.3 Study Characteristics

The type of studies included in the systematic review and meta-analysis were RCT's (n=10), observational cohort studies (n=12; retrospective n=10, prospective n=2) and a Quasi-experimental study (non-randomised trial) (n=1).

Table 23 Characteristics of studies comparing outcomes for continuous versus intermittent infusions of piperacillin-tazobactam.

Study	Study Design/Patient Population	Age (avg)	Gender	Dosage	Clinical Cure n/N (%)	Mortality n/N (%)	Outcome/Suggestions
Grants *et al.,* 2002 (178) *USA*	Prospective cohort study 98 ICU patients	CI - 66 II – 65	F - 37 M - 61	CI (n=47) – 2.25g LD + 9g DD over 24h CI II (n=51) – 3.375g every 6h over 30 min II	CI- 44/47 (94%) II- 42/51 (82%)	CI- 1/47 (2.1%) II- 5/51 (9.8%)	CI provided equivalent clinical and microbiologic to II. CI is a cost-effective alternative to II. CI is well tolerated resulting in CC.
Lau *et al.,* 2006 (179) *USA*	Randomised control trial 167 patients with gram +/- bacteria	CI – NR II – NR	F - NR M - NR	CI (n=81) – 13.5g over 24h CI II (n=86) - 3.375g every 6hrs over 30 min II	CI- 70/81 (86%) II- 76/86 (88%)	CI- 1/130 (0.8%) II- 3/132 (2.3%)	CI are a same and reasonable alternate mode of administration. No differences in bacteriological response by pathogen was noted between CI and II.
Rafati *et al.,* 2006 (172) *Iran*	Randomised control trial 40 Septic, critically ill patients	CI - 50.1 II – 48	F - 13 M - 27	CI (n=20) – 2.25g LD + 9g DD over 24h CI II (n=20) – 3.375g every 6h over 30 min II	CI- 15/20 (75%) II- 16/20 (70%)	CI- 5/20 (25%) II- 6/20 (30%)	Clinical efficacy as a CI is superior to that with II. CI significantly reduces severity of illness resulting in clinical cure.
Lodise *et al.* 2007 (162) *USA*	Retrospective cohort Study 194 ICU patients with Pa	PI - 63.2 II – 63.2	F - 75 M - 119	PI (n=102) – 3.375g every 8hrs over 4hr PI II (n=92) - 3.375g every 4-6hrs over 30 min II	PI- NR II- NR	PI- 5/41 (12.2%) II-12/92 (13%)	No difference in baseline clinical characteristics were noted between the two dosing regimens, however, mortality rates were significantly lower with PI.
Roberts *et al.,* 2009 (263) *Austrailia*	(*) Randomised control trial 16 Critically ill adult patients	CI - 30 II – 41	F - 5 M - 11	CI (n=8) – 4.5g LD + 9g DD over 24h CI II (n=8) – 4g every 6-8h over 20 min II	CI- 8/8 (100%) II- 8/8 (100%)	CI- 0/8 (0%) II- 0/8 (0%)	Administration by CI with initial loading dose achieves superior PD target and CC when compared with conventional II
Lorente *et al.,* 2009 (173) *Spain*	(*) Retrospective cohort study 83 ICU patients suffering VAP	CI - 63.2 II – 61.8	F - 18 M - 65	CI (n=37) – 4.5g LD + 18g DD over 24h CI II (n=46) – 4g every 6h over 30 min II	CI- 33/37 (89.2%) II- 26/46 (56.2%)	CI- 8/37 (21%) II- 14/46 (30.4%)	Higher clinical efficacy achieved by continuous infusion. Higher DD reached target concentration for pathogens with higher MIC's
Li *et al.,* 2010 (288) *China*	Randomised control trial 66 patients with severe pneumonia	PI – NR II – NR	F - NR M - NR	CI (n=28) - 4.5g every 8 hrs over 8hr CI II (n=31)- 4.5g every 8hrs over 30 min II	CI- 24/32 (75%) II- 17/34 (50%)	CI- NR II- NR	Results obtained from the study suggest clinical advantages of CI compared with II administration in patients suffering with severe pneumonia.
Rose *et al.,* 2011 (295) *USA*	Retrospective cohort study 90 ICU patients	PI - 58.4 II – 60.4	F - 13 M - 77	PI (n=54) – 3.375g every 8-12 hrs over 4hr PI II (n=36) - 3.375g every 8-12 hrs over 30 min II	PI- NR II- NR	CI- NR II- NR	PI reduced: (1) days of therapy in ICU, (2) time spent on ventilator, (3) length of ICU and hospital stay and, (4) mortality.
Ye *et al.,* 2011 (289) *China*	Randomised control trial 66 ICU patients, gram (–) bacteria	PI – NR II – NR	F - NR M - NR	PI (n=35) - 4.5g every 8hrs over a 3h PI II (n=31) – 4.5g every 8hrs over 30 min II	PI- 24/35 (68.6%) II- 13/31 (41.9%)	PI- 8/35 (22.9%) II- 8/31 (25.8%)	Prolonged infusion is superior to traditional regimens and should be recommended as empirical therapy for gram (-) bacteria
Yost *et al.,* 2011 (290) *USA*	Retrospective cohort study 270 ICU patients with Pa	PI - 65 II – 62	F - 129 M - 141	PI (n=186) - 3.375g every 8 hrs over 4hr PI II (n=84) - dose not recorded, 30 min II	PI- 171/186 (90.3%) II- 67/84 (79.8%)	PI- 18/186 (9.7%) II- 17/84 (20.2%)	Pharmacodynamic dosing via PI's of piperacillin-tazobactam demonstrated positive outcome compared with II. PRT need to further verify findings.
Fahmi *et al.,* 2012 (291)	Quasi experimental study	PI – NR II – NR	F - NR M - NR	PI (n=31) – 3.375g every 8hrs over a 4h PI II (n=30) - 3.375g every 6hr over 30 min II	PI- NR II- NR	PI- NR II- NR	No significant difference in clinical outcome of PI and II. Suggest administration by PI or II according to MIC of organism.

	61 ICU patients with VAP					
Pereira *et al.,* 2012 (240) *Portugal*	Retrospective cohort study 346 ICU patients	CI – NR II – NR	F - NR M - NR	CI (n= 173) – Majority 18g DD, every 8hr II (n=173) – Majority 18g DD, 30 min II	CI- 124/173 (71.7%) II- 124/173 (71.7%)	CI- 49/173 (28.3%) II- 49/173 (28.3%)	Clinical efficacy of piperacillin-tazobactam dosing was independent of the mode of administration. CI is not associated with a decrease in mortality.
Lee *et al.,* 2012 (187) *USA*	Retrospective cohort study 148 ICU patients	PI – 64 II – 69.6	F - 64 M - 84	PI (n=68) – 3.375g every 8hrs over 4hr PI II (n=80)- 2.25g every 6hr over 30 min II	PI- 55/68 (81%) II- 50/80 (62%)	PI- 13/68 (19.1%) II- 30/80 (37.5%)	Results suggest improved 30-day mortality in ICU patients treated via PI vs CI. Clinical benefits of PI at lower MIC's are less substantial compared with more RO.
Waxier *et al.,* 2012 (292)	Retrospective cohort study 400 ICU patients	PI – NR II – NR	F - NR M - NR	PI (n=200) - dose not recorded, over 4hr PI II (n=200) - dose not recorded, over 30 min II	PI- NR II- NR	PI- NR II- NR	PI patients received fewer doses and demonstrated decreased morbidity and mortality; results however are not SS so larger prospective studies are needed.
Lu *et al.,* 2013 (189) *China*	Randomized control trial 50 patients with HAP	PI – NR II – NR	F - NR M - NR	PI (n=25) - 4.5g every 6hrs over a 3h PI II (n=25) - 4.5g every 6hrs over 30 min II	PI- 22/25 (88%) II- 20/25 (80%)	PI- NR II- NR	PI's of piperacillin-tazobactam for gram negative bacteria with high MIC values, like HAP, provide stable plasma concentration and curative clinical effect.
Cutro *et al.,* 2014 (293) *USA*	Retrospective cohort study 843 patients suffering from sepsis	PI – NR II – NR	F - NR M - NR	PI (n=662) – 2.25-3.375g every 6-12h over 4h PI II (n=181) – 2.25-4.5g every 8-12h over 30 min II	PI- 540/662 (81.6%) II- 145/181 (80.1%)	PI- 72/662 (10.9%) II- 25/181 (13.8%)	No significant difference between the two dosing regimens was observed in terms of mortality or clinical cure however PI resulted in shorter duration of therapy.
Jamal *et al.,* 2015 (171) *Malaysia*	(*) Randomised control trial 16 ICU patients	CI - 44 II – 62.5	F - 4 M - 12	CI (n=8) - 2.25g LD + 9g DD over 24h CI II (n=8) – 2.25g every 6hr over 30 min II	CI- 6/8 (75%) II- 6/8 (75%)	CI- 0/8 (0%) II- 0/8 (0%)	CI is advantageous in the presence of more resistant pathogens as it allows achievement of rapid and consistent piperacillin-tazobactam concentrations.
Abdul *et al.,* 2016 (200) *Malaysia*	(*) Randomised control trial 85 ICU patients	CI - 54 II – 56	F - 27 M - 58	CI (n=38) – dose not recorded II (n=47) – dose not recorded	CI- 22/38 (58%) II- 15/47 (32%)	CI- 7/38 (18.4%) II- 20/47 (42.6)	Results showed that CI piperacillin-tazobactam demonstrated higher clinical cure rates and better PK/PD target attainment compared to II.
Schmees *et al.,* 2016 (294) *USA*	Retrospective cohort study 113 ICU patients	PI - 68 II – 59.4	F - 47 M - 66	PI (n=61) – 3.375-4.5g every 8-12h II (n=52) – dose not recorded	PI-31/61 (50.8%) II-22/52 (42.3%)	PI-9/61 (14.8%) II-11/52 (21.1%)	Mortality rates and length of hospital stay were significantly lower in PI patients. PI improves patient outcomes while maintaining patient safety.
Cortina *et al.,* 2016 (190) Spain	Randomised control trial 78 Patients with suspected Pa	CI - 64.3 II – 63.8	F - 32 M - 46	CI (n=40) – 2.25g LD + 8g DD over 24h CI II (n=38) – 4.5g every 8h over 30 min II	CI- 20/40 (50%) II- 18/38 (47.4%)	CI- 0/40 (0%) II- 1/38 (2.6%)	No SS difference in efficacy between CI & II. Data indicates better performance of II than CI. II cure rates almost doubled CI.
Winstead *et al.,* 2016 (267) *USA*	Retrospective cohort study 181 patients, gram (-) bacteria	PI - 65.1 II – 68.2	F - 99 M - 82	PI (n=86) – 4.5g LD + 3.375g every 6h over 3h PI II (n=95) - 4.5g every 8hrs over 30 min II	PI- NR II- NR	PI- 7/86 (8.1%) II- 6/95 (6.3%)	No SS difference in the primary outcome of mortality and length of hospital stay, however, 30-day hospital re-admission was significantly reduced in PI patients.
Bao *et al.,* 2017 (191) *China*	Randomised control trial 50 patients with HAP	PI - 69.75 II – 67.04	F - 21 M - 29	PI (n=25) – 4.5g every 6h over a 3h PI II (n=25) – 4.5g every 6h over 30 min II	PI- 22/25 (88%) II- 20/25 (80%)	PI- 0/25 (0%) II- 0/25 (0%)	Dosing regimen had no impact on adequacy of treatment and that PI is as effective as II. PI is

							potentially a more cost-effective alternative to II.
Fan *et al.,* 2017 (192) *China*	Prospective cohort study 367 ICU patients	PI - 69 II – 70	F - 120 M - 247	PI (n=182) - 4.5g every 8-12h over 4h PI II (n=185) - 4.5g every 8-12h over 30 min II	PI- NR II- NR	PI- 21/182 (11.5%) II- 29/185 (15.6%)	No significant difference between the two dosing regimens in terms of mortality rate and length of hospital stay

ICU= intensive care unit; CI= continuous infusion; II= intermittent infusion; PI= prolonged infusion; F= female; M= male; MIC= minimal inhibition concentration; LD= loading dose; DD= daily dose; VAP= ventilator-associated pneumonia; PD= pharmacodynamic; CC= clinical cure; Pa= pseudomonas aeruginosa; SS=statistically significant; PRT=prospective randomised trials; RO=resistant organisms; HAP=hospital acquired pneumonia; NR= not recorded; (*)= studies that reported SOFA score.

3.2.4.4 Study Quality

The quality of the majority of RCT's included was moderate to high (**Table 24**). According to the Jadad scale, seven out of ten RCT's (70%) obtained a score of three and above. The studies by Ye (289) and Lu (189) had a score of one and two respectively due to retrieval of only the abstract (full text unavailable). Rafati (172) received a score of two as the article did not describe randomisation method and study was not blinded. All observational studies assessed using the Newcastle Ottawa Scale scored eight or nine stars and are recognised as being of high quality (**Table 25**).

Table 24 Quality assessment of randomised control trials in meta-analysis based on the Jadad Scale.

Quality Assessment of RCTs	Lau (179)	Rafati (172)	Robert (263)	Li (288)	Ye (289)	Lu (189)	Jamal (171)	Abdul (200)	Cotrina (190)	Bao (191)
	2006	2006	2009	2010	2011	2013	2015	2016	2016	2017
[(1)] Described as randomised	1	1	1	1	1	1	1	1	1	1
[(2)] Described as double blind	0	0	0	0	0	0	0	0	1	0
[(3)] Description of withdrawals	1	1	1	1	0	1	1	1	1	1
[(4)] Randomisation method described	1	0	1	1	0	0	1	1	1	1
[(5)] Double blinding method described	0	0	0	0	0	0	0	0	1	0
Score (-/5)	3/5	2/5	3/5	3/5	1/5	2/5	3/5	3/5	5/5	3/5

Randomisation:

Up to two points are given: [(1)] described as randomised (yes = 1) (no = 0) and [(4)] randomisation method described (yes = 1) (no = 0)

Double blinding:

Up to two points are given: [(2)] described as double blind (yes = 1) (no = 0) and [(5)] double blinding method described (yes = 1) (no = 0)

Reports of withdrawals and dropouts:

Up to one point is given: [(3)] description of withdrawals (yes = 1) (no = 0)

Table 25 Quality assessment of observational studies based on the Newcastle-Ottawa Scale

Study	Selection				Comparability	Outcome			Score
	A	B	C	D	E	F	G	H	
Grants 2002 (178) [p]	*	*	*	*	**	*	*	*	9*
Lodise 2007 (162) [r]	*	*	*	*	**	*	*	*	9*
Lorente 2009 (173) [r]	*	*	*	*	**	*	*	*	9*
Rose 2011 (295) [r]	*	*	*	*	**	*	*	*	9*
Yost 2011 (290) [r]	*	*	*	*	*	*	*	*	8*
Pereira 2012 (240) [r]	*	*	*	*	**	*	*	*	9*
Lee 2012 (187) [r]	*	*	*	*	**	-	*	*	8*
Waxier 2012 (292) [r]	*	*	*	*	**	-	*	*	8*
Cutro 2014 (293) [r]	*	*	*	*	*	*	*	*	8*
Schmees 2016 (294) [r]	*	*	*	*	**	-	*	*	8*
Winstead 2016 (267) [r]	*	*	*	*	**	-	*	*	8*
Fan 2017 (192) [p]	*	*	*	*	**	-	*	*	8*

Selection:

*A: representation of the exposed cohort (yes = *) (no= -), b: selection of non-exposed cohort (yes = *) (no= -), c: ascertainment of exposure (yes = *) (no= -), d: demonstration that outcome of interest was not present at start of study (yes = *) (no= -)*

Comparability:

*E: comparability of cohorts based on the design or analysis [controls for: age, sex and marital status (yes = *) (no= -) and for other factors (yes = *) (no= -)]*

Outcome:

*F: assessment of outcome (yes = *) (no= -), g: was follow up long enough for outcome to occur (yes = *) (no= -) and h: adequacy of follow up of cohorts (yes = *) (no= -).*

3.2.4.5 Meta-Analysis of Included Studies

3.2.4.5.1 Clinical Cure

Seventeen of the included studies reported clinical cure rates (**Table 23**) (171,172,210,240,288–290,293,294,173,178,179,187,189–191,200). Patients that received P/CI had a statistically significantly higher clinical cure rate compared to those who received treatment via II (2535 patients; OR 1.56, 95% C.I 1.28-1.90, P = 0 .0001; **Figure 55**). No significant heterogeneity was found among the studies (I^2 = 41%, P = 0.04). The symmetrical funnel plot obtained indicates the absence of publication bias (**Figure 56**).

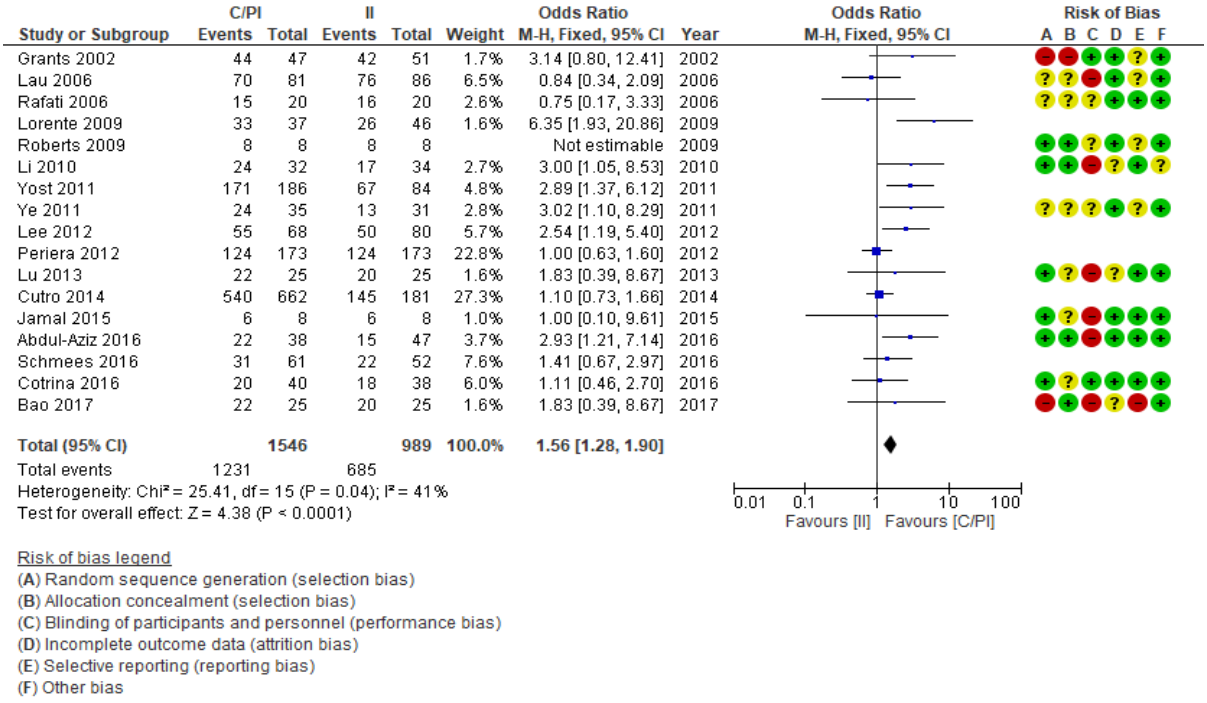


Figure 53 Forest plot representing the odds ratio of clinically cured patients from the P/CI and II patients in included studies

Despite methodological differences among selected studies, patients receiving P/CI displayed higher clinical cure rates compared with patients receiving II; overall, clinical cure rate was 79.62% and 69.26% for P/CI and II respectively. Pooling results from the 17 studies that reported clinical cure showed that the odds of clinical cure was higher in patients receiving P/CI. The pooled OR shows that P/CI piperacillin-tazobactam was 1.56 (95% C.I 1.28-1.90, P = 0 .0001), indicating clinical cure rates are 34% higher than in II with the true population effect between 72% and 10%.

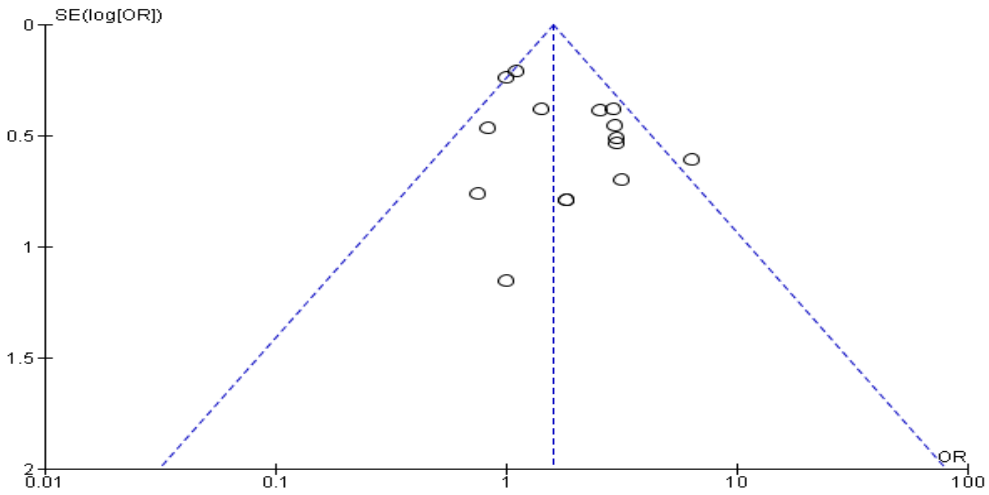


Figure 54 Symmetric funnel plot indicating the absence of publication bias in terms of clinical cure

3.2.4.5.2 Mortality

Eighteen of the included studies reported patient mortality rates (**Table 23**) (162,171,200,263,267,289,290,293,294,296,172,173,178,179,187,190–192). Statistically significantly fewer mortality rates were found among patients receiving P/CI compared with patients receiving conventional II (3100 patients; OR 0.68, 95% C.I 0.55-0.84, P = 0 .0003; **Figure 57**). No significant heterogeneity was found among the studies (I^2 = 0%, P = 0.56). The symmetrical funnel plot obtained indicates the low possibility of publication bias (**Figure 58**).

Results obtained from meta-analysis suggested that P/CI piperacillin-tazobactam resulted in significantly lower mortality rates. Overall, ICU mortality rate was 12.46% and 18.13% for P/CI and II respectively. Combining results from 18 studies that reported mortality, the pooled OR shows that P/CI piperacillin-tazobactam was 0.68 (95% C.I 0.55-0.84), indicating lower mortality rates compared with conventional II. This was statistically significant (P = 0.0003) with the true population effect between 84% and 55%.

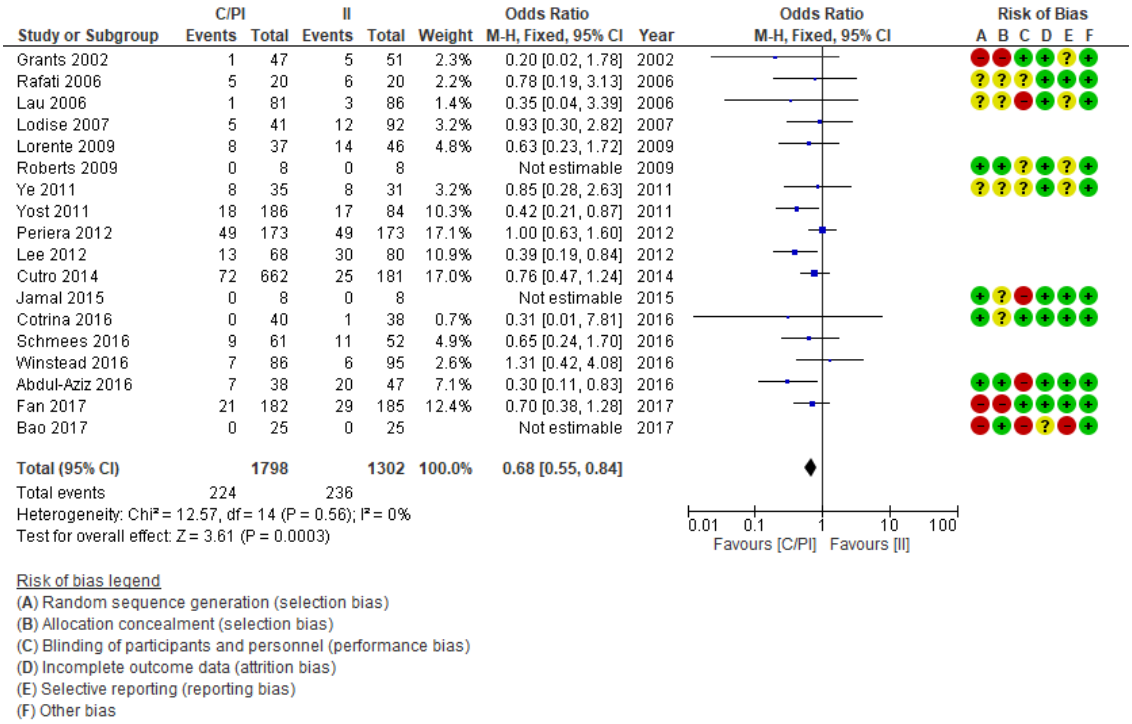


Figure 56 Forest plot representing the odds ratio of mortality patients from P/CI and II patients in included studies

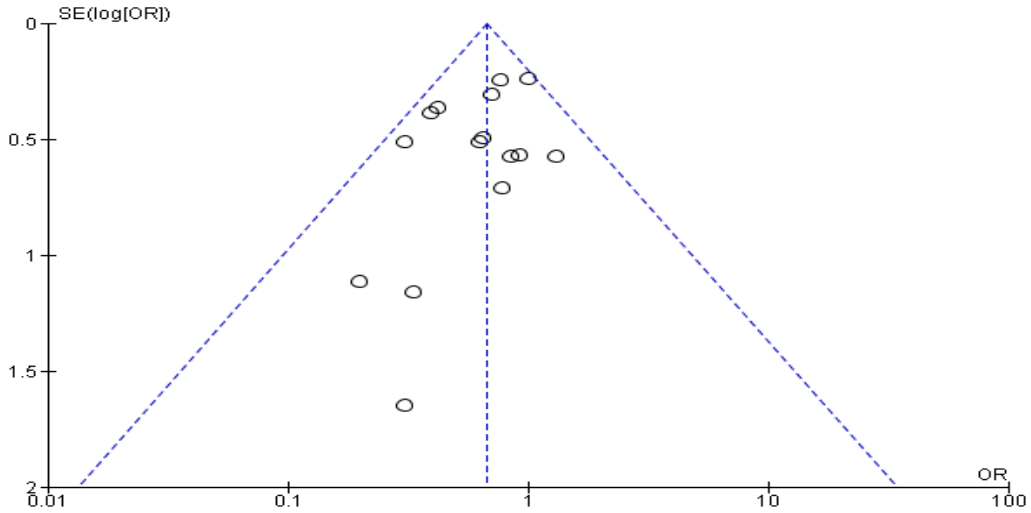


Figure 55 Symmetric funnel plot indicating the absence of publication bias in terms of patient mortality

3.2.4.5.3 Microbiological Cure

Seven of the included studies reported microbiological cure rates (178,179,200,240,288,289,293). Lau *et al.*, (179) found no statistically significant difference between the dosing regimens however, higher microbiological success was seen in patients receiving II. In contrast, Abdul-Aziz *et al.*, (200) found P/CI piperacillin-tazobactam had significantly higher microbiological cure rates compared with II. Pooling of the outcomes of seven studies that reported microbiological cure rates showed that patients receiving P/CI had significantly higher microbiological success rates (920 patients; OR 1.52, 95% C.I 1.10-2.11, P = 0.01; **Figure 59**). No significant heterogeneity was found among studies (I^2 = 0 %, P = 0.48). The symmetrical funnel plot obtained demonstrates the absence of publication bias (**Figure 60**).

The pooled OR shows that P/CI piperacillin-tazobactam was 1.52 (95% C.I 1.10-2.11), indicating P/CI piperacillin-tazobactam achieved higher microbiological cure rates compared to conventional II. Overall, microbiological cure rates were 74.83% and 61.89% for P/CI and II respectively. This was statistically significant (P = 0.01).

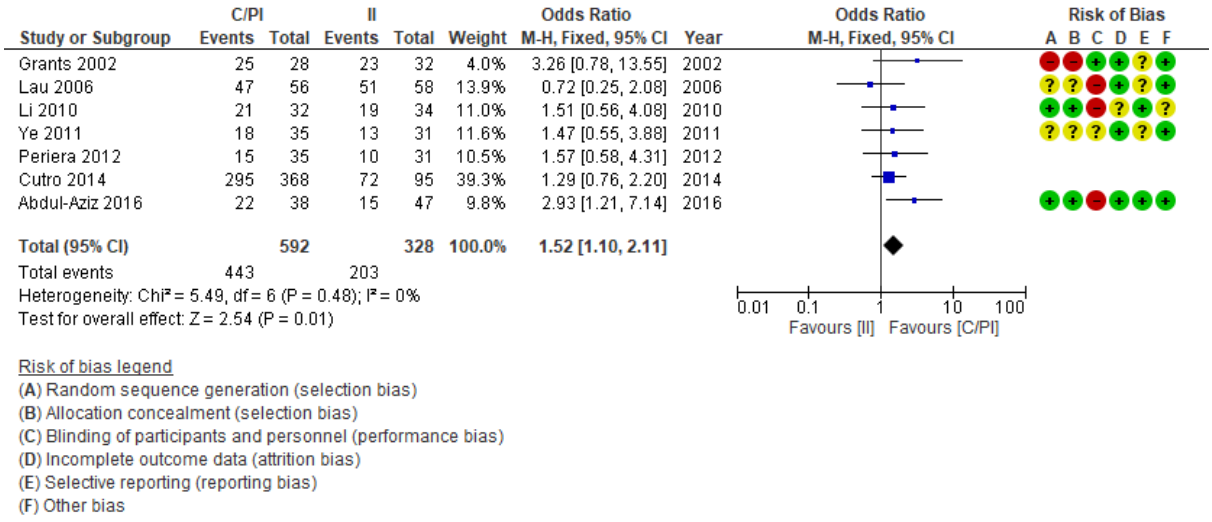


Figure 57 Forest plot representing the odds ratio of microbiologically cured patients from the P/CI and II patients in included studies

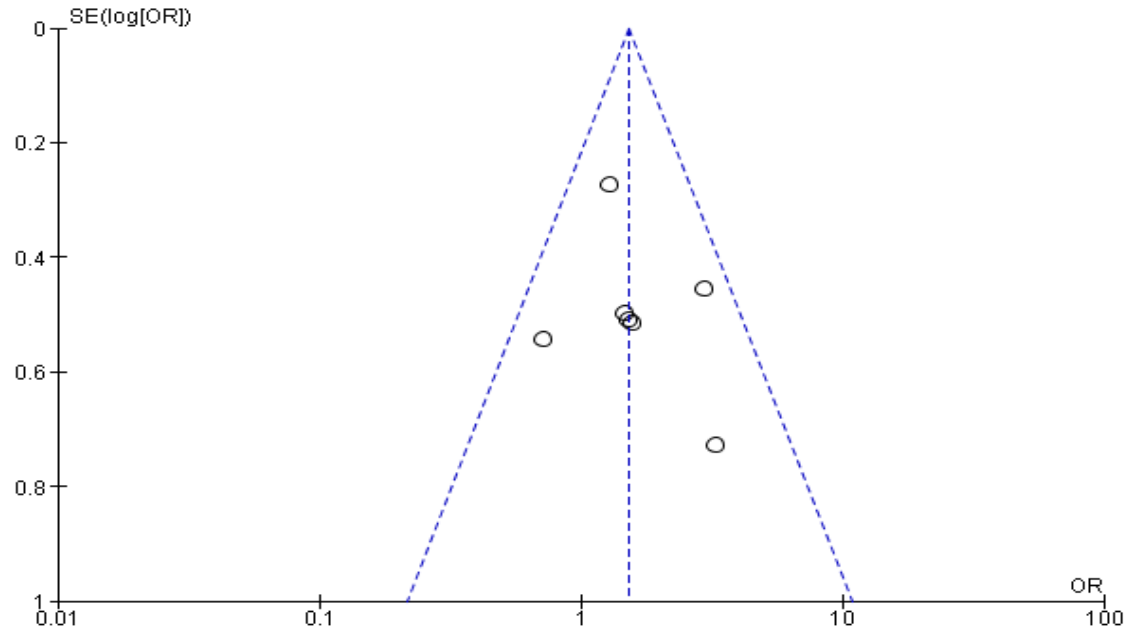


Figure 58 Symmetric funnel plot indicating the absence of publication bias in terms of microbiological cure

3.2.4.5.4 Adverse Events

Six of the included studies reported on adverse events (178,179,191,192,200,294). Three studies reported that no participants experienced adverse events. Participants enrolled in three of these studies experienced adverse event (179,191,294). Lau *et al's*., (179), Bao *et al*., (191) and Schmees *et al*., (294) observed treatment-related adverse events in patients receiving both P/CI and II; CI: 16.9% vs II:13.6%, CI: 47.5% vs II:53.8%, CI: 76% vs II:92%, respectively. Boa *et al*., (191) reported serious adverse events in 9 patients (PI:5 vs II:4), including renal failure, Tachycardia and confusion.

The average occurrence of adverse events was 13.3% for P/CI and 13.4% for II, respectively. Participants in the other three studies did not experience adverse events (178,192,200). Data obtained from studies showed no significant difference between the two infusion strategies (935 patients; OR 0.85, 95% C.I 0.50-1.42, P = 0.53; **Figure 61**). No significant heterogeneity was found among studies (I^2 = 25 %, P = 0.26).

Although adverse events were not observed in the study by Grants *et al*., (178), dosing and administrative errors arose where one patient was administered 13.5g piperacillin-tazobactam dose over a 30 minute II rather than a 24-hour CI. Cortina *et al*., (190) reported that the most common side effects experienced by patients were gastrointestinal and

allergic reactions but the number of patients that experienced these was not reported. The meta-analysis demonstrated that no adverse events that are directly associated to the dosing regimens occurred. P/CI resulted in a lower percentage of adverse events however, the difference between the two groups did not reach statistical significance (935 patients; OR 0.85, 95% C.I 0.50-1.42, P = 0.53; Figure 61).

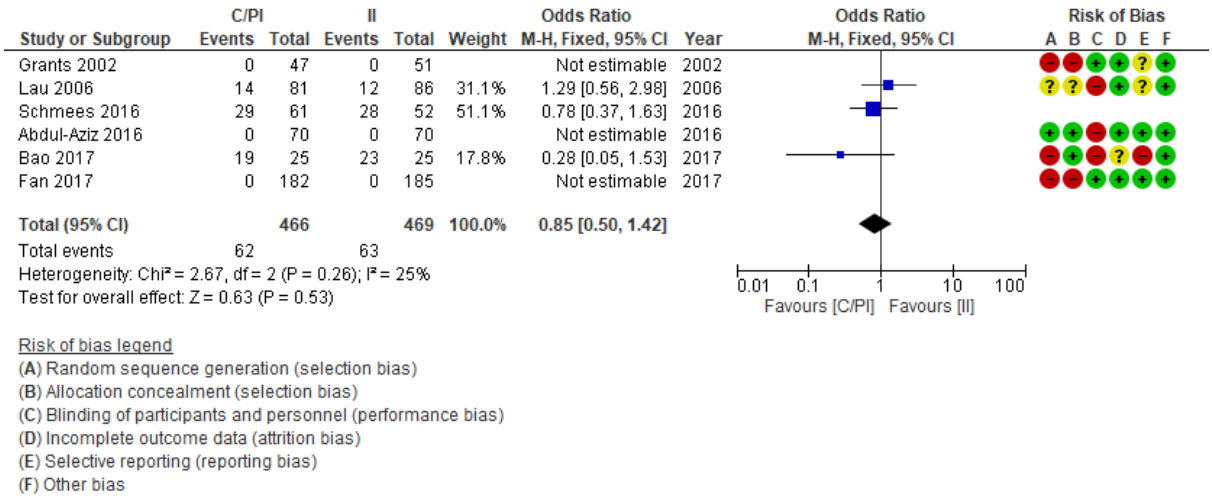


Figure 59 Forest plot representing the odds ratio of adverse events experienced by patients from the P/CI and II groups in included studies

3.2.4.5.5 Length of Hospital Stay

Fifteen of the included studies reported length of hospital stay (162,172,240,267,294,295,297,173,178,179,187,189,190,192,200). Pooling of studies showed that patients receiving P/CI had a significantly shorter length of hospital stay (2101 patients; Mean Difference -1.27, 95% C.I -2.45—0.08, P = 0.04; Figure 62) The meta-analysis suggests there is a significant reduction in the length of hospital stay in patients receiving P/CI compared to those receiving II. Moderate heterogeneity among studies evaluating 'length of hospital stay' (I^2= 65%, P = 0.0003) was observed. This is likely due to clinical heterogeneity in the design and outcomes of the included studies. The length of hospital stay was an independent risk factor for mortality, however the influence of mortality on the length of hospital stay could not be evaluated.

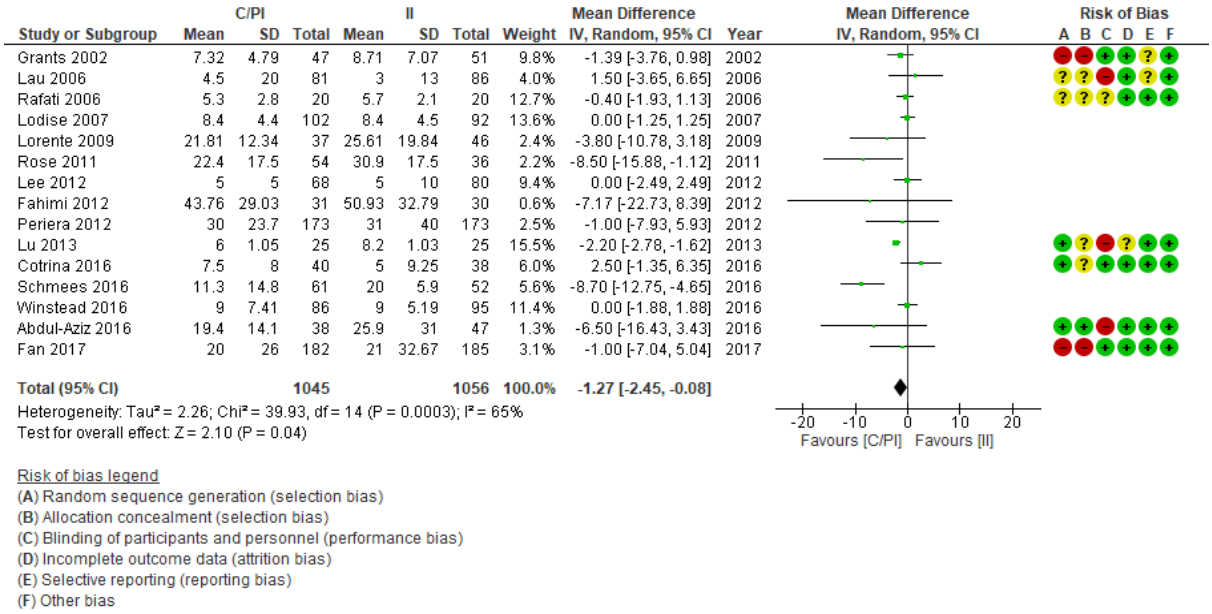


Figure 60 Forest plot representing the MD of length of hospital stay in P/CI and II groups in included studies

3.2.4.5.6 Emergence of Resistance

Data regarding the emergence of resistance was reported in four of the included studies (173,178,179,263). Two resistant pathogens were isolated in one study (178) however, resistant strains were not isolated in three studies (173,179,263) following the initiation of piperacillin-tazobactam treatment. Three studies reported that no resistant pathogen was isolated following the initiation of piperacillin-tazobactam treatment. In the study conducted by Grant *et al*., (178), two resistant strains were isolated from patients receiving CI piperacillin-tazobactam.

3.2.4.5.7 Risk of Bias

The majority of RCT's and prospective studies assessed were judged to have a low risk of bias for random sequence generation, allocation concealment, incomplete outcome data, selective reporting and other biases. However, the blinding of participants and personnel parameter was judged to have a high or unclear risk of bias (**Figure 63**).

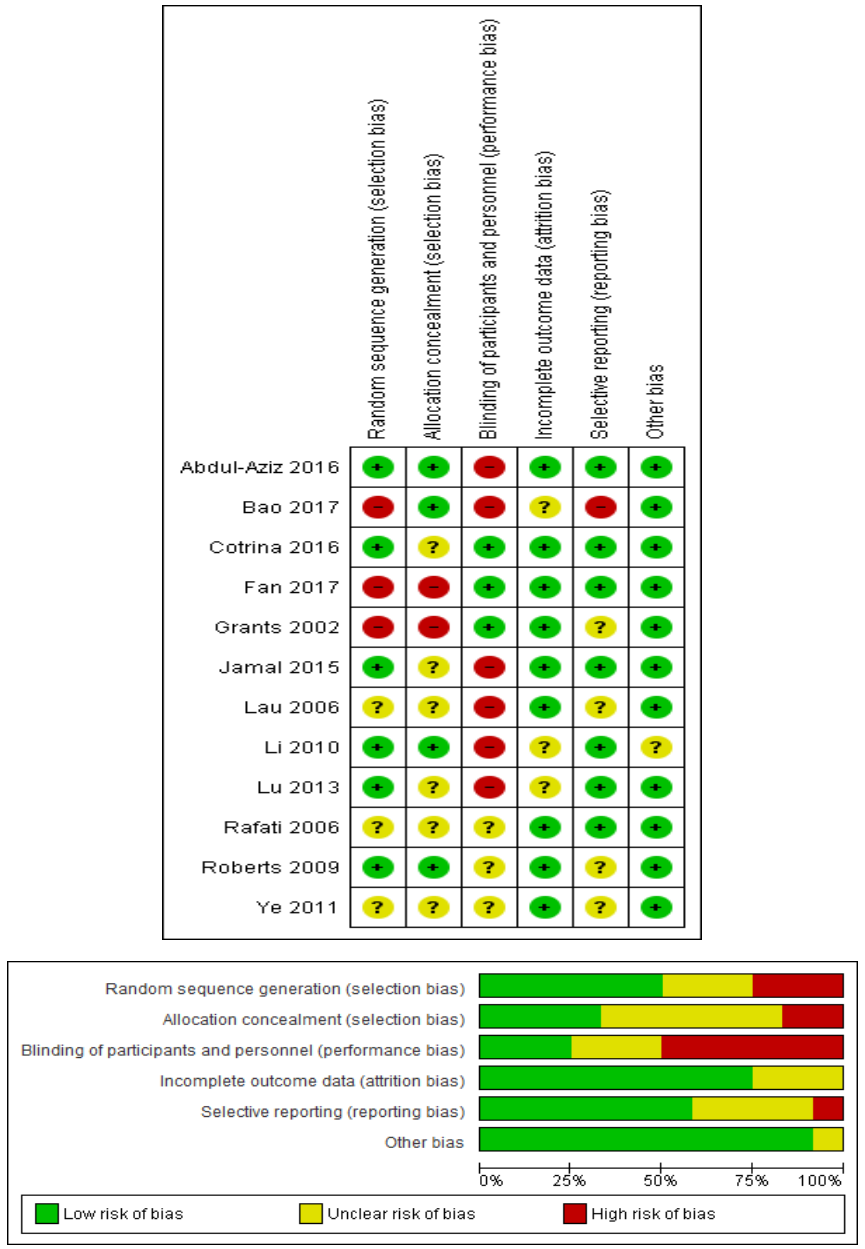


Figure 61 a) Risk of bias summary of included RCT's: displaying details about each risk of bias item for each trial. Green (+) indicates 'low risk', red (-) indicates 'high risk' and yellow (?) indicates 'unclear risk'. b) Risk of bias assessment displaying judgements about each risk of bias item presented as percentages across all RCT's

3.2.5 Discussion

To the best of our knowledge, this systematic review and meta-analysis is the largest study describing clinical outcomes of severely ill patients treated with either P/CI or II piperacillin-tazobactam. The selected studies involved 3828 critically ill adult participants in total (P/CI=2197 and II=1631) from geographically diverse regions.

It is the first meta-analysis that shows P/CI resulted in significantly: (1) higher clinical cure rates (2) lower mortality rates (3) higher microbiological success rates and (4) decreasing the length of hospital stay specifically in critically ill patients. In all the studies, the primary outcome assessed was clinical efficacy. The current study differs from previously published systematic reviews and meta-analyses (202,203,205,207,213,215,216,229) as it specifically focuses on use of piperacillin tazobactam in critically ill ICU patients. The present systematic review and meta-analysis identified a significant clinical cure, mortality, microbiological cure and length of hospital stay benefit for P/CI across all included studies.

In theory, P/CI of piperacillin-tazobactam is a broadly recognised strategy to optimize antibiotic therapy, where concentrations remain above the MIC for a higher percentage of time. Studies have demonstrated that the amount of time in which the free or non-protein bound antibiotic concentration exceeds the MIC (fT > MIC) of the organism is the best predictor of clinical and microbiologic response for β-lactams (235,298). However, data to backup this developing practice have been sparse (205). Twenty-three published studies comparing P/CI and II of piperacillin-tazobactam fit the inclusion criteria (Table 23).

Outcomes of the current study correlate and expand upon previously published reviews including several analyses comparing clinical efficacy of dosing regimens for beta-lactams generally (202,203,213,216). These studies pointed towards a more favourable outcome of P/CI for improved clinical cure and resolution of illness. Falagas *et al*., 2013 (213) and Vardakas *et al*., 2018 (216) reviewed outcomes of P/CI and II beta-lactams. There was a significant reduction in mortality rates among patients receiving P/CI in both studies. Roberts *et al*., 2016 (203) observed higher clinical rates and reduced mortality in P/CI patients and Lal *et al*., 2016 (202) found P/CI to reduce clinical failure rates.

Finding in this study are consistent with published reviews focused specifically on piperacillin-tazobactam (205,207,215,229). Yusuf *et al*., 2014 (229) reviewed literature comparing the effectiveness of P/CI and II administration of piperacillin-tazobactam. They documented P/CI improved clinical cure, mortality and length of hospital stay in comparison to II. Yang *et al*., 2015/6 (207,215) observed similar beneficial effects of P/CI in their systematic reviews. Recently, Rhodes *et al*., 2017 (205) evaluated a wide range of severely ill patients, from hospitalised patients to critically ill patients admitted to ICU. P/CI piperacillin-tazobactam is associated with improved clinical outcome and significantly reduced mortality rates.

Several observations were encountered from reviewing this data which led to reduced comparability among studies. First, clinical heterogeneity was present as selected studies comparing P/CI and II in terms of clinical outcomes have confounding factors including patient sample size, study settings, study design, quality, intervention and outcomes. Second, information regarding monotherapy and combination antibiotic therapy were not reported in the included studies. This reduces the validity of conclusions on P/CI, as agents used possess different antimicrobial spectrum, and drug-drug interactions were unknown hence not considered. Third, assessing safety was challenging due to under-reporting of adverse events. Higher serum concentrations in P/CI patients over a longer period could potentially result in an increased number of adverse events. Fourth, a large number of included studies were RCT's (10/23; 43.5%) with small sample size. Small sample size may result in bias and the probability of small study effects contributing to the favourable outcome for P/CI. However, meta-analyses including small and large studies did not indicate significant discrepancies and similar outcomes were observed with fixed and random effect models. Fifth, duration of piperacillin-tazobactam administration and dosing is not homogenised between studies. CI was administered over the entire dosing interval and the duration of a PI between studies ranged between 3-4 hours which is in line with proposed guidelines (2-4 hours). Traditional II durations between studies ranged between 20-30 minutes (usually 30-60 minutes) (299). Heterogeneity of dosing was also noted. In 7/23 studies, piperacillin-tazobactam treatment was initiated with a loading dose to ensure rapid achievement of therapeutic concentrations. Also, the total daily dose administered differed between CI, PI and II, providing an additional confounding factor as to whether the duration

of infusion or total daily dose attributed to clinical outcome (Table 23). Finally, it wasn't apparent how critically ill the patients within studies were as only four studies reported SOFA scores.

Findings of this meta-analysis should be interpreted in view of certain limitations. First, throughout this review, PI and CI were combined and referred to as P/CI, thus, it is unclear which of the two dosing strategies is most effective for critically ill patients. Additionally, all studies were evaluated for quality and risk of bias and based on the overall assessment of these two factors no studies were excluded (Table 24 and Table 25) (Figure 63). Also, a medical librarian was not involved in this study.

3.2.6 Conclusion

In conclusion, P/CI of piperacillin-tazobactam in critically ill patients was associated with (1) higher clinical cure rates (2) lower mortality rates, (3) higher microbiological success rates and, (4) decreasing the length of hospital stay in critically ill ICU patients. No reduction in 'adverse events' and 'emergence of resistance' has been demonstrated. Results obtained in this study show that clinical outcome in critically ill patients is significantly better in those receiving P/CI. However, the superiority of the benefits and outcome gains achieved with P/CI administration in comparison to II is difficult to deduce as studies selected show considerable heterogeneity in terms of: (1) type of isolated bacteria, (2) piperacillin-tazobactam dose, (3) MIC of pathogen, (4) patient renal function, (5) duration of hospital stay and (6) outcome definitions. More rigorous scientific studies in critically ill patients are warranted to reach a sufficient level of evidence to promote the widespread adoption and further implementation of P/CI piperacillin-tazobactam.

3.3 Aim and Objectives

The route of administration and the correct dose are critical in deciding how to treat a patient appropriately. Several factors such as stability, compatibility, toxicity, contamination, ease of preparation, ease of administration and desired serum antibiotic levels must be considered when choosing the suitable method of administering antibiotics.

Despite all the advantages P/CI piperacillin-tazobactam offers, in order to use this dosing regimen efficiently, an in-depth study of piperacillin-tazobactams stability is required. Concerns regarding the stability of beta-lactam antibiotic solutions present a challenge in practice as most stability information is based on administration via a bolus injection or intermittent infusion. Therefore, the aim of this study is to determine the feasibility of continuous infusion piperacillin-tazobactam in hospital and OPAT settings.

To achieve this aim, the following objectives were set:

1) To develop a stability indicating HPLC method for the quantitative determination of piperacillin-tazobactam concentration over time
2) To determine the specificity of the developed method by conducting a forced degradation study
3) To validate the developed method in compliance with the ICH guidelines
4) To conduct a stability study to determine piperacillin-tazobactam stability when:
    a. Using different diluents
    b. Storing infusion solutions at different temperatures and,
    c. Storing infusion solutions in different infusion devices

3.4 HPLC Method Development

As previously mentioned in **Chapter 1**, a stability indicating method (SIM) is a quantitative analytical technique used to detect a decrease in the amount of API present due to degradation. Due to its remarkable separating abilities, HPLC is an integral analytical tool in assessing drug product stability and it is a prevalent technique adopted to monitor decrease in drug concentration and a corresponding increase in degradation product. The developed HPLC method should however ensure that parent compounds and their degradation products are separated with sufficient resolution and detected appropriately (300,301).

Various stability indicating methods for the determination of piperacillin-tazobactam are reported in the literature; these developed methods define piperacillin-tazobactam in pure drug, pharmaceutical dosage form and in biological samples. A number of analytical methods were utilized including HPLC, LC-MS, micellar electro-kinetic capillary chromatography, TLC and spectrophotometry (3–14).

Campanero *et al*., (304) developed a method to quantify the therapeutic levels of piperacillin and ceftazidime in human plasma. Microbiological assays have been previously employed, however, they are time consuming and potentially subject to interference from concurrently administered antibiotics. The aim was to establish a simple and rapid HPLC method that displays good sensitivity without the time-consuming sample preparation procedures in the form of liquid-liquid extractions and solid phase extraction. The internal standard (para-propionamidophenol) and the plasma were precipitated with 20% trichloroacetic acid. The supernatant was analysed on 5µm Spherisorb octadecyl silyl (ODS) C_{18} column. The mobile phase was composed of acetonitrile and 0.05M phosphate buffer (pH=3.8) (79:21 v/v). Analysis was conducted with UV detection at the wavelength of 254nm (304).

Similarly, a simple and economical HPLC method was developed by McWhinney *et al*., (307) to quantify antibiotic concentration in 200µL of human plasma. Sample preparation involved precipitation of proteins with acetonitrile and the removal of lipid-soluble components by a chloroform wash. The internal standard selected was oxacillin. Chromatographic separation was achieved on a Waters X-bridge C18 column using acetonitrile-phosphate buffer (pH=3.0) (25:75 v/v) mobile phase. UV detection was conducted at the wavelength of 210nm. This

method has been utilised in pathology labs for therapeutic drug monitoring of beta-lactam antibiotics in critically ill patients (307).

A gradient elution HPLC method was developed by Ocampo *et al*., (308) for analysis of human plasma, serum, bile and urine. De-proteinisation of plasma, serum and bile was achieved with the addition of acetonitrile and the removal of lipids involved adding dichloromethane to the supernatant. Urine samples were diluted with 0.05M sodium phosphate buffer solution (pH=6). Chromatographic separation was carried out at ambient temperature using a C_{18} reversed-phase column with detection at UV wavelength 220nm. 0.01M sodium phosphate buffer and acetonitrile was used as a mobile phase and the flowrate was 1.5mL/min (308).

Likewise, Augey *et al*., (303) developed a HPLC-UV method for the analysis of piperacillin-tazobactam in plasma and urine. Separation was achieved using a Hypersil ODS, LiChrosorb, RP-Column (particle size, 5 µm) (250×4.6mm) and guard column (20x4.6 mm). The binary mobile phase was composed of acetonitrile and ammonium acetate buffer; (3.5:96.5, v/v) for the determination of tazobactam in urine and plasma and (18:82, v/v) for the determination of piperacillin in plasma and urine. Chromatographic conditions involved setting the column temperature to 30^{o}C and the flowrate used was 1.0mL/min (run time ~17 minutes). The determination of piperacillin and tazobactam were quantified in separate runs as simultaneous quantification was not possible due to risk of interference with co-administered drugs to critically ill patients. Augey and colleagues concluded that the developed method enables a rapid assay of piperacillin-tazobactam in plasma and urine (303).

Another simple and sensitive HPLC method was developed for the quantification of piperacillin in human plasma by Denooz *et al*., (311). Plasma samples were spiked with the internal standard prior to solid-phase extraction. Separation was performed at 25^{o}C using a Symmetry C8 analytical column (250mm×4.6mm) packed with 5µm diameter particles (Waters), equipped with a guard column (20mm×4.6 mm) containing identical packing material. The run time for sample was 35 minutes and separation were achieved using mobile phase consisting of acetonitrile and phosphate buffer. The developed method was simple, precise, accurate and selective, however wasn't considered rapid (311).

Marselos *et al*., (306) developed a HPLC method for the determination of piperacillin and tazobactam in Tazocin injectable powder. The purpose was to develop a new, reliable, reproducible, simpler, less expensive reversed phase HPLC method. The UV detection wavelength was 220nm and the internal standard used was acetaminophen. Separation was achieved on a Hypersil base deactivated silica (BDS) RS-C_{18} column (250x4.5mm) 5µm column using sodium dihydrogenphosphate-dihydrate, acetonitrile and methanol (pH=5) (70:15:15 v/v/v). Analysis took place at room temperature and the flowrate and injection volume used was 1.0mL/min and 20µL respectively (306).

A more recent study conducted in 2012 by Veni *et al*., (312) developed a method for the simultaneous determination of piperacillin-tazobactam in pharmaceutical formulations. Analysis of samples was completed on a Chromosil, C18 column (250mmx4.6 mm, 5 µm). The mobile phase used was composed of methanol, acetonitrile and 1% orthophosphoric acid (30:50:20, v/v/v) with a final pH of 4. The injection volume selected was 20µL, a flowrate of 1.0mL/min and a run time of 10 minutes. The proposed method is simple, precise, accurate and applicable for the simultaneous quantitative analysis of piperacillin-tazobactam (312).

For the simultaneous determination of piperacillin-tazobactam, Rao *et al*., (313) developed a method to quantify piperacillin and tazobactam in pharmaceutical dosage form. Separation was achieved on a water Nova-Pak HR C18 (300X3.9mm, 6µ) column with a mobile phase composed of methanol and ammonium acetate (35:65, v/v) (pH=4.5). Detection was attained at 225nm, at ambient temperature, with an injection volume of 25µL, at flowrate of 1.0mL/min and a run time of 15 minutes. The developed method offers simplicity, precision and accuracy, producing well resolved peaks (313).

The literature describes numerous HPLC methods for the identification of piperacillin-tazobactam in fatty tissue, human plasma, urine and venous hemofiltration. However, there is little published relating to stability indicating assay methods for piperacillin-tazobactam in solution. Therefore, the aim is to develop a HPLC method that is capable of separating piperacillin and tazobactam from their potential degradation products and measuring their concentration in infusion solutions, accurately and rapidly. The developed HPLC method

should be able to separate, detect and quantify piperacillin-tazobactam and various drug related degradants.

3.4.1 Chemicals

Pharmaceutical dosage form piperacillin-tazobactam, generic brand infusion vials were supplied by St. Georges hospital London. Pure piperacillin sodium salt (analytical standard) 100mg and tazobactam sodium salt (analytical standard) 5mg, aztreonam, cephalothin and nafcillin, ammonium acetate and glacial acetic acid were purchased from Sigma Aldrich. Methanol (HPLC grade) and acetonitrile (HPLC grade) were purchased from Thermo Fisher. NS 0.9% and water for injection (WFI) were purchased from Kingston Pharmacy, 53 Surbiton Road, Kingston, KT1 2HG. IV bags and elastomeric pumps were purchased from Baxter.

3.4.2 Instrumentation and Equipment

Quantitative HPLC analysis was carried out using an Agilent 1260 HPLC instrument with single wavelength UV detection and Chemstation software. Calibrated micropipettes (0.5-10µL, 10-100µL and 100-1000µL) were supplied by Eppendorf Ltd.

3.4.3 Method Development Parameters

Parameters investigated were column, mobile phase, internal standard, detection wavelength, injection volume, column temperature and flowrate..

3.4.3.1 Column

Throughout the HPLC method development process, one of the greatest challenges is selecting a stationary phase that provides desired selectivity, suitable repeatability and stability. Column selectivity as well as injected compounds peak shapes is highly dependent on column characteristics. Selecting a suitable column requires considering many factors that can influence the efficiency and selectivity of a separation including column hardware, support and surface chemistry. The stationary phase was selected based upon previous studies. Eight columns were tested; details of these columns are displayed in Table 26.

Table 26 Displaying details of trialled columns.

Column No.	Column Details			
	Column Manufacturer and Stationary Phase	Column Dimensions Length x Diameter (mm)	Column Pore Size (µm)	Serial Number
1	Phenomenex-Aqua, C18	250 x 3.0 mm	5 µm	417353
2	Zorbax Column, C8	250 x 4.6 mm	5 µm	880967.901
3	Varian-Microsorb, C8	250 x 4.6 mm	5 µm	263503
4	Phenomenex Prodigy, ODS-2	250 x 4.6 mm	5 µm	113500
5	Waters Spherisorb	250 x 4.6 mm	5 µm	102927253 023
6	Zorbax- Dupon, C8	250 x 4.6 mm	5 µm	880952706
7	Acquity, C18	250 x 4.6 mm	1.7 µm	0131301021550
8	Thermoquest hypersil, C-18	150 x 4.6 mm	5 µm	326334

All the columns in Table G were trialled. Columns 1, column 3, column 4 and column 5 were not selected as the back pressure was relatively high (~4000psi) even when the flowrate was reduced to 0.5mL/min. The Zorbax columns, column 2 and column 6 showed three separate peaks (two compound peaks and an internal standard peak) however the resolution achieved was poor; the compounds eluted at similar retention times and when the flowrate was increased the internal standard peak co-eluted with the Piperacillin peak. The column that exhibited optimal resolution was column 7, however, it was not selected due to its small particle size, generating high back pressures making the column prone to blocking. The column selected was column 8 as it displayed the next best resolution and sharp, symmetrical chromatographic peaks were obtained (Table 26).

3.4.3.2 Mobile phase

Literature published on piperacillin/tazobactam method development (303,306,307,311,312) have suggested that both buffered methanol and buffered acetonitrile are suitable mobile phases for the separation and quantification of piperacillin-tazobactam in solution. The majority of published studies previously mentioned use of phosphate or acetate buffer.

Both acetate and phosphate buffers were trialled. Acetate buffer has a long shelf life and is suitable for use in conjunction with mass spectroscopy and sodium phosphate is highly

soluble in water and has an extremely high buffering capacity. Although both buffers presented reproducible results with good resolution, using H_2O produced similar results. The pH of H_2O was reduced from pH 6.5 to pH 4 using phosphoric acid to improve chromatographic peak shape, retention, selectivity, resolution, and detection sensitivity of neutral to basic compounds.

Acetonitrile was selected to be a component of the mobile phase as it is miscible with water and has a low viscosity which reduces back pressure and permits higher flowrates. Acetonitrile also has a lower UV cut of point (200nm) so lower detection wavelengths can be used. Methanol was also used as a mobile phase component as it resulted in a clear baseline between peaks.

The mobile phase components were trialled with numerous v/v/v % compositions. The composition selected was H_2O:MeOH:ACN 55:30:15 running isocratically. Isocratic elution was used as it reduces the run time and increases the throughput as there is no need to re-equilibrate the column between runs.

3.4.3.3 Internal Standard

Using an internal standard is a way of internally standardising analysis. It is a chemical substance that is added in a constant amount to all samples (blanks, standards and samples). An internal standard will be used to correct for possible preparation and instrumental errors such as mis-sampling and drift. The criteria for selecting an internal standard are that it must give good separation without interfering with the analyte peak, be stable, give a good peak shape and have a mutually compatible absorption wavelength with the analyte of interest.

Paracetamol and caffeine were trialled as internal standards. Good peak shapes were achieved for both compounds. However, as caffeine and paracetamol are not physically or chemically similar to piperacillin-tazobactam, they were considered as 'potential' internal standards.

Three chemically similar compounds were also trialled: aztreonam, cephalothin and nafcillin. A 1000ppm stock solution was prepared for all three of these compounds using mobile phase. Noteworthy, whilst transferring the weighed nafcillin into a volumetric flask, it was noticed that yellow residue was left on the weighing boat after rinsing with mobile phase.

Initially all three of the potential internal standards eluted with retention times between 2.4 and 3 minutes (retention times differed from those for piperacillin and tazobactam). The vials were then left in the auto sampler for two days and then rerun to observe whether peak areas, heights and shapes were consistent with previous results. The peak areas, heights and shapes obtained for aztreonam and cephalothin were consistent with results obtained two days prior. However, three peaks were observed when nafcillin was analysed. These new peaks were potentially degradation products, thus, nafcillin was abandoned.

Aztreonam and cephalothin gave similar results. Cephalothin was chosen as the internal standard as it has a longer shelf life (Figure 64).

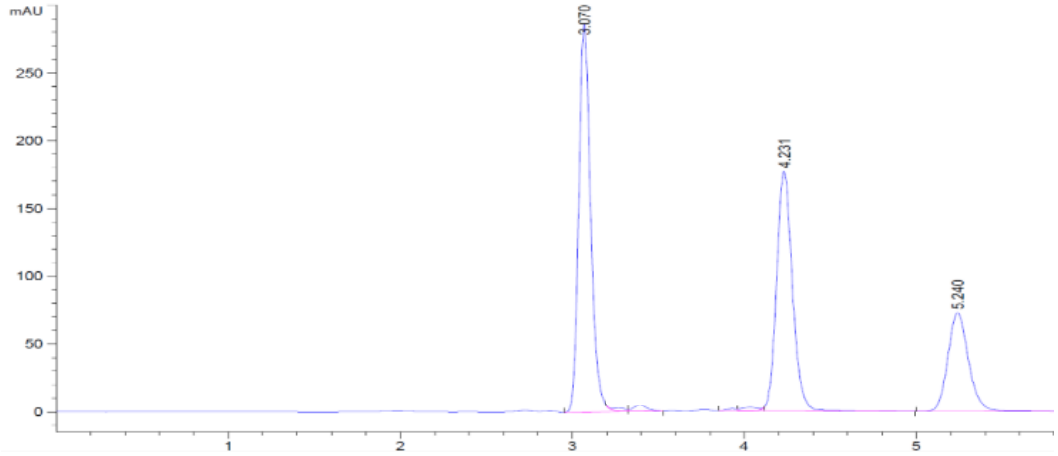


Figure 62 Chromatogram of piperacillin, tazobactam and internal standard, cephalothin (peaks in order of appearance: piperacillin (tR = 3.070 mins), cephalothin (tR = 4.231 mins), tazobactam (tR = 5.240 mins)). Note: flowrate = 0.8mL/min.

3.4.3.4 Wavelength Selection

A UV scan of piperacillin, tazobactam and cephalothin was undertaken to determine which wavelength the target compounds have the maximum absorption (λ_{max}). The UV-Vis scan was carried out from 350-200nm. Piperacillin was observed to have a λ_{max} of 215nm, tazobactam was found to have a λ_{max} of 210nm and cephalothin was found to obtain a λ_{max} of 254nm.

Piperacillin-tazobactam solution was spiked with cephalothin and injected into the HPLC system where the instruments UV detector was set at a range of different wavelengths based on the determined λ_{max}'s. Wavelengths tested include: 205, 210, 215, 220, and 230nm. The wavelength that was selected was 210nm because it accounted for both target molecules piperacillin and tazobactam and was still capable of detecting cephalothin. As cephalothin is the internal standard its concentration can be adjusted to give a distinct and reproducible peak.

3.4.3.5 Injection Volume

From the literature it is apparent that the most common injection volume used when assessing the stability of beta-lactam antibiotics is 10µL. Using an injection volume that is too low will decrease the sensitivity. However, if the injection volume is too big it may result in column overload which distorts peak shape and gives poor quantitative results. Injecting larger volumes of sample can also damage the column. Injection volumes 5, 10, 15 and 20µL were trialled. The injection volume selected was 10µL as it provides adequate detection at a relatively low volume; it avoids distorting the peak shape and reduces the risk of column damage by the sample matrix.

3.4.3.6 Column Temperature

To achieve reproducibility in terms of retention times it is vital to maintain a stable and constant column temperature. The HPLC instrument used was equipped with a column oven that uses a heat balance mechanism to achieve uniform temperature control. To select the optimum column temperature a sample of piperacillin-tazobactam and cephalothin was made up and examined under 4 different temperatures (25, 30, 35 and 40°C); injection volume: 10µL, flowrate: 1.0mL/min and wavelength: 210nm. Results obtained are displayed in Table 27.

Table 27 Showing changes in pressure, peak area and retention time as temperature is increased

Temp	25°C	30°C	35°C	40°C
Pressure (psi)	2400	2300	2170	2040
Peak area (TAZ)	1370	1363	1366	1389
Peak area (CEPH)	1133	1126	1123	1126
Peak area (PIP)	605	614	608	599
Retention time (TAZ)	3.225	3.234	3.198	3.162
Retention time (CEPH)	4.835	4.856	4.718	4.573
Retention time (PIP)	5.505	6.468	6.198	5.898

Table 27 shows that as the temperature increases, the backpressure decreases. It was also observed that when the column temperature is increased, the retention time decreased, giving narrower and taller peaks as well as lowering the detection limit. The higher the column temperature, the faster the exchange of analytes between mobile phase and stationary phase. It is also apparent that with temperature increase, the viscosity of the mobile phase decreases resulting in a decrease of back pressure. The decrease in pressure allows for higher flowrates to be used. The column temperature selected was 30°C as it decreases back pressure and run times as well as obtaining sufficiently resolved peaks without degrading the compounds going through the column.

3.4.3.7 Flowrate

Flowrate was investigated to minimize run times at an acceptable pressure. Lower back pressure is achieved when using slower flowrate; using a slower flowrate is also advantageous as it reduces the probability of co-elution. However, using a faster flowrate allows for quick separation which in turn reduces the retention and run time. A 1000ppm solution was analysed using flowrates 0.6, 0.8, 1.0, 1.2 and 1.4mL/min. From results obtained it was clear that the higher the flowrate the faster the compound eluted, however, as the flowrate increased so did the back pressure. Good separation and resolution of peaks was achieved for all flowrates tested. 1.0mL/min was the chosen flowrate as the goal was to maximise run time at an acceptable pressure while reducing the probability of co-elution. Furthermore, the run time was reduced from six minutes at 0.6mL/min to four and a half minutes at flowrate 1.0mL/min whilst keeping the back pressure around 2000Psi.

3.4.4 HPLC Analytical Conditions

Separation was conducted using a Thermoquest (150×4.6mm) hypersil BDS C-18, 5-micron particle size, Part Number 28105-022, Column Number 326334. The mobile phase consisted of H_2O (pH 4), methanol and acetonitrile (H_2O:MeOH:ACN; 55:30:15) at flowrate of 1.0mL/min. Analysis was performed at 30^oC and detection at 210nm. The injection volume was 10µL with a run time of 4.5 minutes (Table 28).

Table 28 Showing optimized chromatogram conditions for piperacillin-tazobactam.

Method Development Parameter	Selected Condition
Column	Thermoquest hypersil C-18 (150×4.6mm)
Mobile Phase	H_2O (pH 4) : MeOH : ACN (55 : 30 : 15)
Internal Standard	Cephalothin
Flowrate	1.0mL/min
Wavelength	210nm
Injection Volume	10µL
Column Temperature	30^oC
Run Time	4.5 minutes

3.5 HPLC Method Validation

Method validation is a basic requirement to ensure the quality and reliability of obtained results for all analytical procedures (314). It is the process by which performance characteristics of a method are tested by the developer for reliability, accuracy and preciseness. The characteristic of the proposed method should be within prescribed limits and defined standards to confirm its accuracy and authenticity. Characteristics for validation of an analytical method are highly dependent on the understanding of statistical terms and compliance with the requirements for the intended analytical applications. Results obtained from method validation procedure are used statistically to judge the quality, reliability and consistency of analytical data (315).

Previously developed methods for the determination of piperacillin-tazobactam in pharmaceutical formulation, serum, plasma or urine, have been validated as per ICH guidelines (303,304,306–308,311,312). The methods were validated to prove that the method performs as expected under a given set of conditions.

The developed method described in the previous subchapter, was validated according to the ICH validation guidelines (316). The method was tested for its linearity, range, precision, accuracy, specificity, sensitivity and robustness. The method was verified over the period of four days by running three replicates of a standard set of samples once a day for four days.

3.5.1 Linearity and Range

Linearity and Range were examined to observe the developed methods ability to obtain quantitative results which are proportional to the concentration of the analyte. The analytical range is the concentration range over which quantitative results can be obtained without the need for recalibration; this practice is implemented to determine the upper and lower limits of the working range. This range was determined by injecting variable volumes in the range of 2-20µL of a 1000ppm standard solution. This is equivalent to injecting 10µL of a set of standards, concentrations from 200- 2000ppm.

Pharmaceutical formulation is expressed as a combination of piperacillin and tazobactam (both as sodium salts) in a ratio of 8:1. Table 29 and Figure 65 shows the range piperacillin, tazobactam and piperacillin-tazobactam display linearity.

Table 29 Displaying linearity and range of piperacillin-tazobactam, piperacillin and tazobactam.

Compound/s	Analytical Range (PPM)	Linear Equation	R^2
Piperacillin-Tazobactam	250-2000	Y = 124.92x – 14.5	0.9998
Piperacillin	222.2-1777.8	Y = 86.019x – 3.4615	0.9999
Tazobactam	27.8-222.2	Y= 38.896x – 11.038	0.9994

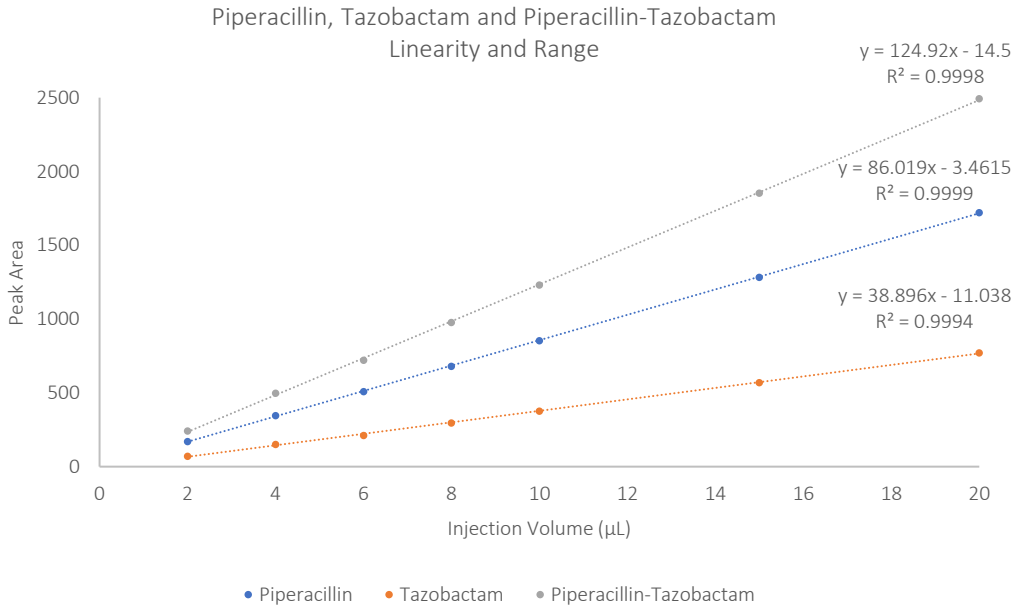


Figure 63 Showing Linearity in the range of 250-2000ppm for piperacillin-tazobactam, 222.2-1777.8ppm for piperacillin and 27.8-222.2ppm for tazobactam

3.5.2 Standard Preparation

3.5.2.1 Preparation of Reference Standard QC Stock

20mg of reference piperacillin was accurately weighed and added to a centrifuge tube with 2.5mg of tazobactam and dissolved with 2.25mL of deionized water (8:1 ratio) (final concentration = 10,000ppm). Calibration standards were prepared from this stock solution (**Table 30**) as well as the high (1825ppm), medium (1125ppm) and low (275ppm) QC's (**Table 31**).

3.5.2.2 Preparation of Internal Standard Stock

Preparation of the internal standard stock solution involved accurately weighing 10mg of reference standard cephalothin and was made to 10mL volume with mobile phase (final concentration = 1000ppm).

Table 30 Showing preparation volumes of standard solution.

Concentration (ppm)	Standard (µl)	Internal Standard (µl)	Mobile Phase (µl)
0 (Blank)	0	250	750
250	25	250	725
500	50	250	700
750	75	250	675
1000	100	250	650
1250	125	250	625
1500	150	250	600
1750	175	250	575
2000	200	250	550

Table 31 Showing preparation volume of standard QC's and LOD

Concentration (ppm)	Standard (µl)	Internal Standard (µl)	Mobile Phase (µl)
Low QC- 275	27.5	250	722.5
Medium QC- 1125	112.5	250	637.5
High QC- 1825	182.5	250	567.5
LOD- 100	10	250	740

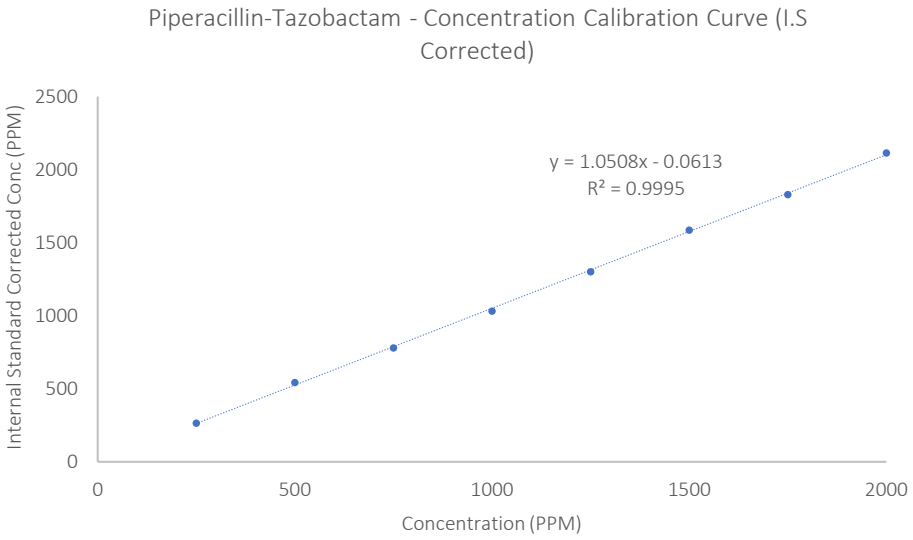
3.5.3 Calibration

In practice, each 4.5g piperacillin-tazobactam vial is reconstituted with 20mL WFI and is further diluted with 50mL of 0.9% sodium chloride for injection; this gives a nominal infusion concentration of 64,286 ppm (4500mg/0.07L=64,286 ppm). This solution was diluted 1 in 50 for analysis, which reduces the amount of sample needed, reduces matrix effects and gives a nominal concentration of 1286ppm piperacillin-tazobactam, in the usual analytical range for quantitative HPLC with UV detection. It was therefore decided to calibrate from 0-2000ppm so that piperacillin-tazobactam (at 1285ppm) would be in the middle of the calibration range. Linearity was assessed using nine standards that ranged in concentration from 250-2000ppm.

All peak areas obtained (raw data) were normalised with the peak area of the internal standard; this was achieved by dividing piperacillin-tazobactam, piperacillin and tazobactam

peak areas by the cephalothin peak area. The internal standard corrected peak area was then calculated and plotted against concentration (Figure 66).

a



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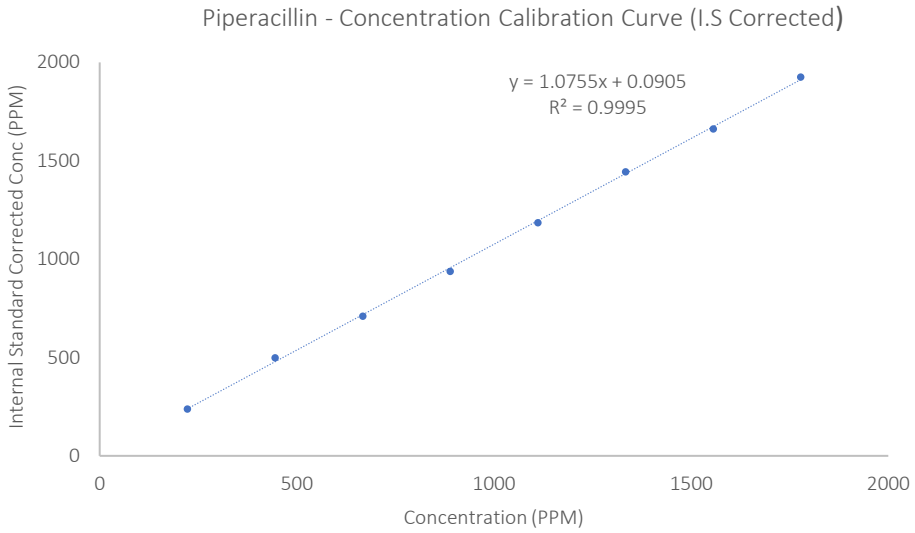


c

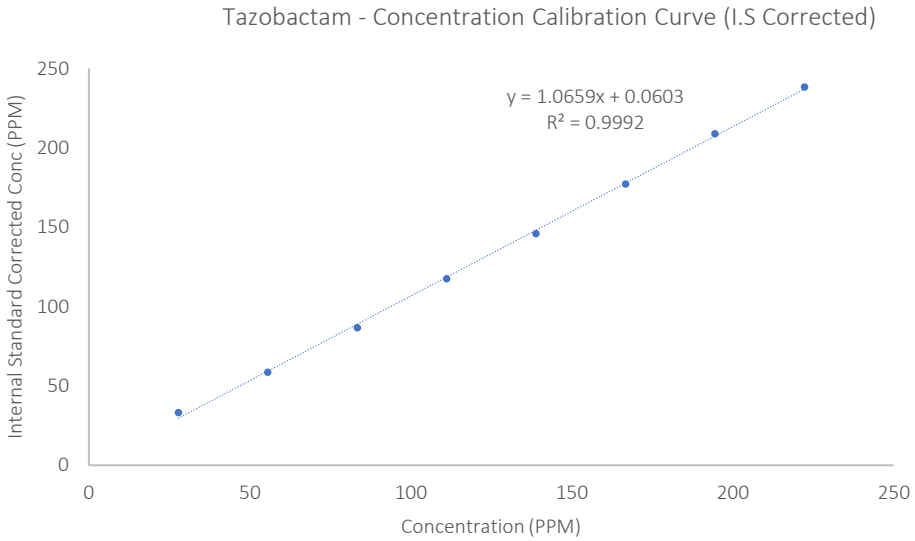


Figure 64 Displaying calibration curves for: a) piperacillin-tazobactam, b) piperacillin and c) tazobactam. Error bars within.

3.5.4 Precision

The precision of obtained results was considered at three levels, these include: (1) repeatability, (2) intermediate precision and, (3) reproducibility. System and method precision were also considered. The precision of the system was considered by the utilization of reference standards to ensure the analytical system is working suitably. Method precision was considered by using piperacillin-tazobactam to observe if the analytical method gave consistent results. Precision was conducted with three sets of three QC reference standards of different concentrations (Table 31).

Three standards were prepared for each QC set and each sample was run three times (nine runs for each QC). The samples were prepared daily for four days and evaluated by generating a calibration curve each day. Repeatability - expressed as precision under the same operating conditions (intra-day) was evaluated via the analysis of the three QC samples of the same concentration; each of the QC samples was sampled in triplicate. Intermediate precision - expressed as the variations with laboratory reproducibility and was assessed by means of comparing assays conducted from the four consecutive days (inter-day).

Good precision was obtained; where (1) intra-sample precision ranged between %RSD 0.00% and 0.2%, (2) intra-day precision attained ranged between %RSD 0.03% and 0.54% and, (3) inter-day precision obtained ranged between %RSD 0.00% and 0.17%, (acceptance criteria: %RSD should not exceed 5%)

3.5.5 Accuracy

Accuracy is the degree of closeness of agreement between an accepted reference value and an obtained value. The accuracy of the method was evaluated by using QC samples within the range of 275ppm and 1825ppm. Reference standard QCs: low (275ppm), medium (1125ppm) and high (1825ppm) were prepared (Table 31) and analysed in triplicate. The percentage error (%Error) calculated ranged between 0.01% and 1.49%, therefore %Recovery values obtained ranged between 99.98 % and 101.49%, (acceptance criteria: recovery should be in the range of 80% to 120%).

3.5.6 Specificity

Specificity was attained through optimal selection of numerous parameters including: (1) column, (2) mobile phase composition, (3) column temperature and, (4) detector wavelengths. Specificity was also evaluated by accelerating the degradation of piperacillin-tazobactam samples to demonstrate resolution of piperacillin-tazobactam and their degradation products (Appendix 4).

3.5.7 Robustness

The validation of the analytical method for this study was not only designed to demonstrate suitability and reliability but also to define its limitations. Robustness is the evaluation of an analytical method wherein the results obtained are found to be reliable even when performed in a slightly varied condition.

Robustness parameters examined include: (1) changes in column temperature (±5°C), (2) changes in the flowrate (±0.2mL/min), (3) changes in mobile phase pH (±0.2 units) and, (4) changes in mobile phase composition (H_2O: MeOH: ACN v/v/v %; 70:20:10, 65:15:20, 70:15:15). Increasing the column temperature decreased the pressure and retention time slightly and decreasing the temperature had the opposite effect on both the pressure and retention time. Changes in the mobile phase pH and composition displayed no difference in terms of both peak area and peak shape. The robustness study demonstrated that the method can optimally perform reproducibly when parameters are slightly changed.

3.5.8 Limit of Detection and Limit of Quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) are two significant performance parameters in method validation. These terms are utilized to designate the smallest concentration of an analyte that can be reliably measured; by HPLC in this case. The LOD is the lowest amount of analyte which can be detected but not necessarily quantitated as an exact value. The LOQ is the smallest amount of analyte that can be quantitatively determined.

ICH guidelines state that the LOD and LOQ may be determined based on the standard deviation of the response and the slope. The calculation used to obtain the limit of detection

was (3.3 x (SD of intercept/Slope)); (3.3 x (25.6/1.0507) = 80.4ppm. The limit of quantitation was calculated using the following equation (10 x (SD of intercept)/Slope]; (10 x (25.6/1.0507) = 243.5ppm. All the calibration standards QC's and LOD samples were above the LOD and LOQ values.

3.6 Determination of Piperacillin-Tazobactams Physicochemical Stability for Administration via Prolonged/Continuous Infusion in hospital and OPAT settings

As highlighted upon in Chapter 2, most commonly, the preparation of P/CI takes place on the wards by nursing staff and is classed as a moderate risk process by the National Patient Safety Agency (NPSA). Risk reduction strategies are recommended to prevent harm to patients through safer use of injectable medicines. One such strategy which may be employed to avoid the dose mismanagement and the microbiological hazard of preparation in clinical areas is the use of a pre-prepared product in validated licenced aseptic facilities under the control of a pharmacist. For this to be viable, batch production needs to be adopted which in turn is only possible if stability data allows sufficient time for manufacture and storage (317)

The frequency at which piperacillin-tazobactam administration takes place via II makes it difficult to use in OPAT settings. Antimicrobials for OPAT services preferably involve once-daily dosing for patient convenience and service delivery considerations (155). Administration via P/CI in EP devices would be advantageous for OPAT services; however, stability should be maintained throughout the infusion time to ensure patients receive adequate piperacillin-tazobactam to achieve cure whilst preventing exposure to degradation products (318).

The National Health Service (NHS) Pharmaceutical Quality and Assurance Committee produced a standardised methodology to establish stability of pharmaceuticals called the Yellow Cover Document (YCD). The YCD specifies the minimum testing requirements needed prior to assigning an expiry date of reconstituted piperacillin-tazobactam. These requirements incorporate the use of a SIM, analysing three samples at each time point, testing the samples in duplication and having at least four time points plus time zero. Moreover, all medicines that are to be administered via infusion devices for 'in use near to body' should be tested at 32^{o}C +/- 1^{o}C. The YCD also stipulates completing physical stability testing e.g. colour, clarity, precipitation and pH (319).

To assign shelf-life to reconstituted piperacillin-tazobactam in IVB and in EP a comprehensive stability test is required. Shelf-life is defined as the length of time required

for a medicines potency to be reduced to some percentage of its original value. For injectable drugs, it is the time from preparation until the original potency of the active ingredient/s has been reduced by 10% (limit of chemical degradation). Enhanced stability would be beneficial for the healthcare professionals preparing and administering solutions for infusion as well the patients. The hospital pharmacy could produce IV solutions in advance which will allow for: (1) wards to have a stock of IVB/EP for acute patients and weekend treatment and (2) home patients could receive more EP at a time.

3.6.1 Stability of Piperacillin-Tazobactam

According to the Summary of Product Characteristics (SmPC), an unopened piperacillin-tazobactam vial has a shelf life of 2 years, in salt form, when stored at <25°C in the marketed packaging prior to reconstitution. After reconstitution and dilution, piperacillin-tazobactam exhibits physical and chemical in-use stability of 24 hrs at 20-25°C and 48 hours at 2-8°C (fridge). However, the manufacturer(s) recommend that it should be used immediately as any storage conditions prior to administration are at the professional's responsibility (320).

The stability of piperacillin and tazobactam have been previously studied (317,319,329–331,321–328). Studies have investigated the stability of piperacillin-tazobactam after reconstitution under a variety of conditions, including different: concentrations, diluents, infusion devices and temperatures. However, published studies mainly reported the stability of antibiotics over 24 hours at 25^{o}C where antibiotic concentrations tested did not always match concentrations commonly prescribed in practice. A review of the literature also highlighted a major lack of data on the stability of piperacillin-tazobactam in portable pumps, particularly regarding the storage temperature.

There is a need for a more prolonged and detailed study that is inclusive of the above conditions to define a maximum shelf-life appropriate for the infusion formulations for both hospital and OPAT settings. Thus, the aim of this study is to establish the stability of piperacillin-tazobactam at a range of temperatures 5°C, 24°C and 37°C, in two infusion devices, IV bags (hospital administration) and elastomeric pumps (OPAT use), using a variety of diluents (0.9% sodium chloride, 5% dextrose and WFI). The intended outcome is to generate new data that comply and meet the standards set out by the NHS Pharmaceutical

Quality Assurance Committee YCD and MHRA, to determine applicability of P/CI for hospital and OPAT use.

3.6.2 Methods

3.6.2.1 Preparation of Admixtures

IV Bags: piperacillin-tazobactam 4.5g vials was reconstituted with 20mL WFI and shaken to dissolve. After reconstitution, the content of the vial was transferred into IV infusion bags containing 50mL of either NS (x9), D5W (x9) or WFI (x9) giving final concentrations of 64mg/mL (64,000 ppm) piperacillin-tazobactam, 57mg/mL (57,000 ppm) piperacillin and 7mg/mL (7000 ppm) tazobactam. Three IV bags were prepared for each diluent – temperature combination (total of 27 IV bags).

Elastomeric Pumps: piperacillin-tazobactam 4.5g vials was reconstituted with 20mL WFI and shaken to dissolve. After reconstitution, the content of the vial was transferred into elastomeric pumps that had been filled with 50mL of either NS (x9), D5W (x9) or WFI (x9) giving final concentrations of 64mg/mL (64,000 ppm) piperacillin-tazobactam, 57mg/mL (57,000 ppm) piperacillin and 7mg/mL (7000 ppm) tazobactam. Three pumps were prepared for each diluent – temperature combination (total of 27 elastomeric pumps).

3.6.2.2 Chemical Stability Study Protocol

Three IV bags and three pumps from each of the diluents (Section 3.7.2.3) were: (1) stored in the fridge at 4-5^{o}C, (2) left at RT 25^{o}C and, (3) incubated at 37^{o}C. 4^{o}C was chosen to reflect the temperature the IVB and EP were likely to be exposed to in a hospital and home refrigerator. 25^{o}C is representative of the average room temperature and the ICH guideline condition for long term stability testing. Solutions investigated at room temperature were exposed to laboratory light conditions to simulate conditions the IVB and EP may be exposed to on a hospital ward. Stability was examined at 37^{o}C as it mimics body temperature as well as high temperatures experienced on hospital wards.

The infusion devices left at room temperature were also exposed to continuous irradiation from daylight and daylight fluorescent lights, also conditions encountered on a hospital ward and in outpatient settings.

Sampling was conducted at 0 hour and then every 24 hours for 240 hours. At each sampling time point around 1mL from each of the infusion devices was taken and tested for piperacillin content, tazobactam concentration and pH.

3.6.2.3 Piperacillin-Tazobactam Stability in EP – 168H (4oC) + 1H (25oC) + 24H (37oC)

Piperacillin-tazobactam 4.5g vials were reconstituted with 20mL WFI and shaken to dissolve. After reconstitution, the content of the vial was transferred into elastomeric pumps that had been filled with 50mL of either NS (x9), D5W (x9) or WFI (x9) giving final concentrations of 64mg/mL (64,000 ppm) piperacillin-tazobactam, 57mg/mL (57,000 ppm) piperacillin and 7mg/mL (7000 ppm) tazobactam. Three pumps were prepared for each diluent (total of 9 elastomeric pumps).

Piperacillin and tazobactam concentrations were measured at 0 hour, then the EPs were stored in the fridge at 4°C for 168 hrs. The quantitative analysis of concentrations was measured every 24 hours. After 168 hrs in the fridge the EPs were left at room temperature for 1 hr so the solution temperature can rise to temperature appropriate for infusion. After 1 hr the concentration of piperacillin and tazobactam was taken again. The EPs were then incubated at 37°C and further measurements were taken over a 24-hr period. Visual inspection and pH analysis were also performed.

3.6.2.4 Sample Solutions for HPLC Analysis

To achieve a nominal concentration of ~1.3mg/mL (1286 ppm), a dilution factor of 50x was used. Samples were diluted 1:50 with mobile phase and internal standard solutions [20µL of sample solution taken from infusion devices, 250µL of internal standard solution, and 730µL of the mobile phase]. Samples were tested in triplicate for optimisation of measurement precision. Blank samples were run for assessing any background signal.

3.6.2.5 Calculation of Piperacillin and Tazobactam Concentration and Shelf-life

The current statistical method outlined in ICH Q1A and Q1E to describe data obtained from a stability study and determine shelf-life is regression analysis.

The concentration of both piperacillin and tazobactam were calculated using the equation for each drug derived from the calibration curve performed on each day of analysis.

The initial piperacillin and tazobactam concentrations were defined as 100% and subsequent concentrations were calculated as percentages of the initial concentration. Acceptance criteria for stability in this study were defined as 90-110% of the initial concentration.

Temperature effects were quantified by calculating the ‘time to degrade by 10%’.

3.6.2.6 pH Profile

The pH of the samples for all conditions was recorded at every sampling interval using a calibrated pH meter. The instrument was calibrated using buffers with known pH of 4.00 and 7.00. pH meter efficiency had to be 100% +/- 5%, to be within equipment specifications. The probe was washed with deionised water between measurements of different samples.

3.6.2.7 Physical Compatibility

Colour and clarity of infusion solutions were monitored at each sampling time point. All samples were checked against: (1) a black background for the observation of particulate matter and, (2) a white background for the observation of colour change.

3.6.3 Results

3.6.3.1 Chemical Stability

The average percentage of initial concentration remaining at all time points for all the combinations are shown in **Table 32 and Table 33** and the influence of diluent and infusion device on piperacillin and tazobactam concentrations at 4°C, 25°C and 37°C over time is shown in **Figure 67**, **Figure 68**, **Figure 69** and **Figure 70**.

Table 32 %Recovery of piperacillin at all sampling intervals in all diluent and temperature combinations in both infusion devices

Conditions			%Recovery at Stability Test Sampling Intervals (hrs)							
Temp	Diluent	Device	T_0	T_{24}	T_{48}	T_{72}	T_{144}	T_{192}	T_{216}	T_{240}
		IVB	100 ± 4.8	107 ± 0.7	96 ± 2.6	91 ± 3.7	88 ± 5.1	94 ± 3.4	95 ± 3.7	90 ± 8.3
	NS	EP	100 ± 2.7	92 ± 4.7	91 ± 3.7	98 ± 4.2	90 ± 2.4	93 ± 3.1	91 ± 4	90 ± 7.6
		IVB	100 ± 2.3	96 ± 30.5	94 ±30.3	90 ±1.6	87 ± 2.5	89 ± 1.8	88 ± 1.2	88 ± 1.4
4°C	D5W	EP	100 ± 4.8	94 ± 2.8	94 ± 3.2	93 ± 2.3	91 ± 3.3	90 ± 3.1	89 ± 2.8	89 ± 3
		IVB	100 ± 36	99 ± 1.6	98 ±2.8	92 ± 33	90 ± 27	90 ± 33	88 ± 31	88 ± 1.7
	WFI	EP	100 ± 0.5	94 ± 0.6	91 ± 1.9	90 ± 0.4	89 ± 1.3	94 ± 0.3	94 ± 0.6	89 ± 2.9
		IVB	100 ± 1.4	93 ± 2.1	91 ± 2	93 ± 1.8	84 ± 0.6	85 ±1.3	80 ± 1.4	77 ± 2
	NS	EP	100 ± 1.7	91 ± 1.1	91 ± 0.7	90 ± 1.2	80 ± 1.8	81 ± 0.9	84 ± 0.5	84 ± 1.9
		IVB	100 ± 1.6	93 ± 0.9	92 ± 2.8	93 ± 2.3	80 ± 1.4	82 ± 1.5	78 ± 1.1	77 ± 0.3
25°C	D5W	EP	100 ± 1.9	92 ± 5	91 ± 3.7	91 ± 4.9	84 ± 4.2	80 ± 3	83 ± 3	77 ± 2.8
		IVB	100 ± 1	99 ± 7.1	95 ± 7.9	90 ± 1.9	82 ± 2.1	82 ± 2.4	79 ± 0.5	78 ± 1.6
	WFI	EP	100 ± 1.3	94 ± 3	90 ± 1	91 ± 2	86 ± 0.7	93 ± 0.8	89 ± 2.2	87 ± 1.9
		IVB	100 ± 1.3	95 ± 15.5	95 ± 13.1	78 ± 0.2	57 ± 2.3	46 ± 2.8	37 ± 3.4	34 ± 4.7
	NS	EP	100 ± 35	92 ± 1.6	93 ± 2	70 ± 3	52 ± 3.8	47 ± 2.1	40 ± 4.3	35 ± 13.2
		IVB	100 ± 1.3	88 ± 1.8	84 ± 1.6	74 ± 0.9	54 ± 1.1	72 ± 2.2	55 ± 4.3	36 ± 0.7
37°C	D5W	EP	100 ± 2.2	73 ± 2.6	83 ± 1.4	78 ± 1.9	58 ± 1.4	49 ± 2.1	46 ± 2	37 ± 1.9
		IVB	100 ± 2.6	95 ± 5.3	92 ± 3.7	79 ± 3.1	62 ± 2.5	50 ± 2.2	46 ± 4	39 ± 2.9
	WFI	EP	100 ± 3.9	93 ± 3.5	93 ± 2.5	81 ± 2.9	57 ± 2.3	54 ± 2.8	48 ± 3.5	42 ± 2.5

D5W = 5% dextrose, NS = normal saline and WFI = water for injection

*Results are the average of three replicates

Table 33 %Recovery of tazobactam at all sampling intervals in all diluent and temperature combinations in both infusion devices

Conditions			%Recovery at Stability Test Sampling Intervals (hrs)							
Temp	Diluent	Device	T_0	T_{24}	T_{48}	T_{72}	T_{144}	T_{192}	T_{216}	T_{240}
		IVB	100 ± 0.3	108 ± 0.1	97 ± 0.5	90 ± 0.3	90 ± 0.5	96 ± 0.3	96 ± 4.4	89 ± 0.2
	NS	EP	100 ± 0.3	92 ± 0.3	92 ± 0.1	100 ± 0.3	92 ± 0.1	97 ± 0.2	95 ± 0.2	95 ± 0.3
		IVB	100 ± 0.2	95 ± 2.4	93 ± 3.1	89 ± 0.1	89 ± 0.6	92 ± 0.2	91 ± 0.1	90 ± 0.1
4°C	D5W	EP	100 ± 0.7	97 ± 0.5	93 ± 0.3	90 ± 4.4	90 ± 1.2	90 ± 0.2	89 ± 1.2	90 ± 7.8
		IVB	100 ± 2.8	99 ± 0.2	98 ± 0.3	92 ± 2.8	92 ± 2.8	93 ± 2.6	91 ± 2.5	84 ± 2.4
	WFI	EP	100 ± 0	93 ± 0.1	91 ± 1.8	89 ± 0.7	91 ± 0.1	98 ± 0.2	99 ± 0	87 ± 0.6
		IVB	100 ± 0.2	93 ± 0.6	91 ± 0.6	95 ± 0.3	93 ± 0.1	97 ± 0.2	92 ± 0.4	84 ± 0.3
	NS	EP	100 ± 0.3	92 ± 0.1	93 ± 0.1	95 ± 0.1	94 ± 0.1	99 ± 0.5	103 ± 0.6	98 ± 6
		IVB	100 ± 0.1	93 ± 0.1	92 ± 0.3	97 ± 0.2	87 ± 0.1	96 ± 1.5	93 ± 0.3	81 ± 0.4
25°C	D5W	EP	100 ± 0.9	91 ±0.4	89 ± 0.4	106 ± 2.1	101 ± 0.8	94 ± 0.3	99 ± 0.3	99 ± 1.1
		IVB	100 ± 0.1	100 ± 0.5	99 ± 0.6	92 ± 0.3	89 ± 0.3	96 ± 0.8	94 ± 0.2	83 ± 0.1
	WFI	EP	100 ± 0.2	92 ± 0.3	90 ± 0.3	94 ± 1	101 ± 0.6	105 ± 0.1	100 ± 0.4	105 ± 0.2
		IVB	100 ± 0.2	97 ± 1.1	101 ± 1.2	91 ± 0.5	79 ± 0.5	92 ± 0.9	72 ± 9.8	71 ± 3.3
	NS	EP	100 ± 0.4	99 ± 0.1	95 ± 0.6	101 ± 1.1	108 ± 1.7	99 ± 1.3	98 ± 1.1	108 ± 0.9
		IVB	100 ± 0.2	91 ± 0.1	89 ± 0.5	88 ± 0.6	69 ± 0.6	69 ± 0.4	69 ± 2.6	64 ± 0.4
37°C	D5W	EP	100 ± 0.1	97 ±0.5	100 ± 2.2	103 ± 0.9	98 ± 0.9	95 ± 0.2	100 ± 0.6	100 ± 0.3
		IVB	100 ±0.3	98 ± 1.3	97 ± 0.4	93 ± 0.2	85 ± 13	78 ± 0.4	78 ± 2.7	72 ± 4.1
	WFI	EP	100 ± 0.3	100 ±1.4	109 ± 1.1	102 ± 1.5	89 ± 0.2	96 ± 0.5	99 ± 0.4	111 ± 0.4

D5W = 5% dextrose, NS = normal saline and WFI = water for injection

*Results are the average of three replicates

3.6.3.1.1 Temperature

Piperacillin-Tazobactam Refrigerated at 4°C:

At 4°C, piperacillin and tazobactam were stable for the same length of time or for longer when stored in EP compared to IVB for all diluents. Refrigerated piperacillin in admixture solutions remained stable for on average 9.35 and 9.1 days in NS and WFI respectively compared to 6.75 days when diluted with D5W. Refrigerated tazobactam in admixture solutions remained stable for on average 31.5 and 20.9 days in NS and WFI respectively compared to 8.25 days when diluted with D5W (**Table 32**, **Table 33**, **Table 34** and **Table 35**). However, there was no statistically significant difference between the diluents studied in terms of piperacillin ($p = 0.401$) and tazobactam stability ($p = 0.412$) when stored in IVB at 4°C. Statistical analysis also shows that no significant difference in piperacillin and tazobactam stability between the three diluents at 4°C in EP, $p = 0.986$ and $p = 0.357$, respectively (**Table 36** and **Table 38**).

Piperacillin-Tazobactam Stored at 25°C:

At 25°C, piperacillin was stable for the same length of time or longer when stored in EP compared to IVB for all diluents. Piperacillin in admixture solutions remained stable for on average 5 and 5.25 days in NS and WFI respectively, compared to 3.3 days when diluted with D5W. Tazobactam in admixture solutions remained stable for on average 9.35 and 7.65 days in NS and D5W respectively compared to 5.5 days when diluted with WFI (Table 32, Table 33, Table 34 and Table 35). However, there was no statistically significant difference between the diluents studied in terms of piperacillin ($p = 0.196$) and tazobactam stability ($p = 0.856$) when stored in IVB at 25°C. Statistical analysis also shows that no significant difference in piperacillin and tazobactam stability between the three diluents at 25°C in EP, $p = 0.362$ and $p = 0.818$, respectively (Table 36 and Table 38).

Piperacillin-Tazobactam Incubated at 37°C:

At 37°C, piperacillin was stable for the same length of time or longer when stored in EP compared to IVB. Piperacillin in admixture solutions remained stable for on average 1.5 and 1.7 days in NS and WFI respectively compared to 14 hours when diluted with D5W. Tazobactam in admixture solutions remained stable for the 10 days of the stability study at elevated temperatures (Table 32, Table 33, Table 34 and Table 35). There was no statistically significant difference between the diluents studied in terms of piperacillin ($p = 0.968$) and tazobactam stability ($p = 0.377$) when stored in IVB at 37°C. Statistical analysis also shows that there is no significant difference in piperacillin and tazobactam stability between the three diluents at 37°C in EP, $p = 0.875$ and $p = 0.699$, respectively (Table 36 and Table 38).

3.6.3.1.2 Diluent:

Piperacillin-Tazobactam in NS:

For solutions diluted with NS and stored at 4°C, piperacillin retained 90% of initial concentration for 9.7 days in IVB and 9 days in EP, making pre-preparation of infusion solutions viable. Dilution in NS provides sufficient stability, up to 3.8 days in IVB and 6.2 days in EP, for administration via a CI when storage temperature of the infusion device does not exceed 25°C. Solution that are exposed to temperatures that exceed 25°C and up to 37°C can be administered via a 24-hour CI in IVB and in EP as solutions retained 90% of initial

concentration for 1.7 days and 1.3 days, respectively (Table 32, Table 33, Table 34 and Table 35). Statistical analysis shows a significant difference in piperacillin stability between the three temperatures studied in NS, p = 0.005 and p =0.007 in IVB and EP, respectively (Table 36).

Piperacillin-Tazobactam in D5W:

Although, not statistically significant, lower stability was demonstrated when using D5W as a diluent. Dilution in D5W provides sufficient stability (3 days in IVB and EP) for administration via a CI when storage temperature of the infusion device does not exceed 25°C. Pre-preparation of infusion solutions using this diluent is also viable. Solutions can be prepared and stored for 5 days in IVB and 7 days in EP at 4°C, prior to administration via conventional II. Pre-prepared solutions stored at ≤25°C can be prepared either 2 days prior to administration via CI or 3 days prior to administration via II. Solution that are exposed to temperatures that exceed 25°C and up to 37°C can be administered via a PI for 5 hours in IVB and in EP (Table 32, Table 33, Table 34 and Table 35). Statistical analysis shows a significant difference in piperacillin and tazobactam stability between the three temperatures studied in D5W in IVB, p = 0.009 and p =0.014, respectively. In EP, stability varies significantly in D5W, p = 0.001 and p = 0.017 for piperacillin and tazobactam, respectively (Table 36 and Table 38).

Piperacillin-Tazobactam in WFI:

Solutions diluted with WFI and stored at 4°C remained stable for 7.5 days in IVB and 10 days in EP, making pre-preparation of infusion solutions viable. Dilution in WFI provides sufficient stability, up to 4.1 days in IVB and 6.4 days in EP, for administration via a CI when storage temperature of the infusion device does not exceed 25°C. Solutions that are exposed to temperatures that exceed 25°C and up to 37°C can be administered via a 24-hour CI in IVB and in EP as solutions retained 90% of initial concentration for 1.7 days (Table 32, Table 33, Table 34 and Table 35). Statistical analysis shows a significant difference in piperacillin stability between the three temperatures studied in WFI, p = 0.019 and p =0.008 in IVB and EP, respectively. However, there was no statistically significant difference between the temperatures studied in terms of tazobactam stability in IVB (p = 0.192) and in EP (P = 0.089) (Table 36 and Table 38).

3.6.3.1.3 Infusion Device

The stability of piperacillin and tazobactam in IVB are equivalent to those in EP. However, they were stable in EP for slightly longer than IVB for most conditions (Table 34 and Table 35). There were no significant differences in piperacillin and tazobactam concentrations when stored in both infusion devices ($p = 0.196$ to $p = 0.491$). Tazobactam stored at 37°C in EP were significantly more stable than tazobactam stored in IVB at the same temperature ($p = 0.001$ to $p = 0.008$) (Table 37 and Table 39).

Table 34 Equation for each condition used to calculate the predicted time at which %recovery of piperacillin falls below 90%

Condition	Linear equation	Predicted Stability (Hours)	Predicted Stability (Days)
4°C NS IVB	y = -0.0431x + 100.07	233.6	9.7
4°C NS EP	y = -0.0273x + 95.936	217.4	9
4°C D5W IVB	y = -0.0471x + 96.549	139	5.8
4°C D5W EP	y = -0.0356x + 96.607	185.6	7.7
4°C WFI IVB	y = -0.0505x + 99.057	179.4	7.5
4°C WFI EP	y = -0.0179x + 94.588	256.3	10.7
25°C NS IVB	y = -0.0797x + 97.256	91	3.8
25°C NS EP	y = -0.0119x + 91.762	148.1	6.2
25°C D5W IVB	y = -0.0884x + 97.078	80.1	3.3
25°C D5W EP	y = -0.0773x + 96.072	78.6	3.3
25°C WFI IVB	y = -0.0945x + 99.223	97.6	4.1
25°C WFI EP	y = -0.0324x + 94.934	152.3	6.4
37°C NS IVB	y = -0.2934x + 102.04	41	1.7
37°C NS EP	y = -0.2815x + 98.407	29.9	1.3
37°C D5W IVB	y = -0.2018x + 94.05	20.1	0.8
37°C D5W EP	y = -0.225x + 91.279	5.7	0.2
37°C WFI IVB	y = -0.2595x + 100.7	41.2	1.7
37°C WFI EP	y = -0.2517x + 100.06	40	1.7

Table 35 equation for each condition used to calculate the predicted time at which %recovery of tazobactam falls below 90%

Condition	Linear equation	Predicted Stability (Hours)	Predicted Stability (Days)
4°C NS IVB	y = -0.0391x + 100.39	265.7	11.1
4°C NS EP	y = -0.005x + 96.147	1229.4	51.2
4°C D5W IVB	y = -0.0291x + 95.692	195.6	8.2
4°C D5W EP	y = -0.0336x + 96.651	197.9	8.3
4°C WFI IVB	y = -0.0506x + 99.547	188.7	7.9
4°C WFI EP	y = -0.0057x + 94.639	813.9	33.9
25°C NS IVB	y = -0.0279x + 96.487	232.5	9.7
25°C NS EP	y = 0.0206x + 94.465	216.7	9
25°C D5W IVB	y = -0.0381x + 96.876	180.5	7.5
25°C D5W EP	y = 0.0121x + 95.889	486.7	17.8
25°C WFI IVB	y = -0.0488x + 99.701	198.8	8.3
25°C WFI EP	y = 0.0466x + 93.006	64.5	2.7
37°C NS IVB	y = -0.1165x + 101.41	97.9	4.1
37°C NS EP	y = 0.0218x + 98.624	395.6	16.5
37°C D5W IVB	y = -0.1406x + 96.442	45.8	1.9
37°C D5W EP	y = -0.0042x + 99.573	2279.3	95
37°C WFI IVB	y = -0.1166x + 101.07	94.9	4.0
37°C WFI EP	y = 0.0003x + 100.6	35333.3	1472

Table 36 Results of piperacillin ANOVA analyses and T-Test performed at the level of diluent and temperature at 95% confidence level

Piperacillin				
Diluent	Variables	ANOVA	T-Test (Individual Analysis)	
4°C - IVB	(NS) vs (D5W) vs (WFI)	Not Significant (p = 0.401)	(NS) vs (D5W) (p = 0.083)	Not Significant
			(NS) vs (WFI) (p= 0.256)	Not Significant
			(D5W) vs (WFI) (p = 0.194)	Not Significant
25°C - IVB	(NS) vs (D5W) vs (WFI)	Not Significant (p = 0.196)	(NS) vs (D5W) (p = 0.387)	Not Significant
			(NS) vs (WFI) (p= 0.478)	Not Significant
			(D5W) vs (WFI) (p = 0.375)	Not Significant
37°C - IVB	(NS) vs (D5W) vs (WFI)	Not Significant (p = 0.968)	(NS) vs (D5W) (p = 0.413)	Not Significant
			(NS) vs (WFI) (p= 0.421)	Not Significant
			(D5W) vs (WFI) (p = 0.496)	Not Significant
4°C - EP	(NS) vs (D5W) vs (WFI)	Not Significant (p = 0.986)	(NS) vs (D5W) (p = 0.438)	Not Significant
			(NS) vs (WFI) (p= 0.450)	Not Significant
			(D5W) vs (WFI) (p = 0.489)	Not Significant
25°C - EP	(NS) vs (D5W) vs (WFI)	Not Significant (p = 0.362)	(NS) vs (D5W) (p = 0.222)	Not Significant
			(NS) vs (WFI) (p= 0.417)	Not Significant
			(D5W) vs (WFI) (p = 0.103)	Not Significant
37°C - EP	(NS) vs (D5W) vs (WFI)	Not Significant (p = 0.875)	(NS) vs (D5W) (p = 0.483)	Not Significant
			(NS) vs (WFI) (p= 0.345)	Not Significant
			(D5W) vs (WFI) (p = 0.313)	Not Significant
Temperature	Variables	ANOVA	T-Test (Individual Analysis)	
NS - IVB	(4°C) vs (25°C) vs (37°C)	Significant (p = 0.005)	(4°C) vs (25°C) (p = 0.031)	Significant
			(4°C) vs (37°C) (p = 0.008)	Significant
			(25°C) vs (37°C) (p = 0.032)	Significant
D5W - IVB	(4°C) vs (25°C) vs (37°C)	Significant (p = 0.009)	(4°C) vs (25°C) (p = 0.131)	Not Significant
			(4°C) vs (37°C) (p = 0.009)	Significant
			(25°C) vs (37°C) (p = 0.030)	Significant

			(4ºC) vs (25ºC) (p = 0.100)	Not Significant
WFI - IVB	(4ºC) vs (25ºC) vs (37ºC)	Significant (p = 0.019)	(4ºC) vs (37ºC) (p = 0.010)	Significant
			(25ºC) vs (37ºC) (p = 0.036)	Significant
			(4ºC) vs (25ºC) (p = 0.259)	Not Significant
NS – EP	(4ºC) vs (25ºC) vs (37ºC)	Significant (p = 0.007)	(4ºC) vs (37ºC) (p = 0.006)	Significant
			(25ºC) vs (37ºC) (p = 0.013)	Significant
			(4ºC) vs (25ºC) (p = 0.045)	Significant
D5W – EP	(4ºC) vs (25ºC) vs (37ºC)	Significant (p = 0.001)	(4ºC) vs (37ºC) (p = 0.002)	Significant
			(25ºC) vs (37ºC) (p = 0.009)	Significant
			(4ºC) vs (25ºC) (p = 0.265)	Not Significant
WFI - EP	(4ºC) vs (25ºC) vs (37ºC)	Significant (p = 0.008)	(4ºC) vs (37ºC) (p = 0.011)	Significant
			(25ºC) vs (37ºC) (p = 0.015)	Significant

Table 37 Results of piperacillin ANOVA analyses and T-Test performed at the level of infusion device at 95% confidence level

Piperacillin			
Infusion Device	Variables	T-Test (Individual Analysis)	
		(IVB NS) vs (EP NS) (p = 0.196)	Not Significant
4ºC	(IVB NS) vs (EP NS)	(IVB D5W) vs (EP D5W) (p = 0.250)	Not Significant
		(IVB WFI) vs (EP WFI) (p = 0.386)	Not Significant
		(IVB NS) vs (EP NS) (p = 0.289)	Not Significant
25ºC	(IVB NS) vs (EP NS)	(IVB D5W) vs (EP D5W) (p = 0.472)	Not Significant
		(IVB WFI) vs (EP WFI) (p = 0.212)	Not Significant
		(IVB NS) vs (EP NS) (p = 0.436)	Not Significant
37ºC	(IVB NS) vs (EP NS)	(IVB D5W) vs (EP D5W) (p = 0.307)	Not Significant
		(IVB WFI) vs (EP WFI) (p = 0.491)	Not Significant

Table 38 Results of tazobactam ANOVA analyses and T-Test performed at the level of diluent and temperature at 95% confidence level

Tazobactam				
Diluent	Variables	ANOVA	T-Test (Individual Analysis)	
4°C - IVB	(NS) vs (D5W) vs (WFI)	Not Significant (p = 0.412)	(NS) vs (D5W) (p = 0.098)	Not Significant
			(NS) vs (WFI) (p= 0.386)	Not Significant
			(D5W) vs (WFI) (p = 0.093)	Not Significant
25°C - IVB	(NS) vs (D5W) vs (WFI)	Not Significant (p = 0.856)	(NS) vs (D5W) (p = 0.233)	Not Significant
			(NS) vs (WFI) (p= 0.392)	Not Significant
			(D5W) vs (WFI) (p = 0.404)	Not Significant
37°C - IVB	(NS) vs (D5W) vs (WFI)	Not Significant (p = 0.377)	(NS) vs (D5W) (p = 0.279)	Not Significant
			(NS) vs (WFI) (p= 0.306)	Not Significant
			(D5W) vs (WFI) (p = 0.123)	Not Significant
4°C - EP	(NS) vs (D5W) vs (WFI)	Not Significant (p = 0.357)	(NS) vs (D5W) (p = 0.068)	Not Significant
			(NS) vs (WFI) (p= 0.430)	Not Significant
			(D5W) vs (WFI) (p = 0.146)	Not Significant
25°C - EP	(NS) vs (D5W) vs (WFI)	Not Significant (p = 0.818)	(NS) vs (D5W) (p = 0.211)	Not Significant
			(NS) vs (WFI) (p= 0.264)	Not Significant
			(D5W) vs (WFI) (p = 0.429)	Not Significant
37°C - EP	(NS) vs (D5W) vs (WFI)	Not Significant (p = 0.699)	(NS) vs (D5W) (p = 0.279)	Not Significant
			(NS) vs (WFI) (p= 0.346)	Not Significant
			(D5W) vs (WFI) (p = 0.280)	Not Significant
Temperature	Variables	ANOVA	T-Test (Individual Analysis)	
NS - IVB	(4°C) vs (25°C) vs (37°C)	Not Significant (p = 0.181)	(4°C) vs (25°C) (p = 0.186)	Not Significant
			(4°C) vs (37°C) (p = 0.082)	Not Significant
			(25°C) vs (37°C) (p = 0.174)	Not Significant
D5W - IVB	(4°C) vs (25°C) vs (37°C)	Significant (p = 0.014)	(4°C) vs (25°C) (p = 0.473)	Not Significant
			(4°C) vs (37°C) (p = 0.013)	Significant
			(25°C) vs (37°C) (p = 0.016)	Significant

			(4°C) vs (25°C) (p = 0.451)	Not Significant
WFI - IVB	(4°C) vs (25°C) vs (37°C)	Not Significant (p = 0.192)	(4°C) vs (37°C) (p = 0.085)	Not Significant
			(25°C) vs (37°C) (p = 0.080)	Not Significant
			(4°C) vs (25°C) (p = 0.232)	Not Significant
NS – EP	(4°C) vs (25°C) vs (37°C)	Significant (p = 0.027)	(4°C) vs (37°C) (p = 0.008)	Significant
			(25°C) vs (37°C) (p = 0.034)	Significant
			(4°C) vs (25°C) (p = 0.039)	Significant
D5W – EP	(4°C) vs (25°C) vs (37°C)	Significant (p = 0.017)	(4°C) vs (37°C) (p = 0.001)	Significant
			(25°C) vs (37°C) (p = 0.210)	Not Significant
			(4°C) vs (25°C) (p = 0.052)	Significant
WFI - EP	(4°C) vs (25°C) vs (37°C)	Not Significant (p = 0.089)	(4°C) vs (37°C) (p = 0.019)	Significant
			(25°C) vs (37°C) (p = 0.254)	Not Significant

Table 39 Results of tazobactam ANOVA analyses and T-Test performed at the level of infusion device at 95% confidence level

Tazobactam			
Infusion Device	Variables	T-Test (Individual Analysis)	
		(IVB NS) vs (EP NS) (p = 0.416)	Not Significant
4°C	(IVB NS) vs (EP NS)	(IVB D5W) vs (EP D5W) (p = 0.069)	Not Significant
		(IVB WFI) vs (EP WFI) (p = 0.008)	Significant
		(IVB NS) vs (EP NS) (p = 0.497)	Not Significant
25°C	(IVB NS) vs (EP NS)	(IVB D5W) vs (EP D5W) (p = 0.068)	Not Significant
		(IVB WFI) vs (EP WFI) (p = 0.001)	Significant
		(IVB NS) vs (EP NS) (p = 0.490)	Not Significant
37°C	(IVB NS) vs (EP NS)	(IVB D5W) vs (EP D5W) (p = 0.088)	Not Significant
		(IVB WFI) vs (EP WFI) (p = 0.007)	Significant

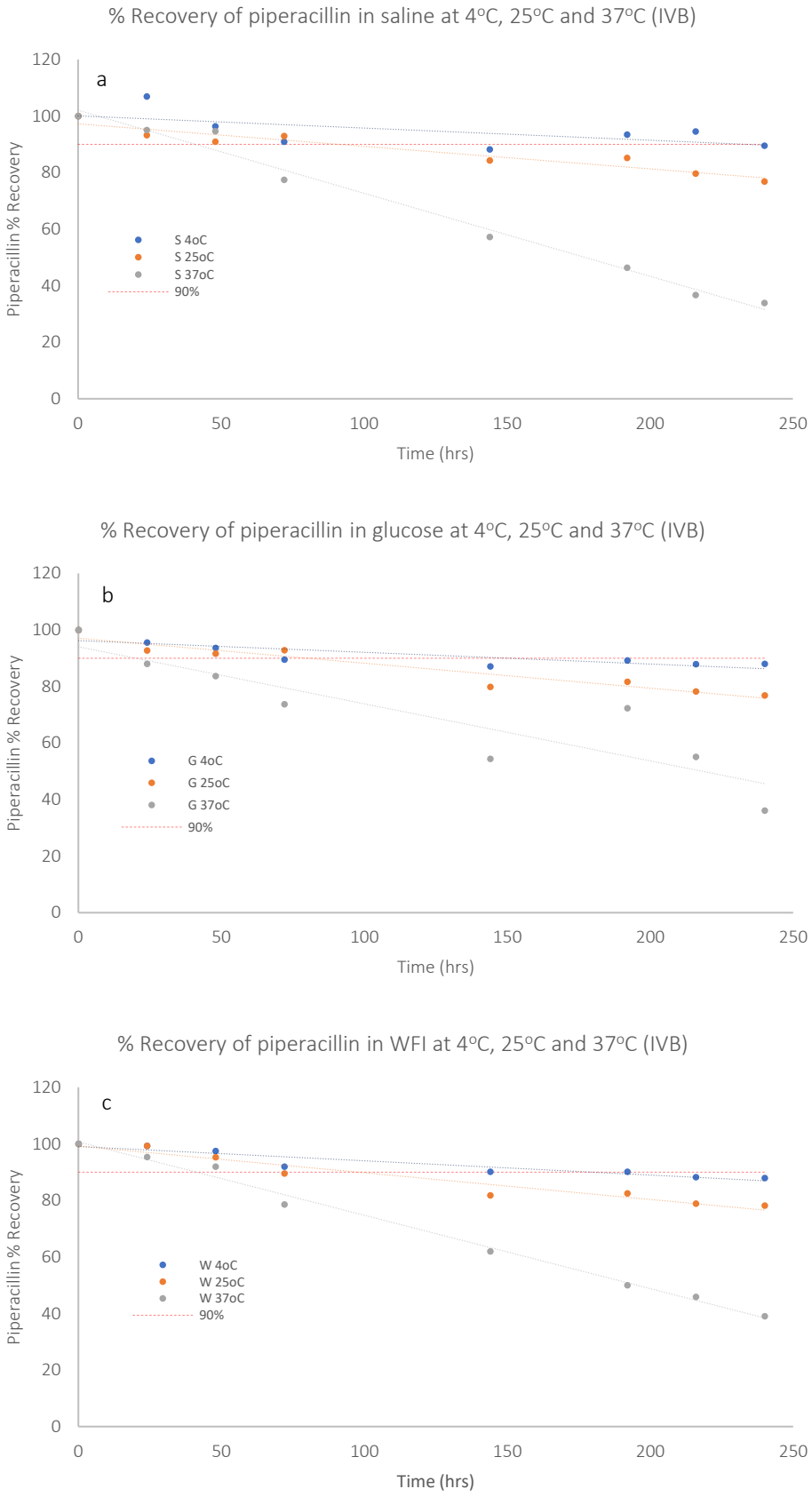


Figure 65 Stability of piperacillin in IVB over time at a) 4ºC, b) 25ºC and c) 37ºC: mean % of intact molecule as a function of time and type of diluent. Dashed line: 90% of initial concentration

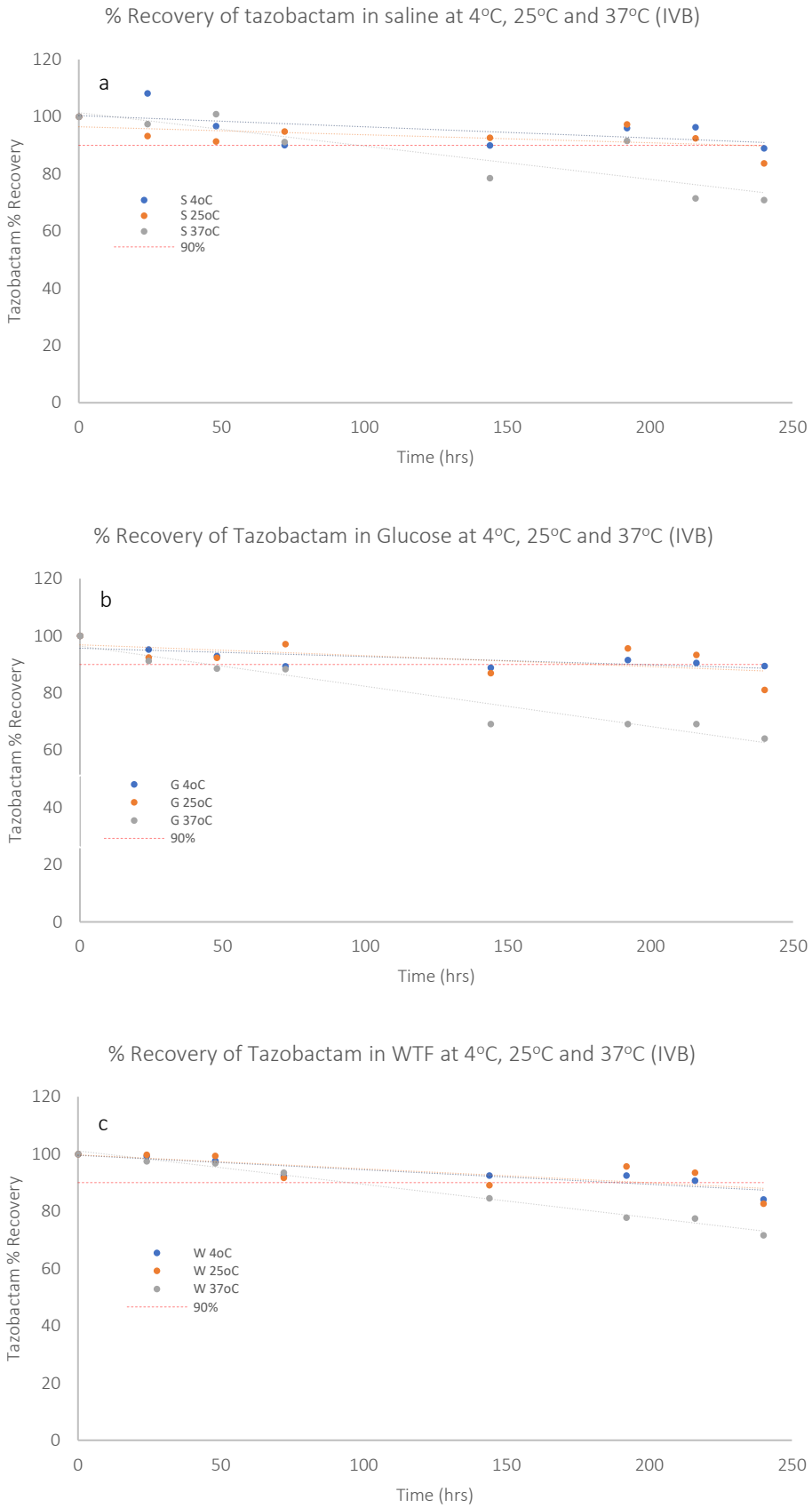


Figure 66 Stability of tazobactam in IVB over time at a) 4ºC, b) 25ºC and c) 37ºC: mean % of intact molecule as a function of time and type of diluent. Dashed line: 90% of initial concentration.

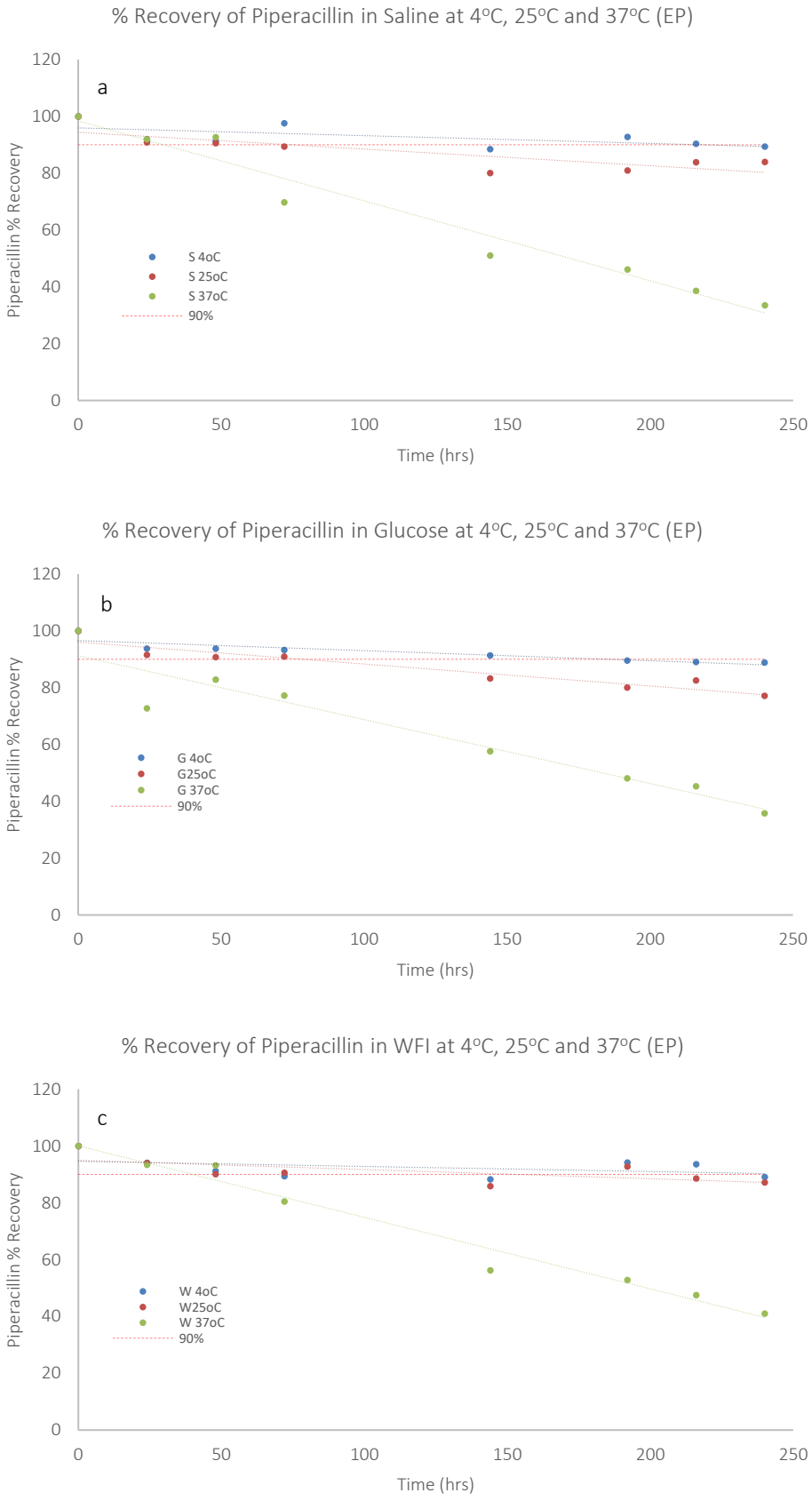


Figure 67 Stability of piperacillin in EP over time at a) 4°C, b) 25°C and c) 37°C: mean % of intact molecule as a function of time and type of diluent. Dashed line: 90% of initial concentration.

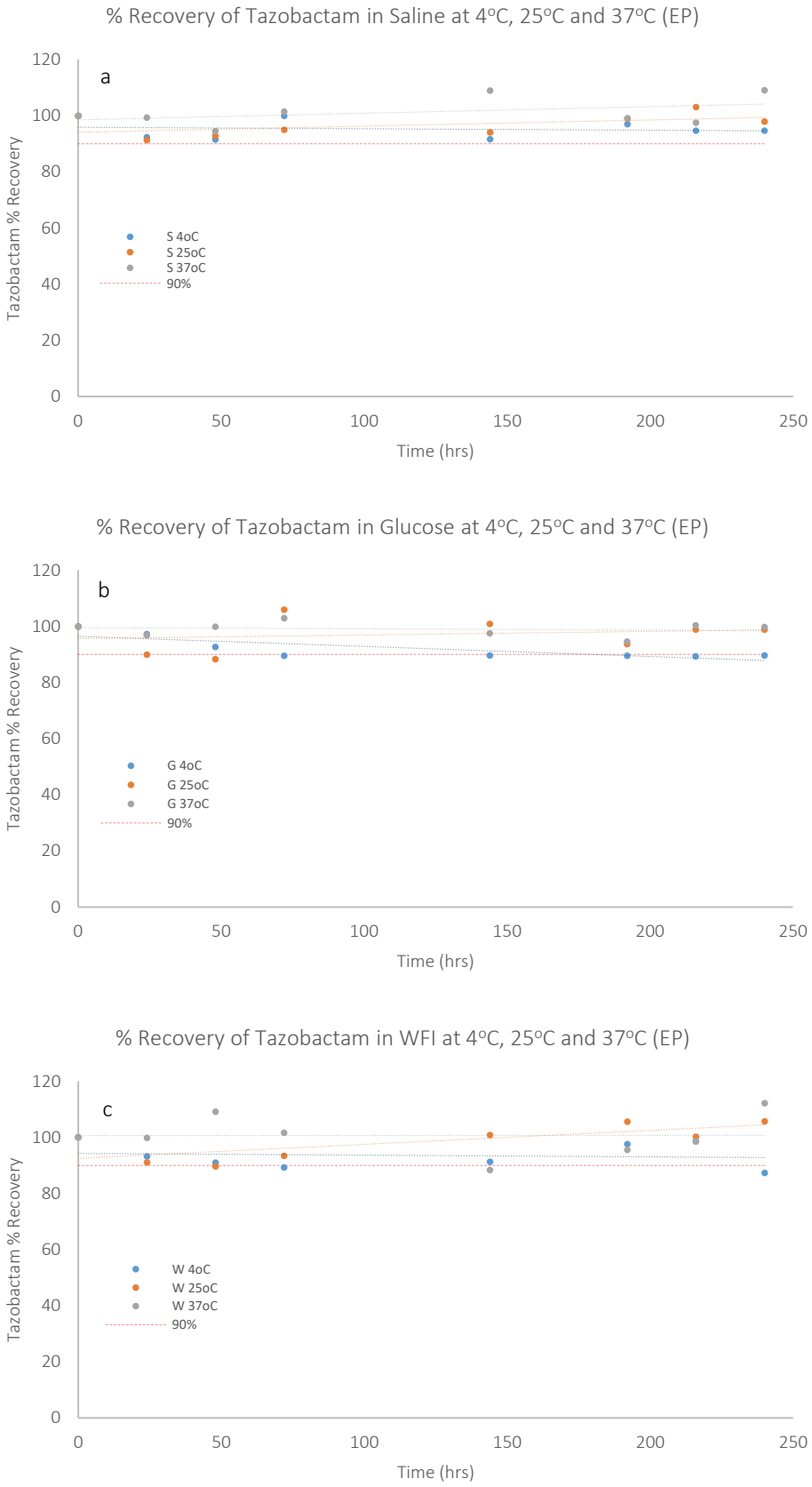


Figure 68 Stability of tazobactam in EP over time at a) 4ºC, b) 25ºC and c) 37ºC: mean % of intact molecule as a function of time and type of diluent. Dashed line: 90% of initial concentration.

3.6.3.2 Piperacillin-Tazobactam Stability in EP - 168H (4oC) + 1H (25oC) + 24H (37oC)

Table 40, **Figure 71** and **Figure 72** show the rate of degradation of piperacillin and tazobactam for the three diluents investigated. The data obtained (**Table 40**) demonstrate extended shelf-life for piperacillin-tazobactam infusions solutions stored for 7 days at 4°C, followed by 1 hour at 25°C to equilibrate the solution temperature prior to infusion, followed by a 24 hour 'in use' period at 37°C.

Table 40 %Recovery of piperacillin and tazobactam stored in EP for all diluents at: 4°C for 168 hours, 25°C for 1 hour and 37°C for 24 hours

Condition	4°C					25°C	37°C	
	T_0	T_{24}	T_{48}	T_{72}	T_{168}	T_{169}	T_{181}	T_{193}
	Piperacillin							
NS	100	101	101	100	98	98	94	92
WFI	100	101	100	99	97	97	95	91
D5W	100	99	99	98	94	94	92	90
	Tazobactam							
NS	100	101	102	99	99	97	95	93
WFI	100	98	98	98	98	98	96	95
D5W	100	97	98	96	96	95	93	92

Piperacillin concentrations after 168 hours (7 days) at 4°C were over 94% in all diluents studied. During the 1-hour temperature equilibration period at 25°C, the percentage of piperacillin concentration lost was 0.5%, 0% and 0.2% for NS, WFI and D5W, respectively. The most pronounced concentration loss for all conditions was during the 24-hours at 37°C, 5.8%, 6.3% and 4.2% for NS, WFI and D5W, respectively. A one-way ANOVA verified that there was no statistically significant difference between the three diluents investigated ($p = 0.469$). Individual t-tests between conditions: (1) NS vs WFI, (2) NS vs D5W and (3) D5W vs WFI also verified that there were no statistically significant differences between the three diluents, $p = 0.422$, $p = 0.133$ and $p = 0.179$, respectively (**Table 41**).

Table 41 Results of piperacillin and tazobactam ANOVA analyses and T-Test performed at the level of diluent at 95% confidence level

Temperature	Variables	ANOVA	T-Test (Individual Analysis)	
Piperacillin	(NS) vs (WFI) vs (D5W)	Not Significant (p = 0.469)	(NS) vs (WFI) (p= 0.422)	Not Significant
			(NS) vs (D5W) (p = 0.133)	Not Significant
			(D5W) vs (WFI) (p = 0.179)	Not Significant
Tazobactam	(NS) vs (WFI) vs (D5W)	Not Significant (p = 0.167)	(NS) vs (WFI) (p= 0.333)	Not Significant
			(NS) vs (D5W) (p = 0.063)	Not Significant
			(D5W) vs (WFI) (p = 0.046)	Significant

Tazobactam concentrations after 168 hours (7 days) at 4^{o}C were over 96% in all diluents studied. During the 1-hour temperature equilibration period at 25^{o}C, the percentage of tazobactam concentration lost was 1.4%, 0.3% and 0.5% for NS, WFI and D5W, respectively. The most pronounced concentration loss for all conditions was during the 24-hours at 37^{o}C, 4.6%, 2.4% and 2.7% for NS, WFI and D5W, respectively. A one-way ANOVA verified that there was no statistically significant difference between the three diluents investigated (p = 0.167). Individual t-tests between conditions: (1) NS vs WFI, (2) NS vs D5W and (3) D5W vs WFI also verified that there were no statistically significant differences between (1) NS vs WFI and (2) NS vs D5W, however a statistically significant difference between (3) D5W vs WFI was found, (1) p = 0.333, (2) p = 0.063 and (3) p = 0.046, respectively (Table 41).

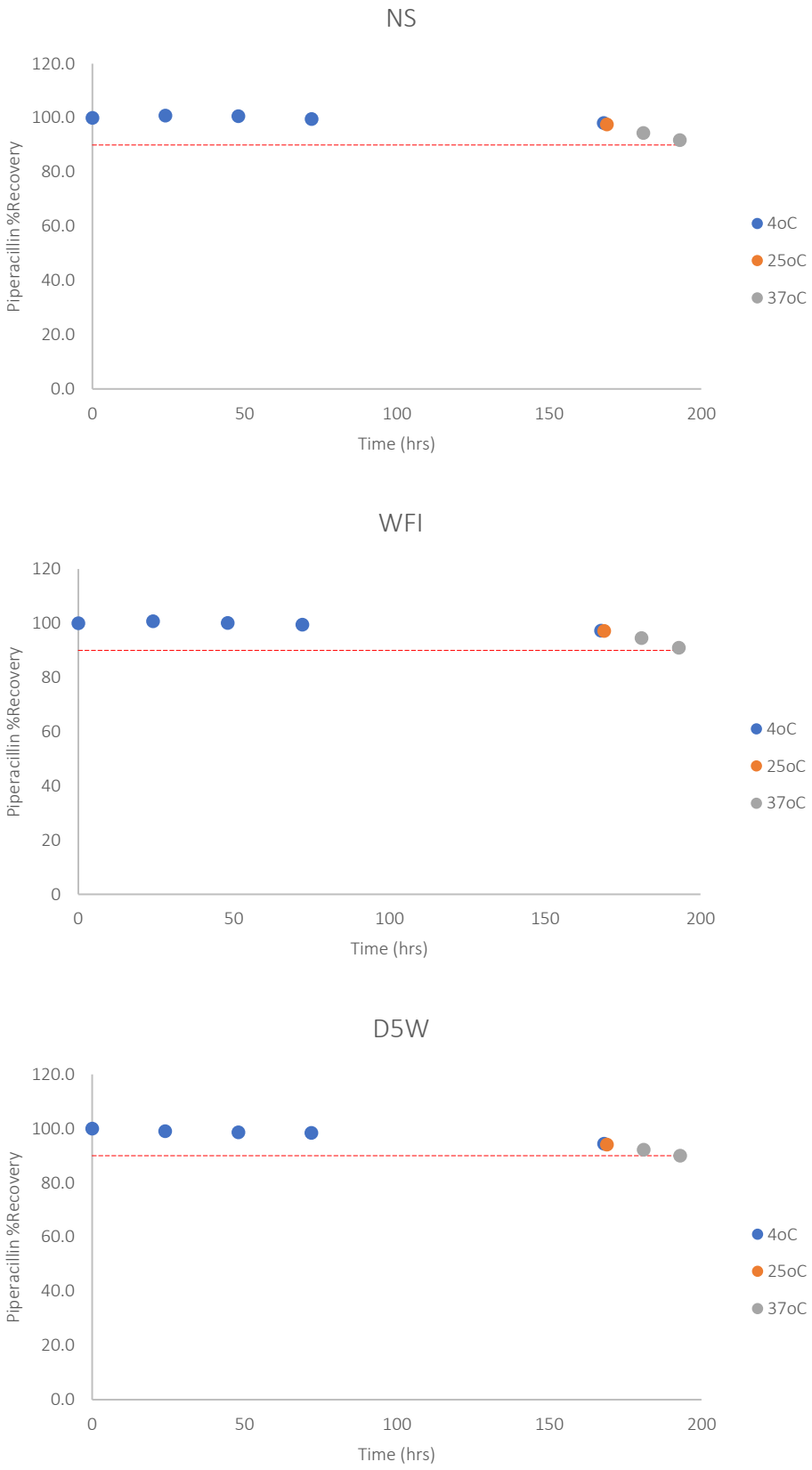


Figure 69 Rate of degradation of piperacillin for the three diluents: a) saline, b) water for injection and c) dextrose, stored for 7 days at 4°C, followed by 1 hour at 25°C, followed by a 24 hour 'in use' period at 37°C.

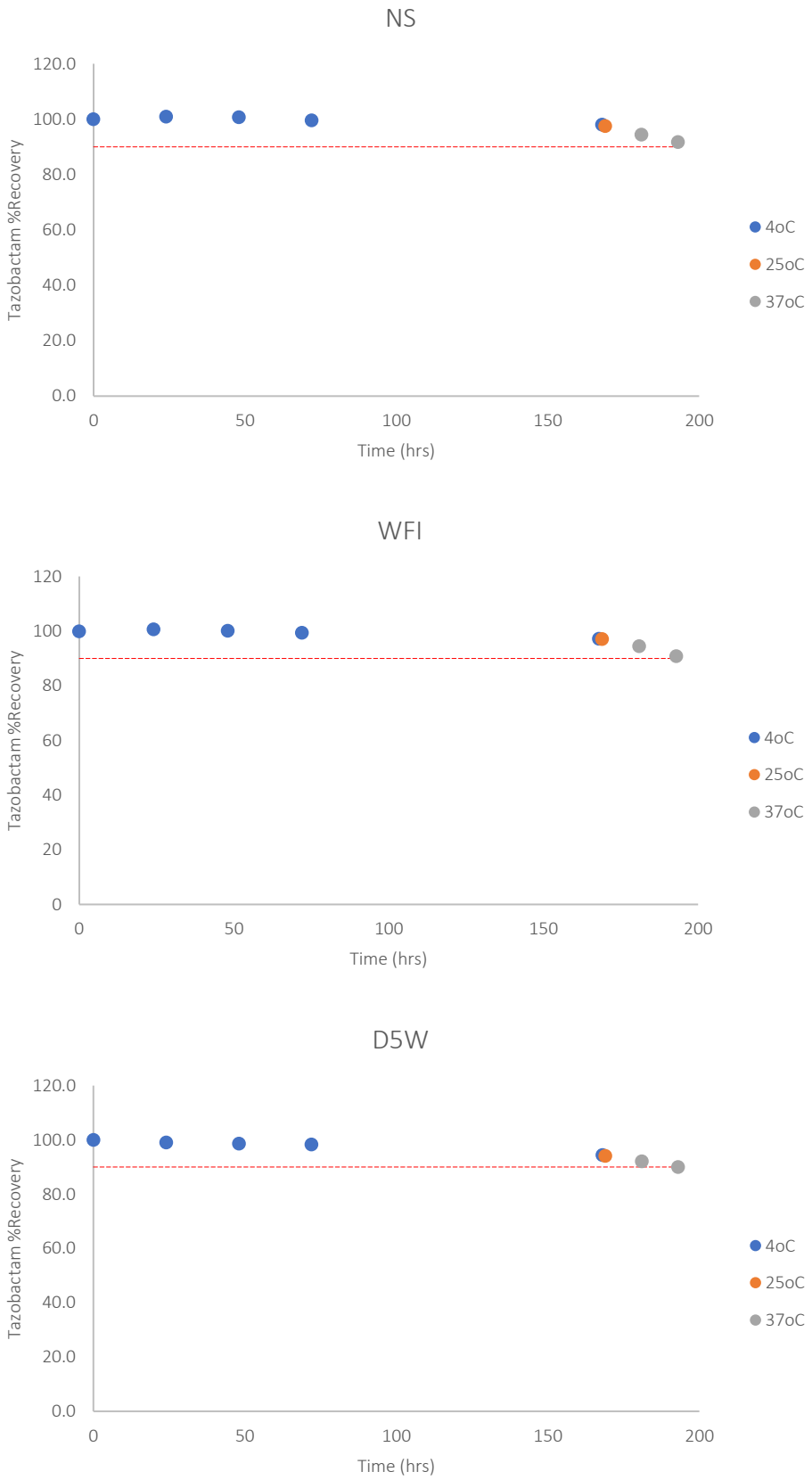


Figure 70 Rate of degradation of tazobactam for the three diluents: a) saline, b) water for injection and c) dextrose, stored for 7 days at 4°C, followed by 1 hour at 25°C, followed by a 24 hour 'in use' period at 37°C.

3.6.3.3 Piperacillin-tazobactam pH profile

Prior to conducting the main stability study, an investigation into the effect of pH on the stability of piperacillin-tazobactam was performed. Both APIs were more labile in basic conditions compared with acidic conditions. Piperacillin appeared to degrade more quickly than tazobactam, especially at higher pH's. Both APIs appeared to be most stable at a more neutral pH (6.5 – 7.0)

While conducting the main stability study, pH stability analysis was performed simultaneously. The level of chemical degradation was compared with pH. A decrease in pH over time in a concentration dependant manner was observed. The pH of the admixture solutions remained within the range of 6.2 – 5.5 in all conditions at 4°C, 6.2 – 5.0 in all conditions at 25°C and 6.2 – 4.7 in all conditions at 37°C over the course of the study (**Table 42**).

Table 42 pH stability profile for piperacillin-tazobactam infusion solutions for all conditions.

			pH								
Temp	Diluent	Device	T_0	T_{24}	T_{48}	T_{72}	T_{144}	T_{192}	T_{216}	T_{240}	Range
	NS	IVB	6.1	6.1	6.1	6.0	5.9	5.9	5.9	5.8	6.1 – 5.8
		EP	6.1	6.1	6.1	6.0	6.0	5.8	5.9	5.9	6.1 – 5.9
	D5W	IVB	6.2	6.1	6.1	5.9	5.9	5.7	5.5	5.5	6.2 – 5.5
4°C		EP	6.2	6.1	6.1	6.0	6.0	5.9	5.8	5.6	6.2 – 5.6
	WFI	IVB	6.0	6.0	6.0	6.0	5.9	5.9	5.8	5.8	6.0 – 5.8
		EP	6.0	6.0	6.0	6.0	6.0	6.0	5.9	5.9	6.0 – 5.9
	NS	IVB	6.1	6.0	6.0	5.9	5.8	5.5	5.4	5.2	6.1 – 5.2
		EP	6.1	6.1	6.1	5.9	5.8	5.8	5.5	5.3	6.1 – 5.3
	D5W	IVB	6.2	6.2	6.0	5.8	5.8	5.4	5.2	5.0	6.2 – 5.0
25°C		EP	6.2	6.2	6.1	5.9	5.9	5.6	5.3	5.1	6.2 – 5.1
	WFI	IVB	6.0	6.0	5.8	5.7	5.5	5.5	5.3	5.2	6.0 – 5.2
		EP	6.0	5.9	5.7	5.7	5.7	5.6	5.4	5.2	6.0 – 5.2
	NS	IVB	6.1	6.1	6.0	5.8	5.5	5.3	5.0	4.9	6.1 – 5.0
		EP	6.1	6.1	6.1	6.0	5.7	5.5	5.2	5.1	6.1 – 5.1
	D5W	IVB	6.2	6.1	6.0	5.8	5.5	5.2	4.9	4.7	6.2 – 4.7
37°C		EP	6.2	6.0	5.9	5.9	5.5	5.1	4.9	4.9	6.2 – 4.9
	WFI	IVB	6.0	6.0	5.7	5.5	5.4	5.2	5.2	5.0	6.0 – 5.0
		EP	6.0	5.9	5.7	5.7	5.5	5.3	5.2	5.2	6.0 – 5.2

*Results are the average of triplicate pH readings samples

3.6.3.4 Physical Compatibility

No significant change in optic density was observed as all solutions remained clear/colourless throughout the analysis.

3.6.4 Discussion

Piperacillin-tazobactam is frequently administered via II promptly after reconstitution and dilution. Routine preparation and administration of piperacillin-tazobactam follow the manufacturer requirements for the duration of time and temperature conditions that the admixture may be exposed to before being infused in the patient (312). Often, the process of preparing the medication and setting up the infusion device is done by a healthcare professional (mainly nurses) on the ward. To avoid dose mismanagement and microbiological hazards associated with preparation in clinical areas, piperacillin-tazobactam infusion solutions can be pre-prepared in approved aseptic facilities under the control of a pharmacist.

3.6.4.1 Chemical Stability Study

Preliminary review of the literature on the stability of piperacillin-tazobactam for administration in both hospital and OPAT settings mainly reported stability over a short duration (24 hours) and at ambient temperature. Published studies on piperacillin-tazobactam in EP investigated, stability in buffered diluents, temperatures that do not comply with YCD or antibiotic concentrations that did not always match patient prescriptions. The literature also lacked data comparing the stability of piperacillin-tazobactam in EP and IVB, particularly regarding the diluents and storage temperature. This detailed study, however, is inclusive of piperacillin-tazobactam stability in two infusion devices when prepared using three diluents and stored at different temperatures.

It is essential that piperacillin-tazobactam solutions demonstrate stability at elevated temperatures, close to body temperature, if it is to be used for continuous 24-hour infusions in hospital settings and via OPAT services. Numerous studies have assessed the stability of piperacillin-tazobactam to optimise its administration. However, most studies were performed over 24 hours at a maximal storage temperature of 25^{o}C. The shelf-life of

piperacillin-tazobactam infusion solutions was determined by conducting numerous stability studies including temperature stability, diluent stability, infusion device stability and pre-preparative stability studies. Results obtained throughout this research provide a greater understanding of piperacillin-tazobactam hydrolysis rates at higher temperatures over a longer duration of time.

Determining the maximum shelf-life of piperacillin-tazobactam also offers an insight into the feasibility of administering via CI, which increases patient response to therapy, thus, can theoretically delay the development of resistance. Stability data generated from this study demonstrate the viability of pre-preparing and storing piperacillin-tazobactam solutions prior to administration. Piperacillin-tazobactam was considerably more stable than what the manufacturer states with a significantly longer maximum shelf-life after reconstitution.

The rate of piperacillin-tazobactam degradation is a function of environmental conditions, with temperature being one of the most important parameters. Hydrolysis rates were highly temperature dependant as greater degradation of piperacillin was observed as the storage temperature increased. It was observed that hydrolysis rates of piperacillin and tazobactam in NS, D5W and WFI typically increase as temperature increases. The slopes of the regression lines for piperacillin in all conditions showed significant deviation from zero at the 99% level of confidence, indicating piperacillin exhibits degradation with time. ANOVA analysis confirmed that there is a statistically significant difference in recovered piperacillin concentration between the temperatures studied when prepared in all diluents (IVB; NS – $p = 0.005$, D5W – $p = 0.009$, WFI – $p = 0.019$) (EP; NS – $p = 0.007$, D5W – $p = 0.001$, WFI – $p = 0.008$). Although, tazobactam degraded faster as storage temperature increased, it appeared less sensitive to temperature and more stable than piperacillin (Table 38).

The most used diluents for IV administration include 0.9% sodium chloride (NS), 5% dextrose (D5W) and water for injection (WFI). The choice of diluent for piperacillin-tazobactam's admixture compatibility and stability should be made according to the requirements of the dosing regimen as well as patient needs. According to manufacturer guidelines, piperacillin-tazobactam is reconstituted with 20mL WFI or NS and then further diluted with one of the reconstitution diluents or with D5W (320). Statistical analysis using one-way ANOVA and one-tailed t-tests showed that there was no statistically significant difference of piperacillin

or tazobactam recoveries when prepared in all three diluents ($p > 0.05$). Although overall there was no statistically significant difference between diluents, piperacillin and tazobactam recoveries were similar when diluted in NS and WFI, however, it was observed that piperacillin was stable for a shorter length of time when diluted in D5W (Table 32 and Table 34).

The stability of piperacillin and tazobactam was studied in two devices, IVB and EP, to establish the shelf life of the combination for inpatient and outpatient therapy. It was found that there was no statistically significant difference between the two infusion devices regarding piperacillin stability. For tazobactam stability, there was no statistically significant difference between infusion device at 4°C and 25°C for all diluents, however, at 37°C, tazobactam stability was significantly enhanced for all diluents when stored in EPs.

The manufacturer indicates that piperacillin tazobactam is stable for 48 hrs at 2-8^{o}C after reconstitution. Results obtained from this study indicate up to 10 days in NS, 8 days in D5W and 9 days in WFI in both IVBs and Eps.

The data obtained from this study open the possibility of CI piperacillin-tazobactam in both hospital and outpatient settings. Increased stability offers the possibility pre-preparing the infusion solutions prior to administration via II or CI. In inpatient settings, wards could have a small stock of IVBs for acute patients and weekend treatments. For OPAT settings, hospital pharmacies could produce pumps in advance and patients can receive more pumps at a time.

Previously published studies reported stability data and expiration dates that were shorter than observed in this study. In IVB, the shelf-life of piperacillin-tazobactam was longer when stored at relevant "in use" temperatures. Mathew *et al*., found that piperacillin-tazobactam (60mg/mL-7.5mg/mL) solutions in PVC bags were stable for 2 days in both saline and D5W at 25^{o}C (324). In 2009, Donnelly *et al*., found that piperacillin to be stable for 72 hours at 23^{o}C (321). Saghir *et al*., 2017 found that piperacillin-tazobactam solutions remained stable for 12 hrs in EP when stored at 37^{o}C (332).

Some studies showed stability with more favourable results than those obtained in this study. Piperacillin is known to degrade in solutions with a high pH and during extended

storage without pH control. Jamieson *et al.*, investigated the effect of pH control by using citrate buffered saline diluent pH 7 on the degradation rate of piperacillin-tazobactam solutions (319). This study presented data of improved chemical stability that supports prolonging their infusion time. Rigge *et al.*, also found piperacillin-tazobactam to be noticeably more stable when diluted in buffered saline (317). Saghir *et al.*, also found that buffered diluents had a positive effect on the stability of piperacillin-tazobactam (332). These studies however, either used more diluted solutions or used buffered diluents. Such diluents are not readily available to clinicians in pharmaceutically validated form and may not be appropriate or compatible for all patient requirements.

3.6.4.2 Piperacillin-Tazobactam Stability – 168H (4°C) + 1H (25°C) + 24H (37°C)

The data obtained from this study open the possibility of CI piperacillin-tazobactam in both hospital and outpatient settings. Increased stability offers the possibility of pre-preparing the infusion solutions prior to administration via II or CI. In inpatient settings, wards could have a small stock of IVBs for acute patients and weekend treatments. For OPAT settings, hospital pharmacies could produce pumps in advance and patients can receive more pumps at a time.

3.6.5 Conclusion

In conclusion, according to the experiments carried out throughout this study, the optimal conditions for the administration of CI piperacillin-tazobactam have been defined. The stability of piperacillin-tazobactam solutions were affected by temperature (with faster degradation at higher temperatures) but not significantly affected by diluent. The shelf-life of piperacillin-tazobactam in solution exceeds the maximum beyond-use dates stated by manufacturer. 24-hr CI piperacillin-tazobactam is feasible when solutions are diluted in NS and WFI and stored in Baxter IVB and EPs when stored at 37°C. Dilution in D5W provides sufficient stability for administration via a 24-hr CI when storage temperature of the infusion device does not exceed 25°C.

CHAPTER 4

SUITABILITY AND FEASIBILITY FOR AMOXICILLIN-CLAVULANIC ACID VIA PROLONGED/CONTINUOUS INFUSION

Publications

Fawaz S, Dixon B, Barton S, Mohamed A, Nabhani-Gebara S. Suitability of amoxicillin–clavulanic acid for administration via prolonged infusion. Drug design, development and therapy. 2020;14:103-109.

Fawaz S, Merzouk M, Barton S, Nabhani-Gebara S. Stability of amoxicillin and clavulanic acid in separate containers for administration via a Y-site. Drug design, development and therapy. 2021;15:3979-3984.

'Drug Design, Development and Therapy 2020;14:103-109 'Originally published by and used with permission from Dove Medical Press Ltd.' & 'Drug Design, Development and Therapy 2021; 15:3979-3984 'Originally published by and used with permission from Dove Medical Press Ltd.'

Conferences

Fawaz S, Barton S, Nabhani-Gebara S. Suitability of Amoxicillin–Clavulanic Acid for Administration via Prolonged Infusion. JPAG, Royal Society of Chemistry, 2019. Poster Presentation.

Fawaz S, Barton S, Nabhani-Gebara S. Suitability of Amoxicillin–Clavulanic Acid for Administration via Prolonged Infusion. Royal Society of Chemistry - Synthesis and Drug Discovery Symposium, Kingston University, 2019. Poster Presentation.

Fawaz S, Barton S, Nabhani-Gebara S. Suitability of Amoxicillin–Clavulanic Acid for Administration via Prolonged Infusion. Royal Society of Chemistry - SE and London Organic Division Meeting, Kingston University, 2020. Poster Presentation.

4.1 Introduction to Amoxicillin-Clavulanic Acid

Amoxicillin is one of the most commonly used BLAs in the primary care settings (333). Amoxicillin-clavulanic acid is indicated for the treatment of infections where amoxicillin alone is insufficient, including severe infections of the ear and nose, respiratory tract infections as well as lung, skin, and urinary tract infections. Amoxicillin-clavulanic acid has a broad-spectrum of activity and is active against most clinically important Gram-negative and Gram-positive bacteria (334,335).

4.1.1 Rationale for the use of Amoxicillin in Combination with Clavulanic Acid

The pharmaceutical formulation contains two active ingredients; (1) amoxicillin, a penicillin antibiotic and, (2) clavulanic acid, a BLI that broadens amoxicillin's spectrum of activity and combats resistance by preventing bacteria from inactivating amoxicillin. Parenterally, amoxicillin alone lacks strong activity against bacteria as the BL ring is hydrolysed by the pathogens BLEs. In practice, parenteral amoxicillin is most administered in conjunction with BLI clavulanic acid as it enhances its effectiveness by inhibiting many BLEs to which it is susceptible, permitting its use for various clinical infections. It is available for parenteral administration only in combination, with a 5:1 ratio of amoxicillin to clavulanic acid by weight (334).

4.1.1.1 Amoxicillin's Mechanism of Action

Amoxicillin exerts bactericidal activity via inhibition of bacterial cell wall synthesis (peptidoglycan) by binding to multiple PBP enzymes that inhibit the process of transpeptidation. This leads to the cross-linking of D-alanine and D-aspartic acid in bacterial cell walls. Without functioning PBPs, bacteria upregulate autolytic enzymes and are incapable of building and repairing their cell walls, leading to bactericidal action (335).

4.1.1.2 Clavulanic Acid Mechanism of Action

Clavulanic acid is a 'suicide' inhibitor that works by irreversibly binding to the catalytic site of a wide variety of pathogens BLEs. (336). It inhibits the destruction of amoxicillin by beta-lactamase enzymes that catalyse the hydrolysis of the beta-lactam ring (334). Alone, clavulanic acid does not have inherent bactericidal activity, however it broadens amoxicillin's spectrum to BLE producing pathogens (333).

4.1.2 Dosage and Administration

Amoxicillin is a time-dependant antibiotic, thus is dosed more frequently than concentration-dependant antibiotics (which can be dosed once daily) to reduce variations in peak and trough serum concentrations (333). Amoxicillin-clavulanic acid is routinely administered intermittently as a bolus injection over 3-5 minutes or by infusion over 20-30 minutes. Each pharmaceutical vial of amoxicillin-clavulanic acid contains 1000mg amoxicillin sodium salt and 200mg clavulanic acid potassium salt. Each 1.2g vial is prepared and administered every 8 hours. Adjustments to dosage are made depending on severity of infection and the demographic of the patient.

4.1.3 Tolerability and Adverse Effects

Amoxicillin-clavulanic acid is generally well tolerated. The most frequent adverse events include gastrointestinal symptoms (e.g., diarrhoea, nausea, vomiting) and urticaria. In rare cases patients may suffer from seizures (e.g., patients with poor renal function) (333).

4.1.4 PK Profile

The distribution of amoxicillin and clavulanic acid is rapid, where peak plasma concentrations are attained immediately upon completion of IV infusion. Upon absorption, both amoxicillin and clavulanic acid display relatively low levels of serum protein binding with around 25% of total plasma clavulanic acid and 18% of total plasma amoxicillin being protein bound (333,337).

The main route of amoxicillin elimination is via the kidney (50-85%). Amoxicillin is partly excreted in the urine as the inactive penicilloic acid metabolite in quantities equivalent to up to 10 to 25% of the initial dose. Clavulanic acid is eliminated by both renal (27-60%) and non-renal mechanisms. It is extensively metabolised in the body and is eliminated in the urine, faeces, and exhalation. The SmPC states that approximately 60 to 70% of amoxicillin and 40 to 65% of clavulanic acid are excreted unchanged in the urine during the first 6 hours after administration of a single 1000 mg/200 mg bolus intravenous injection. Dose adjustments may therefore be necessary in patients with renal insufficiency(333,337).

4.1.5 PD Profile

Amoxicillin-clavulanic acid is a time-dependant antibiotic; hence, its bactericidal activity is closely correlated to the time at which antibiotic concentrations in tissue and serum exceed the MIC threshold of the infecting organism (T > MIC). Periods at which amoxicillin-clavulanic acid concentrations are above the MIC is a major parameter determining efficacy where optimum bactericidal activity is achieved when time above the MIC is approximately 50-60% of the dosing interval. However, amoxicillin has no significant post-antibiotic effect, therefore, when concentrations drop lower than the MIC (T < MIC), bacterial growth resumes immediately, facilitating the development of resistance, especially when serum concentrations fall below the MIC threshold for longer than half of the dosing interval.

4.1.6 Mechanism of Resistance

A major resistance mechanism towards amoxicillin involves its inactivation via BLEs. Clavulanic acid makes amoxicillin effective against BLEs that would normally degrade amoxicillin.

4.2 Aim and Objectives

A review of the literature highlighted a major lack of data on the stability of amoxicillin-clavulanic acid, particularly regarding parenteral formulation. There is a need for more prolonged and detailed studies that are inclusive of the variety of temperature and diluent conditions the infusion solutions are exposed to in clinical practice to define a maximum shelf-life appropriate for the infusion formulations.

Therefore, the overall aim of this chapter was to determine the feasibility of P/CI amoxicillin-clavulanic acid administration. To achieve this aim, the following objectives were set.

1) To develop a stability indicating HPLC method for the quantitative determination of amoxicillin-clavulanic acid concentration over time

2) To determine the specificity of the developed method by conducting a forced degradation study

3) To validate the developed method in compliance with the ICH guidelines

4) To conduct stability studies to determine amoxicillin-clavulanic acid stability when prepared in different diluents and stored at different temperatures.

4.3 Method Development

Numerous SIMs for the quantitative determination of amoxicillin-clavulanic acid are reported in the literature. These methods were specifically developed for the quantification of amoxicillin-clavulanic acid in human plasma (338–340) and in simulated gastric digestion (341). A number of analytical methods were utilized including HPLC, LC-MS, micellar electro-kinetic capillary chromatography, TLC and spectrophotometry. However, very limited studies have focused on the estimation of amoxicillin-clavulanic acid in pharmaceutical dosage form (342–344).

In 2010, Tippa *et al.*, developed a method that detected reconstituted injectable amoxicillin-clavulanic acid. Separation was obtained on a C18 column (250 × 4.0 mm, 4 µm) using a mobile phase composed of sodium dihydrogen phosphate buffer (pH 5) and methanol (95:5, v/v%). The detection wavelength was 220nm at a flowrate of 1mL/min. The method appeared to be suitable for stability studies; however, the total run time was relatively long as each analysis took about 12 minutes to complete (344).

In 2014, Addotey *et al.*, focused on the stability of oral paediatric powder suspensions when different water forms were used. The study used a different mobile phase that consisted of water, sodium acetate buffer (pH 4.4) and methanol (65:20:15, v/v/v%) and investigated the effect of distilled, treated tap water and mineral water on the stability of reconstituted suspensions. The selected: flowrate was 1mL/min, injection volume was 100µL and the detection wavelength was 220nm. The study was set for 7 days only to match the duration of treatment and it concluded that the type of water had no significant detrimental effect on the stability of amoxicillin-clavulanic acid (343).

In 2017, Bellur Atici *et al.*, developed a method to identify the impurities formed when oral formulation amoxicillin-clavulanic acid was subjected to stress testing (thermal degradation, photolytic degradation, neutral hydrolysis, acidic hydrolysis, alkaline hydrolysis, and oxidative degradation). Numerous column types (C8 and C18), with different particle sizes and column lengths were tested to ensure good resolution between impurities and APIs was obtained. The column selected for this study was a C18 column with 3.0 µm particle size and 250 mm column length. Three mobile phases were used for gradient elution; potassium dihydrogen phosphate (pH 4.5), methanol/water (95:5, v/v%) and potassium dihydrogen

phosphate (pH 3.3). The flow rate during each run varied between 0.5 and 0.6mL/min depending on the mobile phase and the detection wavelength used was 215nm (342)

There is little published relating to SIMs for amoxicillin and clavulanic acid in solution for parenteral administration. Therefore, the focus of this subchapter is to develop a HPLC method that is capable of separating amoxicillin and clavulanic acid from their degradation products and measuring their concentration in infusion solutions, accurately and rapidly. The developed HPLC method should be able to separate, detect and quantify amoxicillin and clavulanic acid and various drug related degradants.

4.3.1 Chemicals

Pharmaceutical dosage form amoxicillin-clavulanic acid, generic brand infusion vials NS 0.9% and WFI was purchased from Kingston Pharmacy, 53 Surbiton Road, Kingston, KT1 2HG, UK. Pure amoxicillin sodium salt (analytical standard), clavulanic acid potassium salt (analytical standard), oxacillin, cephalothin, ammonium acetate and glacial acetic acid were purchased from Sigma Aldrich.

4.3.2 Instrumentation and Equipment

Quantitative HPLC analysis was carried out using an Agilent 1260 HPLC instrument with single wavelength UV detection and Chemstation software. Calibrated micropipettes (0.5-10µL, 10-100µL, 100-1000µL and 1-10mL) were purchased from Eppendorf Ltd.

4.3.3 Method Development Parameters

Parameters investigated were column, mobile phase, internal standard, detection wavelength, injection volume, column temperature, and flowrate.

4.3.3.1 Column Selection

The stationary phases were trialled based upon previous studies. Five columns were tested; characteristics of these columns are listed in Table 43.

Table 43 Displaying characteristics of trialled columns.

Column No.	Column Details			
	Column Manufacturer and Stationary Phase	Column Dimensions Length x Diameter (mm)	Column Pore Size	Serial Number
1	Phenomenex, Luna, C18	250 x 4.60 mm	5 µm	300204
2	Alltech, C18	250 x 4 mm	5 µm	3352
3	Phenomenex, Aqua, C18	250 x 3 mm	5 µm	417353
4	Phenomenex, Prodigy	150 x 4.6 mm	5 µm	247775
5	Phenomenex, Spherisorb	100 x 4.6 mm	5 µm	84362

Column 1 and Column 3 were not selected as the obtained chromatograms displayed a single peak (Column 1) or two co-eluting peaks (Column 3). Columns 2 was not selected as the back pressure was relatively high (around 2800psi) even when the flowrate was reduced to 0.5mL/min. Column 4 separated the three compounds (two compound peaks and an internal standard peak), however, the resolution achieved was relatively poor. Column 5 was selected as separation of all compounds was achieved with good resolution. Furthermore, the column did not generate high backpressure when the variable parameters were changed, allowing for flexibility and ease of use when the flow rate was increased.

4.3.3.2 Mobile phase

Literature published on amoxicillin-clavulanic acid method development have suggested that buffered methanol and buffered acetonitrile (ACN) are suitable mobile phases for the separation and quantification of amoxicillin-clavulanic acid in solution. Published studies mentioned above used potassium dihydrogen phosphate, tetramethyl ammonium chloride, sodium phosphate, and ammonium acetate buffers. Most buffers were combined with methanol.

ACN was selected to be the organic component of the mobile phase as its low UV cut-off, makes it suitable for high sensitivity analysis at short UV wavelengths. It is also miscible with water and has a low viscosity which reduces back pressure and permits for the use of higher flowrates. ACN was used in conjunction with ammonium acetate buffer (5mM), the aqueous component of the mobile phase. The buffer was adjusted to pH 4 using glacial acetic acid. The composition of the mobile phase was optimised by altering the ratio of ACN to

ammonium acetate (Table 44). The selected composition was 50 : 50 (ACN : ammonium acetate (5mM), v/v%).

Table 44 The different compositions of ACN and ammonium acetate tested.

% ACN	% Ammonium Acetate Buffer (5mM)
70	30
65	35
60	40
50	**50**
40	60

4.3.3.3 Internal Standard

Three compounds, oxacillin, cephalothin and caffeine, were tested to determine their suitability for use as an internal standard. Oxacillin and cephalothin were contenders due to the chemical and physical similarities they have with amoxicillin and clavulanic acid. However, they were deemed unsuitable as they co-eluted with clavulanic acid (Figure 73 and Figure 74). Caffeine appeared to be the most suitable internal standard as it eluted after the analytes with good resolution and remained stable during the whole testing duration (Figure 75).

4.3.3.3.1 Oxacillin

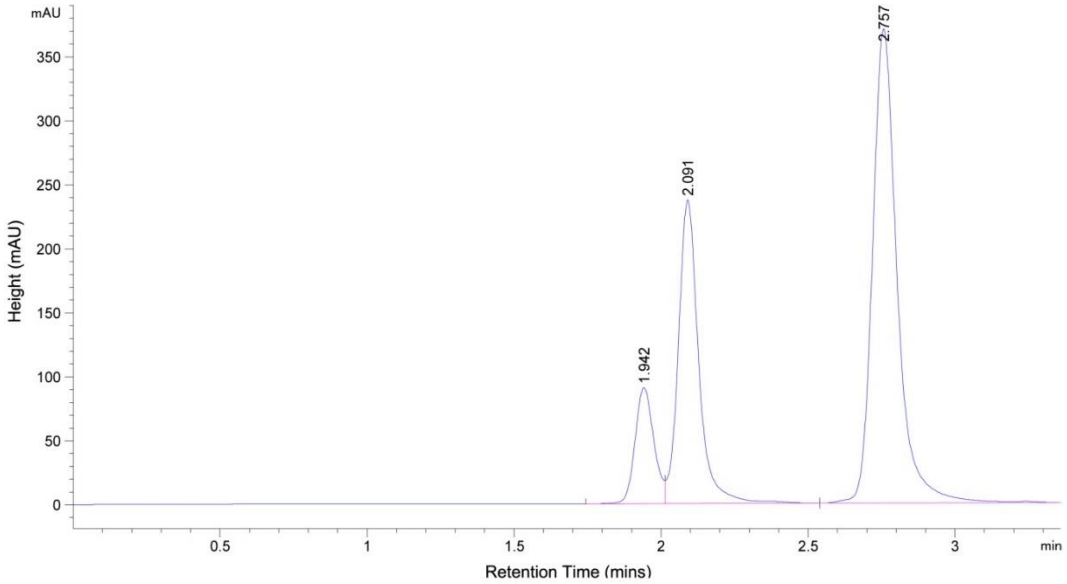


Figure 71 Chromatogram of amoxicillin, clavulanic acid and potential internal standard, oxacillin (peaks in order of appearance: clavulanic acid (tR = 1.942 mins), oxacillin (tR = 2.091 mins), amoxicillin (tR = 2.757 mins)).

Figure 72 Chromatogram of amoxicillin, clavulanic acid and potential internal standard, cephalothin (peaks in order of appearance: co-eluted clavulanic acid and cephalothin (tR = 1.971 mins), amoxicillin (tR = 2.760 mins)).**Figure 73** Chromatogram of amoxicillin, clavulanic acid and potential internal standard, oxacillin (peaks in order of appearance: clavulanic acid (tR = 1.942 mins), oxacillin (tR = 2.091 mins), amoxicillin (tR = 2.757 mins)).

4.3.3.3.2 Cephalothin

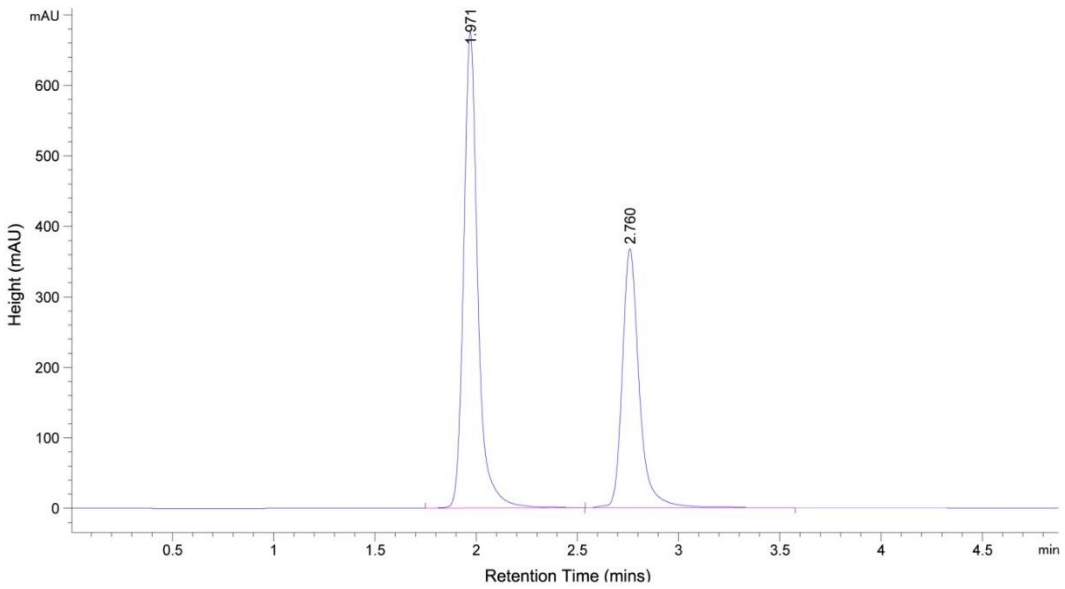


Figure 74 Chromatogram of amoxicillin, clavulanic acid and potential internal standard, cephalothin (peaks in order of appearance: co-eluted clavulanic acid and cephalothin (tR = 1.971 mins), amoxicillin (tR = 2.760 mins)).

Figure 75 Chromatogram of amoxicillin, clavulanic acid and internal standard, caffeine (peaks in order of appearance: clavulanic acid (tR = 1.935 mins), amoxicillin (tR = 2.702 mins), caffeine (tR = 3.211 mins)).**Figure 76** Chromatogram of amoxicillin, clavulanic acid and potential internal standard, cephalothin (peaks in order of appearance: co-eluted clavulanic acid and cephalothin (tR = 1.971 mins), amoxicillin (tR = 2.760 mins)).

4.3.3.3.3 Caffeine

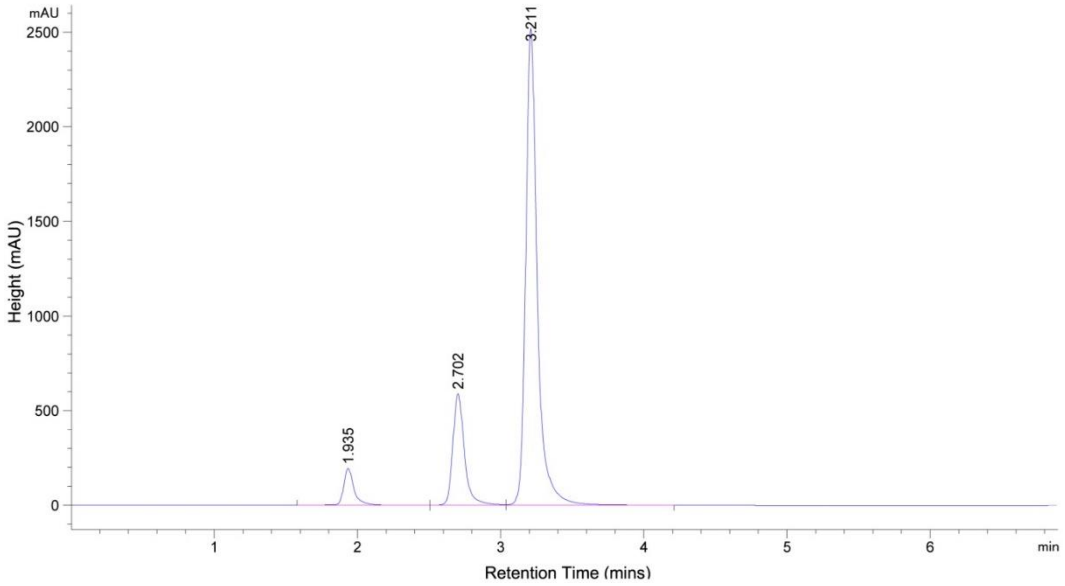


Figure 77 Chromatogram of amoxicillin, clavulanic acid and internal standard, caffeine (peaks in order of appearance: clavulanic acid (tR = 1.935 mins), amoxicillin (tR = 2.702 mins), caffeine (tR = 3.211 mins)).

Figure 78 Showing linearity in the range of 10-80ppm for amoxicillin-clavulanic acid, 8.33-66.67ppm for amoxicillin and 1.67-13.33ppm for clavulanic acid.**Figure 79** Chromatogram of amoxicillin, clavulanic acid and internal standard, caffeine (peaks in order of appearance: clavulanic acid (tR = 1.935 mins), amoxicillin (tR = 2.702 mins), caffeine (tR = 3.211 mins)).

4.3.3.4 Wavelength Selection

A UV scan of amoxicillin, clavulanic acid and caffeine was undertaken from 350-200nm to determine which wavelength the target compounds have the max absorption. Amoxicillin was observed to have a λ_{max} of 270nm, clavulanic acid was found to have a λ_{max} of 205nm and caffeine was found to obtain a λ_{max} of 260nm.

Amoxicillin-clavulanic acid solution was spiked with caffeine and injected into the HPLC system where the instruments UV detector was set at a range of different wavelengths based on the determined λ_{max}'s. Wavelengths tested include: 205, 215, 225, 235, and 245nm. The wavelengths 225 and 235nm gave optimal absorbance. Higher and lower wavelengths resulted in poor baselines or a smaller clavulanic acid peak. The wavelength 225nm was selected as optimal peak shape, height and area were achieved as well as good resolution and baseline.

4.3.3.5 Injection Volume

From the literature it is apparent that the most common injection volume used when assessing the stability of beta-lactam antibiotics is 10µL. Injection volumes 5, 10, 15 and 20µL were trialled. The injection volume selected was 10µL as it provides adequate detection at a relatively low volume; it avoids distorting the peak shape and reduces the risk of column damage.

4.3.3.6 Column Temperature

To achieve reproducibility in terms of retention times it is vital to maintain a stable and constant column temperature. To select the optimum column temperature a sample of amoxicillin-clavulanic acid and caffeine was prepared and examined under 4 different temperatures (25, 30, 35 and 40°C) using the same flowrate and injection volume. As the temperature increases, the backpressure decreases. It was also observed that when the column temperature is increased, the retention time decreased, giving narrower and taller peaks as well as lowering the detection limit. The higher the column temperature, the faster the exchange of analytes between mobile phase and stationary phase. It is also apparent that with temperature increase, the viscosity of the mobile phase decreases resulting in a decrease of back pressure. The decrease in pressure allows for higher flowrates to be used. The column temperature selected was 30°C as it decreases back pressure and run times as

well as obtaining sufficiently resolved peaks without degrading the compounds going through the column.

4.3.3.7 Flowrate

To attain the optimal flowrate, a sample of amoxicillin-clavulanic acid and caffeine was prepared and left at room temperature for three days. This degraded solution was run using flowrates 0.5, 1, 1.5, 1.75 and 2 mL/min. The higher the flowrate, the faster the compound eluted, however, as the flowrate increased so did the back pressure. Good separation and resolution of peaks was achieved for all flowrates tested. 1.75mL/min was the chosen flowrate as the goal was to shorten the run time while maintaining an acceptable pressure and avoiding co-elution (with other compounds of interest or degradation products).

4.3.4 Selected HPLC Analytical Conditions

Separation was conducted using a Phenomenex Spherisorb 5 µm, 100 x 4.6 mm column. The mobile phase consisted of ammonium acetate buffer (5mM) pH 4 : ACN (50:50, v/v%) at flowrate of 1.75mL/min. Analysis was performed at 30°C and detection at 225nm. The injection volume was 10µL with a run time of 2.5 minutes (**Table 45**).

Table 45 Showing optimized chromatogram conditions for amoxicillin -clavulanic acid

Method Development Parameter	Selected Condition
Column	Phenomenex Spherisorb 5 µm, 100 x 4.6 mm
Mobile Phase	Ammonium Acetate Buffer (5mM) pH 4 : ACN (50:50 v/v)
Internal Standard	Caffeine
Flowrate	1.75mL/min
Wavelength	225nm
Injection Volume	10µL
Column Temperature	30°C
Run Time	4 minutes

4.4 Method Validation

The developed method described in the previous section, was validated according to the ICH validation guidelines (316). The method was verified by running three replicates of a standard set of samples once a day for three days. The method was tested for its linearity, range, precision, accuracy, specificity, sensitivity, and robustness.

4.4.1 Linearity and Range

Linearity and the analytical range were assessed through analysis of a range of reference standards (0-80 ppm) that were prepared using a 1000ppm (1mg/mL) stock solution. The stock solution was prepared using the 5:1 (amoxicillin : clavulanic acid) concentration ratio utilised in practice. The range was determined by injecting variable volumes in the range 0.1-0.8µl of a 1000ppm standard solution. This is equivalent to injecting 10µL of a set of standards, concentrations from 10 to 80ppm. **Table 46** and **Figure 76** shows the range amoxicillin-clavulanic acid, amoxicillin and clavulanic acid display linearity.

Table 46 Displaying linearity and range of amoxicillin-clavulanic acid, amoxicillin and clavulanic acid.

Compound/s	Analytical Range (PPM)	Linear Equation	R^2
Amoxicillin-clavulanic acid	10-80	Y = 761.79x + 4.6429	1
Amoxicillin	8.33-66.67	Y = 637.48x + 3.0964	1
Clavulanic acid	1.67-13.33	Y = 124.31x + 1.5464	0.9996

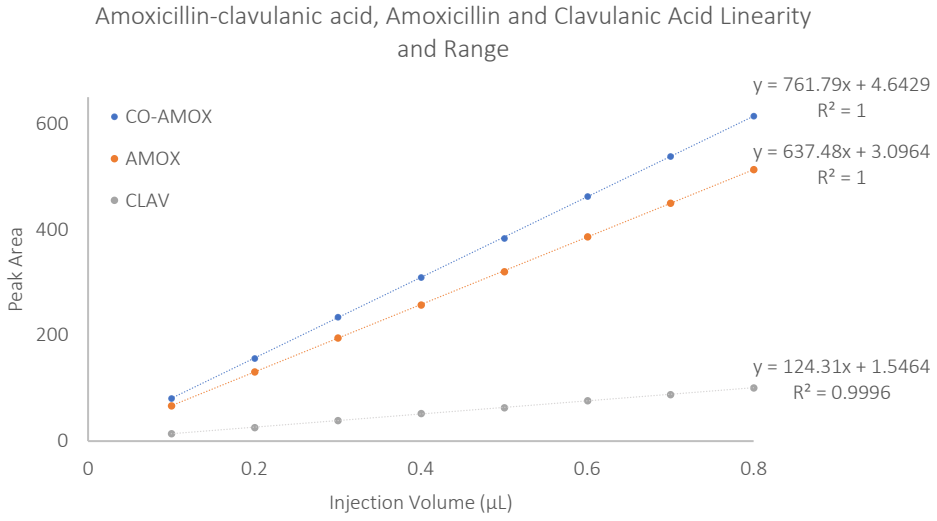


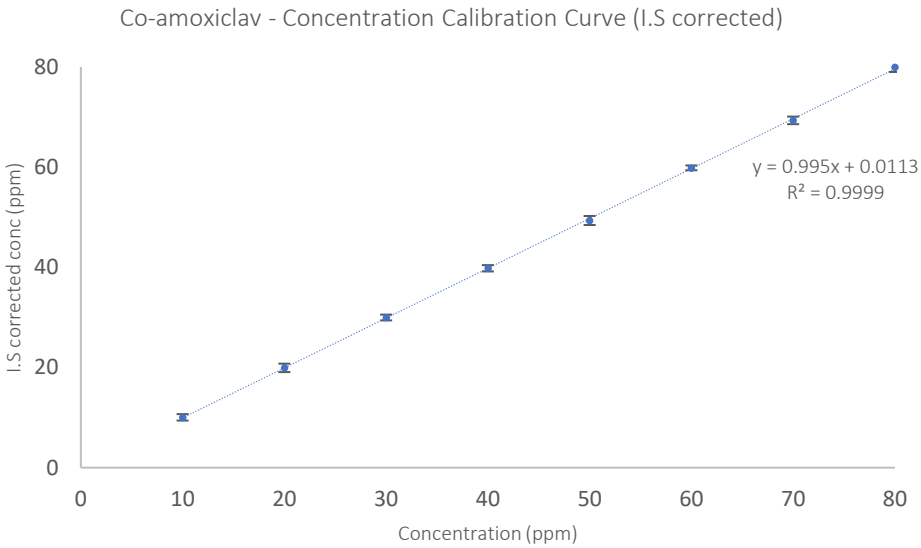
Figure 80 Showing linearity in the range of 10-80ppm for amoxicillin-clavulanic acid, 8.33-66.67ppm for amoxicillin and 1.67-13.33ppm for clavulanic acid.

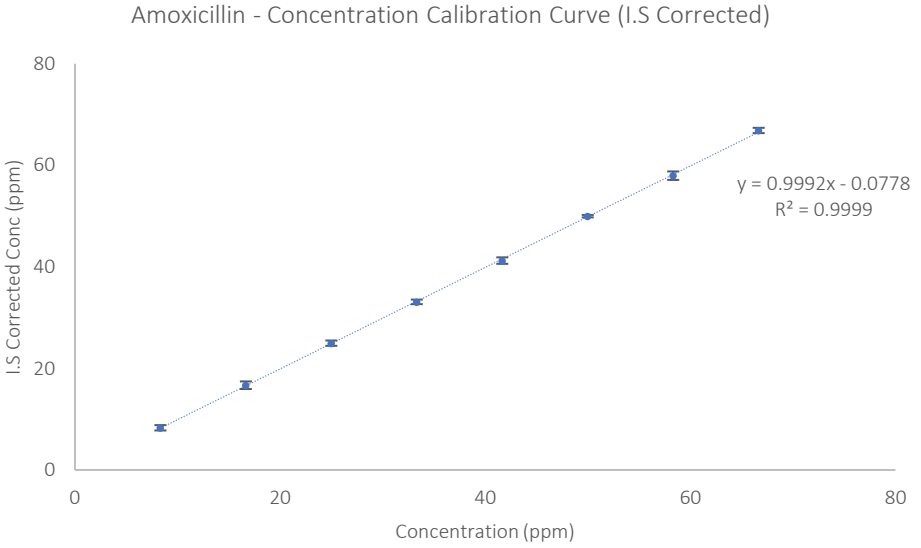
Figure 81 Calibration curves for a) amoxicillin-clavulanic acid, b) amoxicillin and c) clavulanic acid.**Figure 82** Showing linearity in the range of 10-80ppm for amoxicillin-clavulanic acid, 8.33-66.67ppm for amoxicillin and 1.67-13.33ppm for clavulanic acid.

4.4.2 Calibration

In practice, each 1.2g amoxicillin-clavulanic acid vial is reconstituted with 20mL WFI and is further diluted with 50mL of injection diluent; this gives a nominal infusion concentration of 17,143ppm (1200mg/0.07L=17,143ppm). This solution was diluted 1 in 500 for analysis, which reduces the amount of sample needed, reduces matrix effects and gives a nominal concentration of 34.29ppm amoxicillin-clavulanic acid, in the usual analytical range for quantitative HPLC with UV detection. It was therefore decided to calibrate from 0-80ppm so that amoxicillin-clavulanic acid (at 34.29ppm) would be roughly in the middle of the calibration range. Linearity was assessed using nine standards that ranged in concentration from 10-80ppm.

All peak areas obtained (raw data) were normalised with the peak area of the internal standard; this was achieved by dividing amoxicillin-clavulanic acid, amoxicillin and clavulanic acid peak areas by the caffeine peak area. The internal standard corrected peak area was then calculated and plotted against concentration (**Figure 77**).





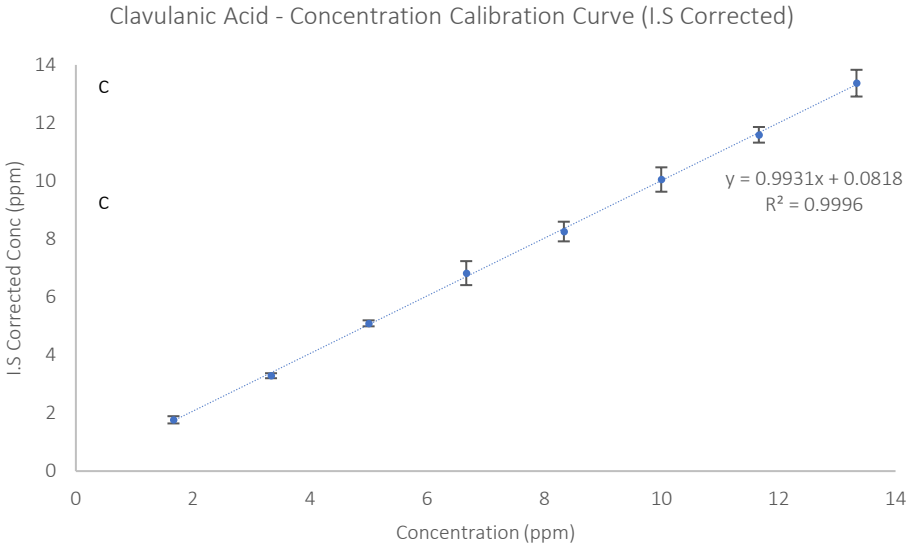


Figure 83 Calibration curves for a) amoxicillin-clavulanic acid, b) amoxicillin and c) clavulanic acid. Error bars within.

4.4.3 QC Sample Preparation

Low (15 ppm), medium (45 ppm), and high (75 ppm) QC samples corresponding to each quartile of the working range were used to determine the accuracy (%error and %recovery) and precision (%RSD) of developed method.

4.4.4 Precision

As previously explained in Section 3.5.4, precision was considered at three levels, these include: (1) repeatability, (2) intermediate precision and, (3) reproducibility. Good precision was obtained; where (1) intra-sample precision ranged between %RSD 0.07% and 1.66%, (2) intra-day precision attained ranged between %RSD 0.65% and 5.63% and, (3) inter-day precision obtained ranged between %RSD 1.16% and 1.80%, (acceptance criteria: %RSD should not exceed 5%)

4.4.5 Accuracy

The accuracy of the method was evaluated by using QC samples defined in Section 4.4.3. The percentage error (%Error) calculated ranged between 0.07% and 8.78%, therefore %Recovery values obtain ranged between 91.22 % and 105.23%, (acceptance criteria: recovery should be in the range of 80% to 120%).

4.4.6 Robustness

Robustness was assessed by varying chromatographic: flow rate (+/-0.5mL/min), column temperature (+/-5°C), wavelength (+/-10nm), injection volume (+/-5µL) and mobile phase composition. The method resumed optimal performance when parameters were slightly changed, with marginal differences in retention time, peak areas and heights observed. Retention time decreased with increased flow rate and temperature. Increasing injection volume resulted in increased peak areas relative to the change in volume. Changes in wavelength also altered peak areas slightly depending on each compound's absorption. Lastly, increasing the ratio of organic component in mobile phase gave decreased resolution, while increasing the aqueous phase slightly augmented resolution and run time. The differences observed did not significantly alter the method performance.

4.4.7 Specificity

Specificity was attained through optimal selection of numerous parameters including: (1) column, (2) mobile phase composition, (3) column temperature and, (4) detector wavelengths. Good resolution between the three peaks of interest was attained.

Method specificity was demonstrated by analysing blank samples (containing only internal standard and mobile phase) to observe any interferences at the determined amoxicillin and clavulanic acid retention times. When sample blanks were analysed, the method showed good specificity for the compounds of interest.

A forced degradation study was conducted to determine whether the developed method could separate amoxicillin and clavulanic acid from their degradation products. Four conditions that were pharmaceutically relevant to degradation mechanisms were tested: oxidative stress, hydrolytic stress, photolytic stress, and thermal stress.

4.4.8 LOD and LOQ

The calculation used to obtain the LOD was (3.3*(SD of intercept/Slope)); (3.3*(0.47/0.9951) = 1.57ppm. The LOQ was calculated using the following equation (10*(SD of intercept)/Slope]; (10*(0.47/0.9951) = 4.76ppm. All the calibration standards, QC standards and LOD samples prepared were above the calculated LOD and LOQ values.

4.5 Determination of Amoxicillin-Clavulanic Acid Physicochemical Stability for Administration via Prolonged/Continuous Infusion

According to the SmPC, an unopened amoxicillin-clavulanic acid vial has a shelf life of 2 years, in salt form, when stored at <25°C in the marketed packaging prior to reconstitution. After reconstitution and dilution, amoxicillin-clavulanic acid exhibits physical and chemical in-use stability of 2 hrs at 25°C and 8 hours at 5°C (fridge). However, the manufacturer(s) recommend that it should be used immediately as any storage conditions prior to administration are at the professional's responsibility (337).

A review of the literature highlighted a major lack of data on the stability of amoxicillin-clavulanic acid, particularly regarding parenteral formulation. There is a need for more prolonged and detailed studies that are inclusive of the variety of temperature and diluent conditions the infusion solutions are exposed to in clinical practice to define a maximum shelf-life appropriate for the infusion formulations. This subchapter consists of two published studies that determine the stability of amoxicillin and clavulanic acid and define the suitability of their administration via P/CI.

4.5.1 Suitability of Amoxicillin-Clavulanic Acid for Administration via Prolonged Infusion

4.5.1.1 Abstract

Previously, we have been able to outpace bacterial mutation by replacing increasingly ineffective antibiotics with new agents. However, with the discovery of new antibiotics diminishing, optimising the administration of existing broad-spectrum antibiotics such as co-amoxiclav has become a necessity.

A stability indicating HPLC method was developed and validated in compliance with International Council for Harmonisation (ICH) guidelines. Stability of co-amoxiclav at clinical concentration was evaluated at three temperatures (4°C, ambient (23-25^{o}C) and 37°C) in three diluents (water for injection (WFI), 0.9% w/v NaCl and Ringer's solution). To establish whether there were significant differences at the level of both diluent and temperature, results were analysed using analysis of covariance (ANCOVA) to assess differences between the attained slopes of regression.

Data obtained indicated co-amoxiclav stability superior to that previously proposed making it suitable for extended infusion therapy. The degradation of amoxicillin appeared to follow a linear trend, with the rate of degradation elevated at higher temperatures, demonstrated by the magnitude of the regression slopes in these conditions. Analysis of regression slopes via ANCOVA demonstrated that diluent and temperature both significantly affected co-amoxiclav stability. Amoxicillin retained 90% of its initial concentration for 7.8 to 10 hrs when stored at 4^{o}C, 5.9 to 8.8 hrs at ambient and 3.5 to 4.5 hrs when incubated at 37^{o}C.

Co-amoxiclav is suitable for administration via prolonged infusion. Findings from this study aid in ameliorating current dosing regimens to optimise antibiotic efficacy. Other valuable applications conferred from these findings include the ability to pre-prepare solutions for use in bolus administration, minimising preparation time and workload.

4.5.1.2 Introduction

While the rate of antibiotic discovery has plummeted, the global burden of antimicrobial resistance (AMR) is on the rise and shows no signs of receding (345–347). Urgent action is required to address this public health threat and halt the advent of a post-antibiotic era.

Recently, the World Health Organisation (WHO) identified significant gaps in the present status of surveillance and information on AMR and confirmed that treatments for commonly acquired infections are becoming less effective (348). Reduced susceptibility to antibiotics, coupled with the lack of new agents has led to a renewed interest in optimising currently available antimicrobials. One growing area for reducing the development of AMR involves differential dosing regimens such as prolonged or continuous infusions of time-dependent antibiotics (76–80). However, this may not be possible for all antibiotics due to varying stability profiles.

The European Pharmacopeia considers pharmaceuticals stable providing they maintain 90% of their initial concentration (349). Uncertainty regarding β-lactam antibiotic stability after reconstitution and dilution presents a challenge in practice when assigning a shelf-life to injections that are pre-prepared and stored in ready-to-administer containers (349). These antibiotics display a time-dependent nature whereby maintaining concentrations above the minimum inhibitory concentration (MIC) promotes maximal bactericidal activity (350).

One such drug is amoxicillin-clavulanic acid (co-amoxiclav), a combination β-lactam antibiotic/β-lactamase inhibitor that exhibits broad-spectrum activity against a wide variety of bacterial infections. Currently, parenteral administration of co-amoxiclav is via bolus intermittent infusion. A proposed dosing strategy for enhancing co-amoxiclav's efficacy involves extending the time at which concentrations are maintained above the MIC via continuous/prolonged infusions (77). Prolonging infusion from 0.5 to 2 hours has previously been associated with improvements in time above the MIC (T>MIC) (351).

Literature indicates that the main constraints of co-amoxiclav stability include infusion diluent and storage temperature. Co-amoxiclav has been found to be less stable at higher temperatures, with data suggesting that shelf-life ranges between 1-5.5 hours at room temperature in water for injection (WFI) and up to 8 hours at 4°C (146–148,352).

To expand the breadth of current knowledge, this study utilises the bench-to-bedside approach, where challenges experienced in practice are addressed in the laboratory. Co-amoxiclav stability is a crucial parameter that needs to be determined to assess the feasibility of administration via continuous/prolonged infusions. To address this, a high-performance liquid chromatography (HPLC) stability indicating method (SIM) was developed and validated in compliance with International Council for Harmonisation (ICH) guidelines. Quantitative analysis of co-amoxiclav stability was then conducted in a range of temperatures and diluents to determine their effect on degradation.

4.5.1.3 Materials and Methods

4.5.1.3.1 Materials

GSK pharmaceutical dosage form co-amoxiclav (1000mg/200mg) infusion vials were provided by St George's Hospital, London, UK. Amoxicillin sodium, potassium clavulanate and caffeine reference standards were purchased from Sigma Aldrich, as were ammonium acetate and glacial acetic acid. Water for injection (WFI), 0.9% sodium chloride, and Ringer's solution were purchased from The Pharmacy, Kingston, UK. Methanol (HPLC grade) and acetonitrile (HPLC grade) were purchased from VWR and distilled water was generated in the laboratory at Kingston University, London, UK.

4.5.1.3.2 Instrumentation

Quantitative analysis of amoxicillin-clavulanic acid was carried out using an Agilent 1260 HPLC system with single wavelength UV detection and Chemstation software.

4.5.1.3.3 HPLC-SIM Development & Validation

A SIM was developed and validated in accordance with ICH guidelines. Parameters investigated included column, mobile phase and internal standard selection. The method was optimised through selection of suitable flowrate, wavelength, injection volume and column temperature.

To determine the developed method's specificity, a forced degradation study was conducted. Co-amoxiclav solutions were exposed to oxidative, hydrolytic, photolytic and

thermal stress. Stressed solutions were analysed to assess the method's ability to separate the parent compounds from their degradation products.

Validation was conducted over the span of three days. The analytical range was determined by running reference standard amoxicillin-clavulanic acid at various concentrations (0, 10, 20, 30, 40, 50, 60, 70, and 80ppm).

Quality control (QC) solutions equating to approximately 25%, 50% and 75% of the working range i.e. 15ppm, 45ppm and 75ppm, were prepared prior to each validation run to assess the accuracy. Precision was considered at three levels: repeatability, intermediate precision and reproducibility through analysis of prepared QC solutions. Three sets of each sample were prepared daily and each sample was run in triplicate.

Method selectivity was demonstrated by analysing 'sample blanks' to observe any interferences at the determined amoxicillin and clavulanic acid retention times. Robustness was examined by marginally varying the following parameters: flow rate (+/-0.5mL/min), column temperature (+/-5°C), wavelength (+/-10nm), injection volume (+/-5µL) and mobile phase composition.

4.5.1.3.4 Quantitative HPLC Assay

In clinical settings, a 1.2g amoxicillin-clavulanic acid vial is reconstituted with 20mL WFI and is diluted further with 50mL of diluent (1200mg/70mL = 17 143ppm). Nine pharmaceutical formulation vials were reconstituted with 20mL WFI, further diluted with either 50mL of 0.9% sodium chloride (n=3), Ringer's solution (n=3) or WFI (n=3) and stored at 4^{o}C, ambient and 37^{o}C in vials.

Standard practice involves dilution of co-amoxiclav with sodium chloride solution. Other compatible diluents including Ringer's solution and WFI were trialled to examine the influence of diluent on co-amoxiclav stability. To evaluate preparation and storage feasibility, the stability of co-amoxiclav solutions was assessed at 4°C. Ambient and 37°C temperature conditions were considered in order to mimic average and high temperatures experienced in hospital wards due to seasonal variations.

To achieve a nominal concentration of 34.3ppm, a dilution factor of 500x was used. Sampling was undertaken every 2 hrs and the infusion solution was considered stable while the percentage recovery of amoxicillin remained above 90%.

4.5.1.3.5 Data and Statistical Analysis

Raw data obtained was corrected for drift and internal standard. Results are reported as the residual ratio of amoxicillin concentration from three replicates. To determine whether amoxicillin concentration decreased significantly over time, the slope of the linear regression line for each condition was tested against the null hypothesis (H_0 = no deviation from zero) using a one tailed t-test at the 99% level of significance. To establish whether there were significant differences at the level of both diluent and temperature, results were analysed using analysis of covariance (ANCOVA) to assess differences between the attained slopes of regression.

4.5.1.4 Results

4.5.1.4.1 HPLC-SIM Development & Validation

Amoxicillin, clavulanic acid and caffeine separation was attained using conditions presented in **Table 45**. The method demonstrated specificity with parent compounds separated from their degradation products with sufficient resolution. A representative chromatogram is displayed in **Figure 75**.

The calibration curve (**Figure 77**) obtained demonstrated good linearity over the concentration range of 0-80ppm. The representative calibration curve had correlation coefficient (R^2) of 0.9999. Good intra-sample precision was achieved with %RSD ranging between 0.07-1.66%. Intra-day precision ranged between 0.65 and 5.63%.

Good inter-day precision was also obtained with %RSD ranging between 1.16-1.80% (ICH acceptance criteria: %RSD ≤ 5%). The percentage error ranged between 0.07 and 8.78% and percentage recovery between 91.22 and 105.23% (ICH acceptance criteria: %Recovery: 80-120%), demonstrating good accuracy.

The method continued to perform optimally when parameters were varied, exhibiting slight changes in retention time, peak areas and heights. LOD and LOQ were calculated to be 1.57ppm and 4.76ppm, respectively.

4.5.1.4.2 Quantitative HPLC Assay

In general, amoxicillin-clavulanic acid solutions retained more of the initial drug concentration at lower temperatures compared with solutions stored at higher temperatures. The influence of diluent on amoxicillin concentrations at 4^{o}C, ambient and 37^{o}C over time are shown in **Figure 78**. The slopes of the regression lines for each condition showed significant deviation from zero at the 99% level of confidence, indicating amoxicillin exhibits degradation with time (**Table 47**).

Amoxicillin retained 90% of its initial concentration for 7.8 to 10 hrs when stored at 4^{o}C, 5.9 to 8.8 hrs at ambient and 3.5 to 4.5 hrs when incubated at 37^{o}C. Stability data for all conditions are displayed in **Table 47**. Significant differences between regression slopes of temperature conditions for each diluent were observed, as were differences between diluents for each temperature condition (**Table 48**). Clavulanic acid appeared to maintain concentrations within 90% of initial concentration for the entirety of the sampling duration in all conditions analysed.

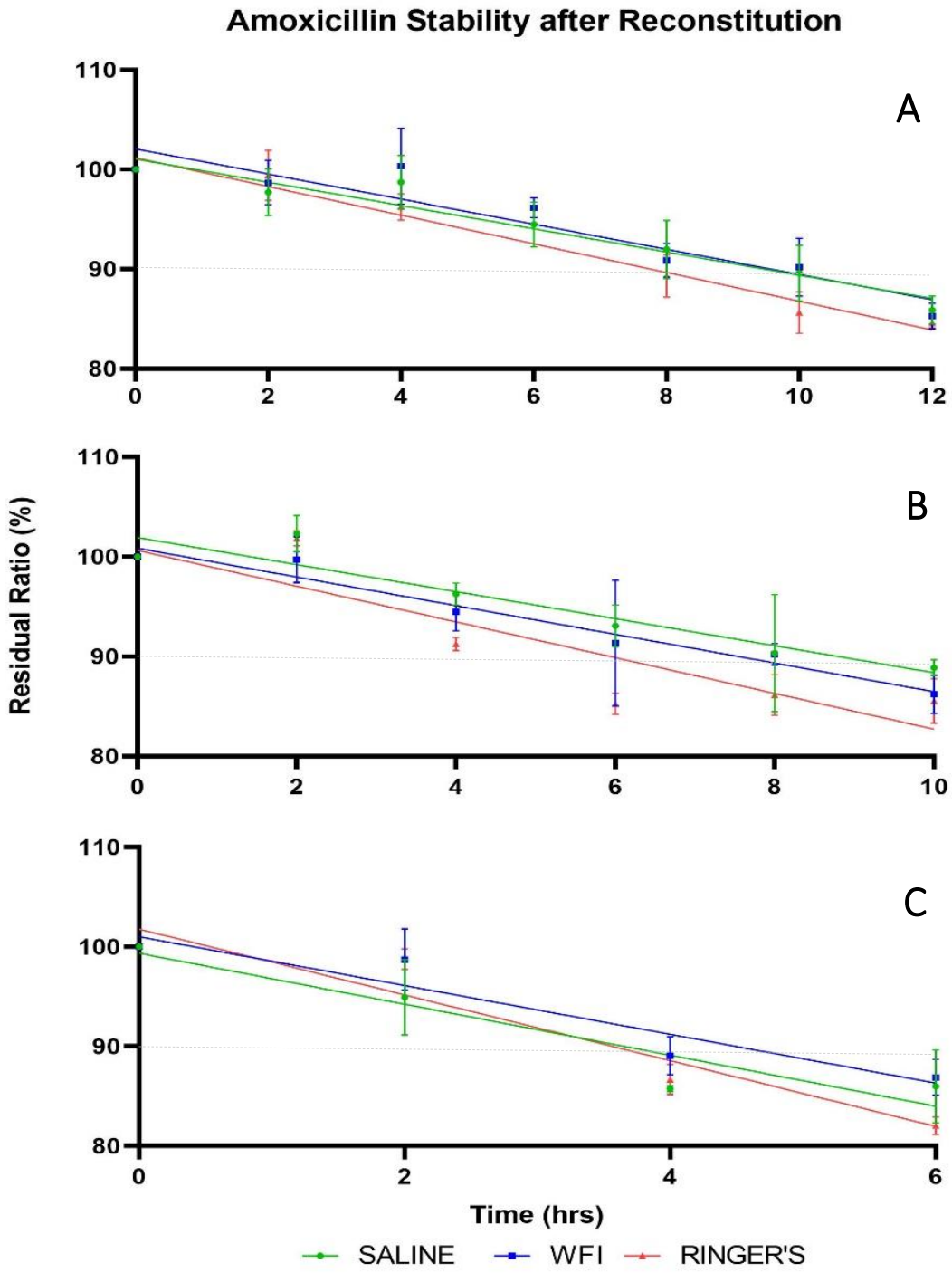


Figure 86 Stability of amoxicillin over time at a) 4°C, b) ambient and c) 37°C: mean % of intact molecule as a function of time and type of diluent. Error bars: ± standard deviation. Dashed line: 90% of initial concentration

Figure 87 Preliminary NMR analysis of amoxicillin, clavulanic acid and co-amoxiclav after 72 hours of reconstitution.**Figure 88** Stability of amoxicillin over time at a) 4°C, b) ambient and c) 37°C: mean % of intact molecule as a function of time and type of diluent. Error bars: ± standard deviation. Dashed line: 90% of initial concentration

Table 47 Displaying the linear regression equations for each condition used to calculate the predicted time at which residual ratio of amoxicillin falls below 90%

Condition	Deviation of Slope from Zero	Linear Equation	Predicted Stability (hrs)
4°C Saline	Significant $p < 0.0001$	$y = -1.167x + 101.1$	10.03
4°C WFI	Significant $p < 0.0001$	$y = -1.261x + 102.1$	9.60
4°C Ringer's Solution	Significant $p < 0.0001$	$y = -1.440x + 101.2$	7.78
Ambient Saline	Significant $p < 0.0001$	$y = -1.355x + 101.9$	8.78
Ambient WFI	Significant $p < 0.0001$	$y = -1.438x + 100.8$	7.51
Ambient Ringer's Solution	Significant $p < 0.0001$	$y = -1.792x + 100.6$	5.92
37°C Saline	Significant $p = 0.0001$	$y = -2.560x + 99.35$	3.65
37°C WFI	Significant $p < 0.0001$	$y = -2.449x + 101.0$	4.49
37°C Ringer's Solution	Significant $p < 0.0001$	$y = -3.299x + 101.8$	3.58

Table 48 Results of ANCOVA analyses performed at the level of diluent and temperature. (S = significant, NS = not significant at 75% confidence level)

Diluent	ANCOVA (All Temperature Conditions)	ANCOVA (Individual Analyses)
Saline (4°C, Ambient, 37°C)	Significant $p = 0.019$	4°C vs Ambient: $p = 0.875$ (NS) 4°C vs 37°C: $p = 0.007$ (S) Ambient vs 37°C: $p = 0.009$ (S)
WFI (4°C, Ambient, 37°C)	Significant $p = 0.004$	4°C vs Ambient: $p = 0.048$ (S) 4°C vs 37°C: $p = 0.024$ (S) Ambient vs 37°C: $p = 0.063$ (S)
Ringer's Solution (4°C, Ambient, 37°C)	Significant $p = 0.026$	4°C vs Ambient: $p = 0.160$ (S) 4°C vs 37°C: $p = 0.047$ (S) Ambient vs 37°C: $p = 0.184$ (S)
Temperature	**ANCOVA (All Diluent Conditions)**	**ANCOVA (Individual Analyses)**
4°C (Saline, WFI, Ringer's Solution)	Significant $p = 0.135$	Saline vs WFI: $p = 0.629$ (NS) Saline vs Ringers: $p = 0.07$ (S) Ringers' vs WFI: $p = 0.065$ (S)
Ambient (Saline, WFI, Ringer's Solution)	Significant $p = 0.023$	Saline vs WFI: $p = 0.125$ (S) Saline vs Ringers: $p = 0.07$ (S) Ringers' vs WFI: $p = 0.233$ (S)
37°C (Saline, WFI, Ringer's Solution)	Not significant $p = 0.919$	Saline vs WFI: $p = 0.289$ (NS) Saline vs Ringers: $p = 0.925$ (NS) Ringers' vs WFI: $p = 0.408$ (NS)

4.5.1.5 Discussion

The emergence of resistance threatens our capacity to treat common infectious diseases as antibiotics progressively become less effective (345–347). Optimising dosing regimens of antibiotics has shown potential for controlling the spread of resistance (76–80).

The utilisation of the bench-to-bedside approach enables outcomes to be translated directly from the laboratory to the clinical setting, integrating these advancements into practice. Sustaining serum concentrations above the MIC by employing methods which exploit the

time-dependent nature of co-amoxiclav such as prolonged infusion could improve its clinical effectiveness (353). Optimising co-amoxiclav efficacy serves not only to improve treatment strategies but also to minimise the further development of antimicrobial resistance.

To our knowledge, this is the first study of its kind to utilise a multifactor analysis for determination of co-amoxiclav stability. By concomitantly examining drug stability at clinical concentrations in multiple diluents across a range of temperatures, a more comprehensive evaluation of multi-parameter stability was attained. The consequent understanding of the molecular stability of co-amoxiclav allows for a greater evaluation of its suitability for administration via prolonged infusion, aiding in the development of novel treatment strategies. Co-amoxiclav has not previously been considered for prolonged infusion, however, findings from this study demonstrate its feasibility.

Results obtained confirm that reconstitution diluent and storage temperature significantly influence co-amoxiclav stability. The degradation of amoxicillin appeared to follow a linear trend, with the rate of degradation elevated at higher temperatures as demonstrated by the magnitude of the regression slopes in these conditions. Storage at lower temperatures correlated with increased shelf-life, which is concurrent with previous studies on other β-lactam antibiotics (77,354,355) (Table 47).

Dilution with Ringer's solution demonstrated the least stability, exhibiting significant differences compared to saline and WFI when stored at both 4°C and ambient conditions (Table 48). Greatest stability of amoxicillin was achieved in WFI and saline solutions at 4°C, where shelf-lives of 9.6 and 10.0 hrs, respectively, were determined (Table 47).

Data obtained indicated co-amoxiclav stability superior to that previously proposed (146–148,352) making it suitable for extended infusion therapy. Prolonged co-amoxiclav infusion would improve the effectiveness of therapy without altering the dose or dosing schedule, giving no increase in toxicity. Insight into this greater stability paves the way for further investigation of differential dosing regimens and optimisation of current treatment strategies, which have potential to improve and enhance clinical efficacy.

Another valuable application conferred from these findings includes the ability to pre-prepare solutions for use in bolus administration, minimising preparation time and

workload. Furthermore, unused reconstituted solutions are typically discarded after use, however, this study demonstrates that these may be utilised for subsequent administrations, reducing wastage and costs.

Future assays investigating the stability of co-amoxiclav should consider analysis at a range of concentrations to account for patient populations with specific dosing requirements, such as those on fluid restriction due to reduced renal clearance. Further investigations are warranted to understand the stability of co-amoxiclav in various infusion devices such as elastomeric pumps and intravenous infusion bags.

4.5.1.6 Conclusion

Resistance to common infections has previously been mitigated by the discovery of novel antibiotics. However, with the current scarcity of newly developed compounds, this study focused on optimising the administration of broad-spectrum co-amoxiclav by determining its shelf-life in a range of temperatures and diluents. Results suggest co-amoxiclav shelf-life is longer than previously determined, rendering it suitable for administration via prolonged infusion in terms of stability. Multifactor analysis indicated that co-amoxiclav stability was significantly influenced by diluent and storage temperature. Findings from this study aid in ameliorating current dosing regimens to optimise antibiotic efficacy.

4.5.2 Stability of Amoxicillin and Clavulanic Acid in Separate Containers for Administration via a Y-Site

4.5.2.1 Abstract

With the discovery of new antibiotics diminishing, optimising the administration of existing antibiotics such as amoxicillin-clavulanic acid has become a necessity. At present, the optimal approach for enhancing the effectiveness of time-dependant antibiotics involves extending the time at which antibiotic concentrations are maintained above the minimal inhibitory concentration by prolonging the infusion time. This pharmacodynamic rationale cannot be applied to co-amoxiclav because of poor stability at room temperature. The aim of this study was to establish the shelf-life of amoxicillin and clavulanic acid prepared in separate containers to determine the feasibility of 24-hr continuous infusion therapy.

A previously developed and validated stability-indicating HPLC method was used to establish the shelf-life of reconstituted amoxicillin and clavulanic acid when prepared in separate containers. Stability at clinical concentration was evaluated at three temperatures. To establish whether there were significant differences at the level of both active ingredients and temperature, results were analysed using analysis of covariance (ANCOVA) to assess differences between the attained slopes of regression.

Data obtained indicated amoxicillin and clavulanic acid stability superior to that previously proposed making it suitable for continuous infusion therapy. Analysis of regression slopes via ANCOVA showed that temperature significantly affected amoxicillin and clavulanic acid stability. Amoxicillin retained 90% of its initial concentration for 80.3 hrs when stored at 4°C, 24.8 hrs at 25^{o}C and 9 hrs when incubated at 37°C. Clavulanic acid retained 90% of its initial concentration for 152 hrs when stored at 4°C, 26 hrs at 25^{o}C and 6.4 hrs when incubated at 37°C.

Amoxicillin and clavulanic acid are suitable for administration via continuous infusion when prepared, stored, and administered in separate containers. Results obtained from this study aid in ameliorating current dosing regimens to optimise antibiotic efficacy, however, more in-depth amoxicillin and clavulanic acid y-site compatibility studies are warranted.

4.5.2.2 Introduction

Despite advances in modern medicine, antibiotic dosing regimens have remained largely unchanged since their discovery. Previously, antibiotic dosing schedules were empirically designed based on *in vitro* data and clinical experience. By failing to encompass characteristics including dose response, dosing interval, optimal duration of therapy or post-antibiotic effects, dosing regimens based on an understanding of pharmacodynamics (PD) were therefore not established (142,143).

The rise of antimicrobial resistance has prompted investigation into optimising the administration of antibiotics currently used in practice. β-lactams are the most extensively utilised antibiotics due to their relatively high effectiveness, low cost, ease of delivery and minimal side effects. Currently, parenteral administration of β-lactam antibiotics is via bolus dosing which produces unnecessary erratic peak plasma and low trough concentrations below the minimal inhibitory concentration (MIC) between dosing intervals (356). It has been established that maintaining serum concentrations above the MIC of the respective organism for ≥50% of the dosing interval promotes maximal PD activity (357,358).

Amoxicillin is widely used for the treatment of uncomplicated penicillin-sensitive infections; however, its use alone is limited as beta-lactamase producing bacteria can easily destroy it. Concomitant administration with clavulanic acid broadens the antibacterial spectrum by exerting a pronounced synergistic effect (148).

Administration regimens including more frequent dosing or continuous infusion have been found to optimise the PD profile of amoxicillin. Furthermore, continuous infusion of β-lactam antibiotics has demonstrated a reduction in the total daily dose of drug required (356), shorter treatment duration (359), as well as a reduction in the formation of resistant bacteria (356,360,361). Although administration by continuous infusion maximises β-lactam's PD properties, uncertainty regarding amoxicillin's stability after reconstitution and dilution presents a challenge in practice when assigning a shelf-life to infusion solutions (37,77,362).

The literature suggests that amoxicillin and clavulanic acid undergo hydrolytic degradation after reconstitution (37,352). Kinetic studies have reported the catalytic effect of clavulanic acid on amoxicillin (363,364). The two species, and possibly their decomposition products, interact in solution where an enhancement of the catalytic effect of one reacting species

upon another due to proportional increased concentration of the catalyst is likely (363). The catalysis of amoxicillin by clavulanic acid or vice versa in infusion solutions prompts further investigation.

To expand the breadth of current knowledge, a proposed strategy for enhancing amoxicillin and clavulanic acids stability involves the preparation and administration of the parenteral amoxicillin and clavulanic acid via separate infusion devices. Little is known about the physicochemical stability of amoxicillin and clavulanic acid alone in comparison to the combination of amoxicillin with clavulanic acid infusion solutions at clinically relevant concentrations. The logistical advantages of simultaneous administration in separate devices (e.g., improved stability), will pave the way for optimising current treatment strategies (i.e. via concurrent Y-site administration) which have potential to improve and enhance clinical efficacy.

4.5.2.3 Materials and Methods

4.5.2.3.1 Materials

Amoxicillin sodium, potassium clavulanate and caffeine reference standards were purchased from Sigma Aldrich, as were ammonium acetate and glacial acetic acid. Water for injection (WFI) was purchased from The Pharmacy, Kingston upon Thames, UK. Methanol (HPLC grade) and acetonitrile (HPLC grade) were purchased from VWR and deionised water was generated in the laboratory at Kingston University, London, UK.

4.5.2.3.2 Instrumentation

Qualitative analysis of amoxicillin-clavulanic acid was carried out using 600mHz base frequency Bruker Advance III Two-channel FT-NMR spectrometer.
Quantitative analysis of amoxicillin-clavulanic acid was carried out using an Agilent 1260 HPLC system with single wavelength UV detection and Chemstation software.

4.5.2.3.3 Qualitative NMR Investigation

Preliminary examinations of amoxicillin, clavulanic acid and co-amoxiclav were carried out by the means of NMR spectroscopy to provide an indication of how fast they degraded and inform the development of the quantitative high performance liquid chromatography (HPLC) stability indicating method (SIM). Amoxicillin, clavulanic acid and co-amoxiclav solution at the relevant concentrations were prepared and transferred into NMR tubes. The instrument was programmed to periodically analyze throughout a 72hr period using a 1D (proton) H NMR using the "noesy1d presaturation" water suppression method.

4.5.2.3.4 HPLC SIM

To quantify amoxicillin, clavulanic acid and co-amoxiclav concentrations with respect to time, HPLC was used. A previously developed and validated method (in accordance with ICH guidelines) was utilised (37). Separation was conducted using Phenomenex Spherisorb 5µm, 100 x 4.6 mm column with a binary mobile phase composition consisting of Ammonium Acetate (5mM) pH4 and acetonitrile (50:50 v/v). Analysis was performed at detection wavelength 225nm (ME). Flow rate was 1.75mL/min (37).

The calibration curves obtained for amoxicillin (range = 0 – 66.7 ppm) and clavulanic acid (range = 0 – 13.3 ppm) demonstrated good linearity (**Figure 77**)

4.5.2.3.5 Quantitative HPLC Assay

In clinical settings, a 1.2g amoxicillin-clavulanic acid vial is reconstituted with 20mL WFI and is diluted further with 50mL of diluent (1200mg/70mL = 17 143ppm). To mimic these conditions, 1g of amoxicillin sodium and 0.2g of potassium clavulanate were accurately weighed and transferred into separate containers. Amoxicillin and clavulanic acid were each reconstituted using 70mL WFI. The two solutions were each split into three subsamples and stored at the relevant temperature condition. Noteworthy is the concentrations may vary with differing dilution practices. The concentration used in this study falls within the higher range of clinical concentrations, therefore, the stability of more dilute solutions, commonly used in practice, are expected to exhibit lower degradation rates (i.e., higher stability).

To evaluate preparation and storage feasibility, the stability of solutions was assessed at 4°C. Ambient and 37°C temperature conditions were selected in order to mimic average and high temperatures experienced in hospital wards due to seasonal variations. Sampling was undertaken at 2, 4, 6, 8, 10, 12, 24, 30, 36, 50, 56, and 152 hrs and the infusion solution was considered stable while the percentage recovery of amoxicillin and clavulanic acid remained above 90%.

4.5.2.3.6 Data and Statistical Analysis

Raw data obtained was corrected for the internal standard. Results are reported as the residual ratio of amoxicillin and clavulanic acid concentration from three replicates. To determine whether amoxicillin or clavulanic acid concentration decreased significantly over time, the slope of the linear regression line for each condition was tested against the null hypothesis (H_0 = no deviation from zero) using a one tailed t-test at the 99% level of significance. To assess whether there was a significant difference between amoxicillin and clavulanic acids rate of degradation, results were analysed using analysis of variance (ANOVA) at the 95% level of significance. One tailed t-tests and ANOVA analysis was undertaken using Microsoft Excel 365. To establish whether there were significant differences at the level of temperature, results were analysed using analysis of covariance

(ANCOVA) at the 75% level of significance, to assess differences between the attained slopes of regression. ANCOVA analysis was undertaken using IBM SPSS 26.

4.5.2.4 Results

4.5.2.4.1 NMR

The preliminary NMR method for studying amoxicillin, clavulanic acid and co-amoxiclav degradation, detected a considerable difference in the rate at which degradation occurs in separate solutions compared to the combined solution. The spectra relating to the combined solution (after 72 hours of dilution) in **Figure 79**, displays the appearance of new peaks, most prominently in circled regions, suggesting that the rate of degradation is accelerated when amoxicillin and clavulanic acid are prepared in combination. Qualitative data was retrieved from NMR as this technique lacks sensitivity and suffers from signal overlapping when analysing mixtures.

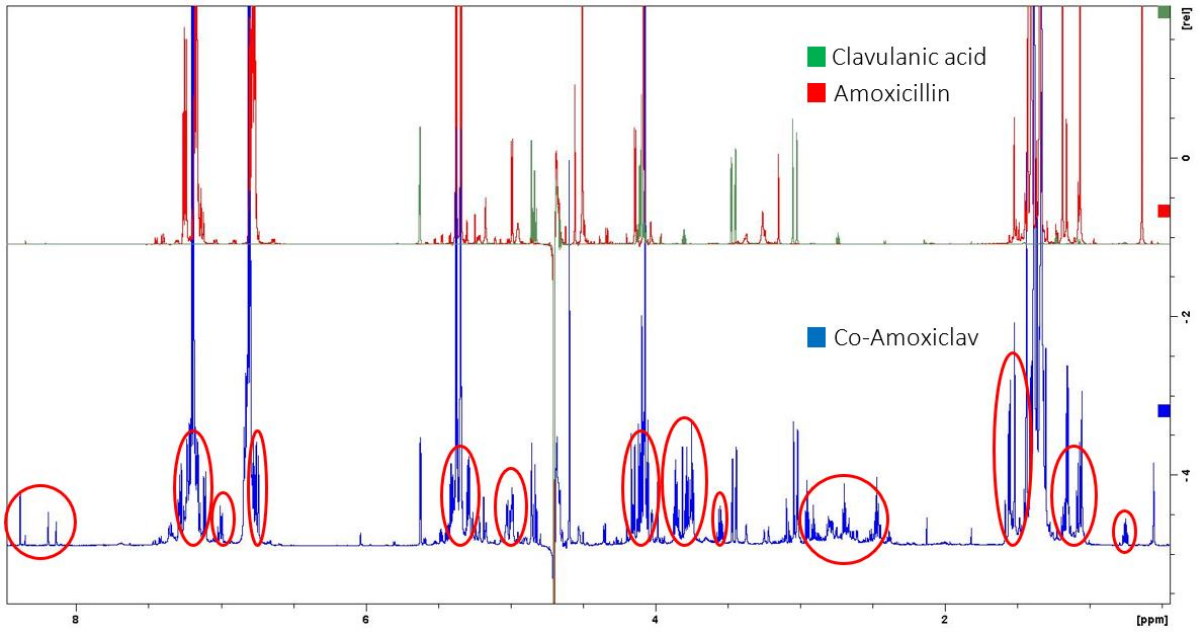


Figure 89 Preliminary NMR analysis of amoxicillin, clavulanic acid and co-amoxiclav after 72 hours of reconstitution.

Figure 90 Stability of amoxicillin and clavulanic acid over time at (a) 4oC, (b) 25oC and (c) 37oC: mean % of intact molecule as a function of time. Error bars: ± standard deviation.**Figure 91** Preliminary NMR analysis of amoxicillin, clavulanic acid and co-amoxiclav after 72 hours of reconstitution.

4.5.2.4.2 HPLC

Amoxicillin and clavulanic acid solutions retained more of their initial concentration for longer than reported in previously published results where they were prepared in combination (37). The slopes of the regression lines for both active pharmaceutical

ingredients (APIs) at the three temperatures studied showed significant deviation from zero at the 99% level of confidence, indicating amoxicillin and clavulanic acid exhibit degradation with time (Table 49). Similarly, to previously published data (37), ANCOVA showed that solutions stored at lower temperatures remained stable for significantly longer than solutions at higher temperatures (Table 50). The influence of temperature of both APIs is displayed in Figure 80.

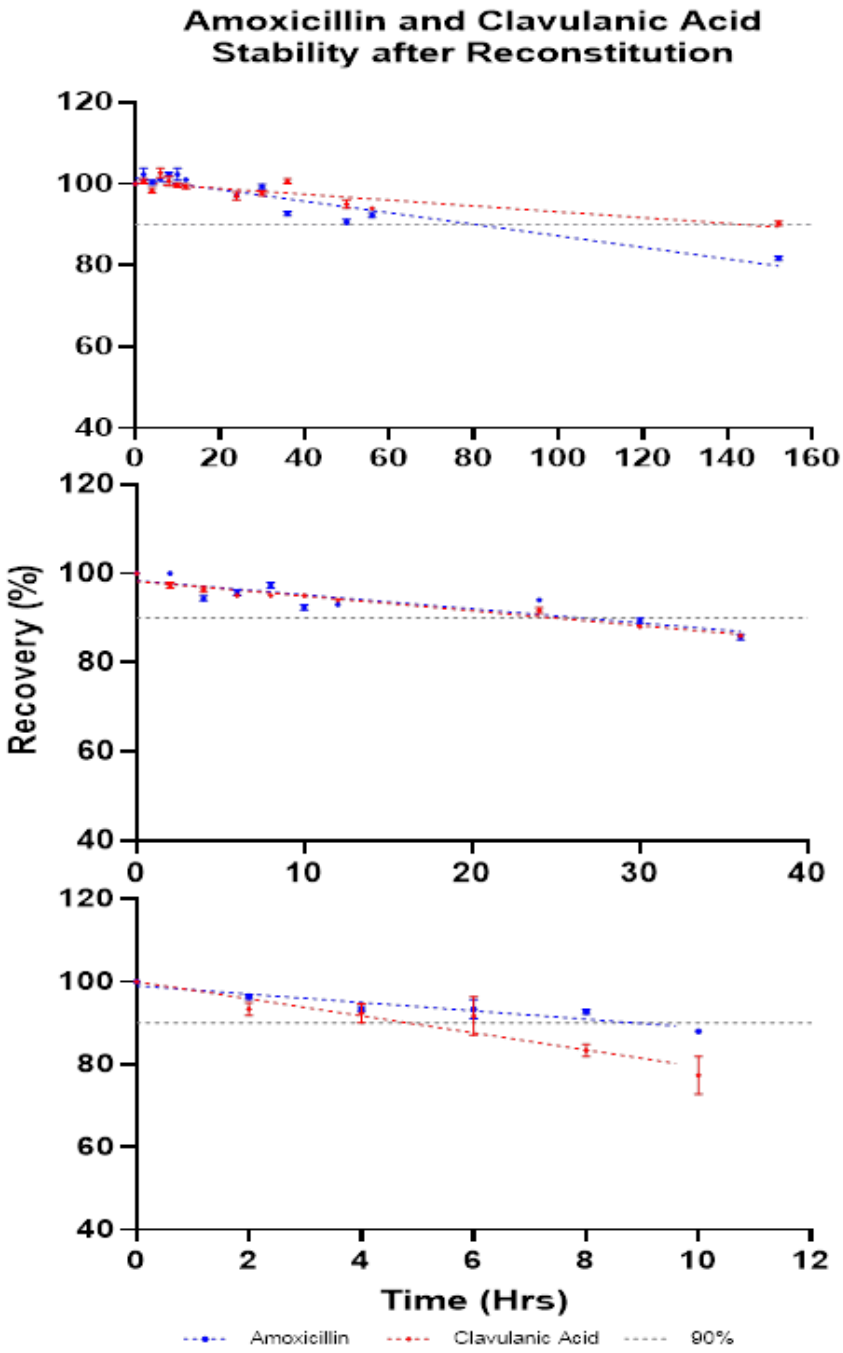


Figure 92 Stability of amoxicillin and clavulanic acid over time at (a) 4oC, (b) 25oC and (c) 37oC: mean % of intact molecule as a function of time. Error bars: ± standard deviation.

Figure 93 Stability of amoxicillin and clavulanic acid over time at (a) 4oC, (b) 25oC and (c) 37oC: mean % of intact molecule as a function of time. Error bars: ± standard deviation.

Stability data for all studied concentrations are displayed in **Table 49**. Amoxicillin retained 90% of its initial concentration for 80.3, 24.8 and 9 hours at 4°C, 25°C and 37°C (%recovery at 6 hours = 95% and at 8 hours = 95% at 25°C), respectively. Clavulanic acid retained 90% of initial concentration for 152, 26 and 6.4 hours at 4°C, 25°C and 37°C (%recovery at 6 hours = 96% and at 8 hours = 98% at 25°C), respectively. Significant differences between regression slopes of temperature conditions for each API were observed (**Table 49**).

Table 49 Displaying the linear regression equations for amoxicillin and clavulanic acid conditions used to calculate the predicted time at which residual ratio of amoxicillin falls below 90%. Previously reported predicted stability data for co-amoxiclav is displayed in RED.

Condition	Deviation of Slope from Zero	Linear Equation	Predicted Stability (hrs)
Amoxicillin 4°C	Significant ($p < 0.0001$)	y = -0.1419x +101.4	80.3
Amoxicillin 25°C	Significant ($p < 0.0001$)	y = -0.3315x + 98.21	24.8
Amoxicillin 37°C	Significant ($p < 0.0001$)	y = -1.014x + 99.02	9.0
Clavulanic Acid 4°C	Significant ($p < 0.0001$)	y = -0.07180x + 100.3	152
Clavulanic Acid 25°C	Significant ($p < 0.0001$)	y = -0.3162x + 98.34	26.4
Clavulanic Acid 37°C	Significant ($p < 0.0001$)	y = -1.2727x + 98.166	6.4
Combination 4°C	Significant ($p < 0.0001$)	y = -1.261x + 102.1	9.6
Combination 25°C	Significant ($p < 0.0001$)	y = -1.438x + 100.8	7.5
Combination 37°C	Significant ($p < 0.0001$)	y = -2.449x + 101.0	4.5

Table 50 Results of ANCOVA analyses performed at the level of active ingredient at the 75% confidence level

Active Ingredients	ANCOVA (All Temperature Conditions)	ANCOVA (Individual Analyses)
Amoxicillin	($p \leq 0.001$)	4°C vs 25°C: ($p \leq 0.001$) 4°C vs 37°C: ($p \leq 0.001$) 25°C vs 37°C: ($p = 0.020$)
Clavulanic Acid	($p < 0.001$)	4°C vs 25°C: ($p < 0.001$) 4°C vs 37°C: ($p < 0.001$) 25°C vs 37°C: ($p = 0.010$)

Table 51 Results of one tailed t-tests at the 99% confidence level and ANOVA analyses performed at the level of temperature at the 95% confidence level.

	Amoxicillin vs Clavulanic Acid	
Temperature	**One-Tailed T-Test**	**ANOVA**
4°C	($p = 0.292$)	($p = 0.582$)
25°C	($p = 0.429$)	($p = 0.858$)
37°C	($p = 0.144$)	($p = 0.280$)

4.5.2.5 Discussion

To the best of our knowledge, this is the first study of its kind to concomitantly examine amoxicillin and clavulanic acid stability at clinical concentrations across a range of temperatures. The consequent understanding of the molecular stability of amoxicillin and clavulanic acid in separate solutions allows for a greater evaluation of its suitability for administration via prolonged and continuous infusion, aiding in the development of novel treatment strategies. Amoxicillin and clavulanic acid have not previously been considered for separate continuous infusion when administered in combination, however, findings from this study demonstrate its feasibility.

The methods of antibiotic administration have remained unchanged since their discovery. This poses the question of whether antibiotics are being used to their greatest potential? Dosing regimens were established from data describing in vitro antibacterial activity as well as clinical experience, thus leading to intermittent bolus dosing. These regimens were infrequently validated yet have been standard clinical procedure for numerous decades. Therefore, it is time for re-evaluating conventional practice. Current administration methods should be challenged based on the advanced knowledge of the pharmacodynamics of these antibiotics.

Effective antibiotic treatment modalities mainly consist of two variables: the dose and duration of treatment. Drug efficiency studies have determined the effective dose and duration for co-amoxiclav treatment regimen, although, a limitation of this approach is that it only provides information for the regimen being analysed and offers no indication for other potential dosing regimens. Traditional antibiotic dosing consists of administering a fixed dose for a specified duration. However, the increase in antibiotic resistant bacteria poses a threat to the effectiveness in treating bacterial infections (144). With the lack of new antibiotics and the increase in antibiotic resistance, strategies to improve the utility of existing antibiotics, like co-amoxiclav, are mandatory (365).

Finding optimal treatment regimens is critical in ensuring the prolonged effectiveness of antibiotics like co-amoxiclav. Optimising amoxicillin and clavulanic acids potential for successful clinical outcomes requires consideration of PD attributes to maximise bacterial eradication as well as minimise the capability for further resistance. If traditional dosing

regimens are modified to deliver amoxicillin and clavulanic acid doses where concentrations are maintained above the MIC of infecting organism for 50% of the dosing interval then the initial facilitation of resistant bacteria will disappear (144).

Even though clavulanic acid is not available in pharmaceutical formulation and is not licenced to be administered alone, this study paves the way for innovation to overcome stability concerns. Data obtained indicated stability superior to that previously proposed (37,146–148,352) rendering it suitable for extended or continuous infusion therapy. Results obtained are in alignment with those recently published, suggesting that storage temperature significantly influences the stability of amoxicillin and clavulanic acid (Table 49, Table 50 and Table 51) (37).

Studies have previously confirmed that there is no difference between the pharmacokinetic data derived from serum level determinations of amoxicillin or clavulanic acid after administration of the single substances or of the combination (366). Therefore, simultaneous administration of amoxicillin and clavulanic acid as single substances via a y-site would improve the effectiveness of therapy without altering the dose or dosing schedule, giving no increase in toxicity (37).

Stability data should be specific to in-use conditions and the dosing regimen, thus, clinically relevant concentrations, diluent, and temperatures of solutions during storage and administration were studied to establish shelf-life of amoxicillin and clavulanic acid after reconstitution and dilution. Compatibility study carried out, including visual inspection for precipitation when two solutions come in contact with each other showed no changes solution colour or clarity, however, more in-depth y-site compatibility studies are warranted.

4.5.2.6 Conclusion

This study demonstrates that amoxicillin and clavulanic acid demonstrate stability for longer than that stated by manufacturers and previous stability studies when prepared as separate solutions. Results obtained suggest amoxicillin and clavulanic acids shelf-life is longer when they are prepared as separate solutions, rendering it suitable for administration via continuous infusion or for outpatient settings in terms of stability. Multifactor statistical analysis indicated that the stability of both APIs was significantly influenced by storage

temperature. Findings from this study aid in improving current dosing regimens to optimise amoxicillin and clavulanic acid efficacy.

CHAPTER 5

CONCLUSION

5 Conclusion

This thesis has addressed a range of topics in relation to differential antibiotic dosing. Considering the conflicting perspectives reported in the literature regarding the benefits of differential antibiotic dosing, the data gathered throughout this research are supportive of the beneficial role of P/CI BLAs. The findings of this research indicate that P/CIs are feasible, advantageous and could potentially improve patient clinical outcomes. Further work to expand on the findings of this PhD research have been categorised into literature, practice and laboratory-based research. The specific gaps in knowledge addressed, implications, limitations, and future work for each of these categories are summarised in the following subchapters.

5.1 Literature -Based Research

5.1.1 Knowledge Gaps Addressed

To date there is no comprehensive evidence available on the clinical outcomes of CI ampicillin and no SRs had been conducted to evaluate the efficacy of CI vs II temocillin. To address this gap in the literature, SRs were conducted to assess (1) the clinical efficacy and safety of CI ampicillin and (2) compare the clinical outcomes of II and CI temocillin administration. These are the first SRs to investigate and describe the clinical outcomes (in terms of clinical cure, mortality, adverse events, and length of hospital stay) of differential dosing parenteral ampicillin and temocillin.

5.1.2 Implications

The studies included in the SR (even though limited) focused on ampicillin demonstrated that ampicillin administered via CI is associated with improved clinical outcomes. Considering the scarcity of available studies comparing CI vs II temocillin, the included studies showed favourable outcomes when temocillin was administered via CI (Section 2.2).

5.1.3 Limitations

Findings of these SR should be interpreted in view of certain limitations. First, clinical heterogeneity was present as selected studies examining the clinical efficacy of CI ampicillin and CI vs II temocillin have confounding factors including patient sample size, study settings, and study design. Also, the duration of ampicillin and temocillin administration and total daily dose was not homogenised between studies providing an additional confounding factor as to whether the duration of infusion or total daily dose attributed to clinical outcome. Second, information regarding whether the patients received ampicillin or temocillin as a monotherapy or as a combined therapy with other antibiotic/s was not reported in the included studies, reducing the validity of conclusions. Third, a medical librarian was not involved to aid in searching for the evidence needed to create the SR. Article searching, source selection, citation management, document supply and critical appraisal was based on the experience of the authors. Fourth, the small number of studies

and the small sample size may result in bias and the probability of small study effects contributing to the favourable outcome for P/CI.

5.1.4 Future Work

The conclusions drawn from this study provides a good starting point for further research. Two recommendations for future research include:

- Studying ampicillin and temocillin dose-exposure-response relationships to develop input profile models that offer the optimal compromise between benefits and risks for a given patient population. Ultimately, the model-based predictions can then be validated using data from future clinical trials.
- Conduct studies that determine the shelf-life of ampicillin and temocillin after reconstitution to establish the feasibility of pre-preparation, storage, and CI administration for both in-patient and out-patient settings. Studies that establish ampicillin and temocillin stability at a range of temperatures, in a variety of diluents and stored in different infusion containers that mimic in-use conditions will aid practice.

5.2 Practice-Based Research

5.2.1 Knowledge Gaps Addressed

5.2.1.1 Retrospective Practice Review of Prolonged Infusion in Critical Care

There is uncertainty regarding whether dose optimisation of BLA therapy, guided by PK and PD principles achieves desired clinical outcomes in ICU patients. Despite this, in recent years, PI is increasingly used in ICU. This study was the largest real-world study evaluating the use of PI piperacillin-tazobactam and meropenem in practice. The purpose of this study was to provide insight into prescribing patterns of PI BLAs in critical care.

5.2.1.2 Differential Antibiotic Dosing in Critical Care: Survey on Nurses' Perceptions

Statistics on nurses' own perspectives regarding antibiotic knowledge contributes significantly to educational preparation and quality in healthcare. The lack of studies in the literature investigating nurses' clinical practice in the AMS and particularly in antibiotic administration suggests that this nursing practice has been overlooked. To address this gap in the literature, a survey on nurses' knowledge, perceptions and experience was conducted. The purpose of this study was to gain a better understanding of nurse's knowledge and perceptions regrading P/CI antibiotic therapy and to provide an evidence base to support future needs in terms of education and training.

5.2.2 Implications

With the growing interest and recent increase in the use of differential antibiotic dosing, evidence shows that there is a lack of formal training for HCP regarding P/CI BLAs. Some healthcare settings are implementing the use of P/CI as a dosing strategy to improve patient outcomes, however, many HCPs practicing in these institutions have not received training on the quality and safety of these infusions. This was evident from the retrospective practice review (Section 2.3) conducted that discloses the use of meropenem for longer than its suggested shelf-life, potentially indicating that patient/s received sub-therapeutic doses. The need for tailored education and training in continuing professional development programs for HCPs to improve their knowledge of P/CI is further verified from results obtained from the survey of nurses' perception (Section 2.4).

5.2.3 Limitations

5.2.3.1 Retrospective Practice Review of Prolonged Infusion in Critical Care

Firstly, with the retrospective nature of this study, data is limited to the depth and accuracy of the documented medical records. Secondly, this study addressed a heterogeneous population that was limited to ICUs in a single centre. Thirdly, data on bacteria MIC was not available for analysis, thus, not permitting the identification of patients that did not attain piperacillin-tazobactam and meropenem PD targets or patients with toxic antibiotic concentrations. Lastly, the number of patients receiving meropenem was relatively small compared to those receiving piperacillin-tazobactam making it difficult to compare or draw conclusions on the efficacy of PI meropenem.

5.2.3.2 Differential Antibiotic Dosing in Critical Care: Survey on Nurses' Perceptions

First, hard copies of the survey tool were distributed in-person, limiting the exposure of the survey to a wider audience. Second, the survey was disseminated in a single-centre, SGH. Although the obtained data is representative and included a wide range of nurses from three ICU wards within the hospital, there is a need for multicentre studies to provide a stronger basis for subsequent generalisation. Third, the survey was disseminated to and completed by day-shift nurses. Day-shift nurses typically support and work alongside doctors and surgeons and tend to have more experience than night-shift nurses. The gathered data, however, do not account for the difference in experience between day- and night-shift nurses.

5.2.4 Recommendations

Recommendations for future practice-based studies include:

- Real-world, pragmatic studies with rigorous methodologies are vital for providing evidence of P/CI treatment effectiveness in clinical practice. These studies will aid in gaining a wider understanding of current usage in practice, potential benefits and potential risks associated with P/CI of BLAs in ICU patients as well as those on general wards. These studies will investigate antibiotic doses, dosing frequency, infusion time, resistance breakpoint profiles with a diverse, large and unrestricted patient population.

- Upskilling the workforce is an essential area for further exploration. Educating HCPs is important to ensure that they have the skills needed to improve the quality of healthcare and boost their motivation to do so. This can be a feasible intervention to enhance their confidence and practice in prescribing, preparing and administering BLAs via P/CI as well as heighten their awareness of their importance, benefits and associated risks.

- Collaboration with professional bodies to increase the reach and impact of such trainings.

5.2.5 Future Work

Further research could prove quite beneficial to the literature. A few points that could be considered in the future include:

5.2.5.1 Retrospective Practice Review of Prolonged Infusion in Critical Care

- Conducting multicentre studies on a national scale to investigate the prescribing patterns of BLAs in ICU as well as general wards. The valuable information gathered will allow for greater generalisation of findings and potentially direct subsequent prospective studies.

- Conducting a real-world study on the use of other BLAs commonly used in ICU (e.g., amoxicillin) to gain a wider understanding of their current use in practice and the impact of differential dosing regimens on patients in critical care as well as those in general wards.

5.2.5.2 Differential Antibiotic Dosing in Critical Care: Survey on Nurses' Perceptions

- Conduct a similar study on a national level in collaboration with professional organisations to reach a wider audience. This will allow for access to new and broader populations and greater generalisation of findings collected from larger quantities of responses.

- Conducting studies using the same survey tool to assess the knowledge, perceptions, and experience of other HCPs (e.g., doctors and pharmacists) on differential antibiotic dosing. Also, carry out semi-structured interviews to gain more insight and a wider understanding of nurses and other stakeholders' viewpoints regarding differential

antibiotic dosing. Semi-structured interviews will encourage two-way communication, where the participants can express views openly, providing in-depth information of their opinions. Findings can provide as an evidence base to support needs in terms of education and training.

- Develop and conduct a survey asking nurses about any prior training received on infectious diseases. This study will:
    - Explore how and what infectious disease topics are taught
    - Summarise the perceived successes and challenges in learning about infectious diseases
    - Investigate whether the education received aided their practice being antimicrobial stewards

5.3 Laboratory-Based Research

5.3.1 Knowledge Gaps Addressed

With the discovery of new antibiotics diminishing, optimising the administration of existing broad-spectrum antibiotics such as piperacillin-tazobactam and amoxicillin-clavulanic acid has become a necessity. To expand the breadth of current knowledge, the bench-to-bedside approach was utilised, where challenges experienced in practice were addressed in the laboratory. BLA stability is a crucial parameter that needs to be determined to assess the feasibility of administration via P/CIs. To address this, HPLC-SIMs were developed and validated in compliance with ICH guidelines. Quantitative analysis of BLA stability was conducted at a range of temperatures and diluents to determine their effect on degradation.

5.3.2 Implications

Findings from these studies aid in ameliorating current dosing regimens to optimise antibiotic efficacy. Results obtained from stability studies assist in resolving challenges experienced in practice in terms of preparation, storage, and administration as they indicate the effects of temperature, diluent, and pre-preparation of infusion solutions. Studies demonstrated that stability data generated in all studies are an improvement to the stability data presented in the British, American, and European pharmacopoeias.

5.3.3 Limitations

Findings of this study should be interpreted in view of some limitations. Firstly, the results are only applicable to the antibiotics, diluents, temperatures and temperature cycling used. Secondly, data obtained from stability studies are only relevant for clinical concentrations used. Higher concentrations and more dilute solutions were not tested.

5.3.4 Recommendations

As previously mentioned in Chapter One, the lack of YCD-compliant studies is impeding OPAT expansion as services are compelled to use broad-spectrum, once daily agents. Therefore, there is a need to generate stability data for narrow-spectrum (e.g., flucloxacillin) BLAs in EPs that entirely conform to the UK national standards specific to OPAT.

5.3.5 Future Work

These laboratory-based studies could constitute the object of future work. Recommendations for further research include:

- Randomised clinical trials to determine feasibility and clinical efficacy of P/CI amoxicillin-clavulanic acid as this is a relatively unexplored area.
- Conduct stability studies for piperacillin-tazobactam and amoxicillin-clavulanic acid diluted in other diluents (e.g., Ringer's and Lactated Ringer's).
- Conduct a stability study of amoxicillin-clavulanic acid that conform to OPAT YCD specifications to establish the feasibility of P/CI amoxicillin-clavulanic acid in outpatient settings.
- PK/PD studies that involve blood sample analysis for patients that will receive P/CI piperacillin-tazobactam and amoxicillin-clavulanic acid to investigate PK/PD properties of differential dosing regimens.
- Future assays investigating the stability of piperacillin-tazobactam, and amoxicillin-clavulanic acid should consider analysis at a range of concentrations to account for patient populations with specific dosing requirements, such as those on fluid restriction due to reduced renal clearance.
- Further investigations are warranted to understand the stability of amoxicillin-clavulanic acid in various infusion devices such as elastomeric pumps. These studies should conform to OPAT YCD specifications to establish the feasibility of P/CI amoxicillin-clavulanic acid in outpatient settings.

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Appendices

Appendix 1

Kingston University London
Faculty of Science, Engineering and Computing

Patient Details

Age: ..... DOB: ..... Gender: ..... Weight: .....

Susceptible Bacteria

Source:

Community acquired ☐
Hospital acquired ☐

Indication:

Empirical ☐
Definitive ☐

Cultures:

Yes ☐
No ☐

Diagnosis::

Type of infection acquired:

Respiratory ☐
Abdominal ☐
Soft tissue ☐
Urinary Tract ☐
Other _____

Isolated Pathogen

Yes ☐ No

If yes, list organism: _____

If yes, list sensitivities: _____

Agent Administration

Agent: 1

Agent used:

Name: .....
Single ☐ Combined ☐
Name other agent/s:
• .....

Administration:

Dose: .....
Volume: .....
Frequency: .....
Infusion Time: .....

Duration:

Start Date:
...../...../.....
End Date:
...../...../.....

Prior Antibiotics:

Yes ☐ No ☐
If yes, please state:

Reason for changing dose?

Reason for changing antibiotic?

Agent: 2

Agent used:

Name: .....
Single ☐ Combined ☐
Name other agent/s:
• .....

Administration:

Dose: .....
Volume: .....
Frequency: .....
Infusion Time: .....

Duration:

Start Date:
...../...../.....
End Date:
...../...../.....

Reason for changing dose?

Reason for changing antibiotic?

Agent: 3

Agent used:

Name: .....

Single ☐ Combined ☐

Name other agent/s:

- .....

Administration:

Dose: .....

Volume: .....

Frequency: .....

Infusion Time: .....

Duration:

Start Date:

...../...../.....

End Date:

...../...../.....

Reason for changing dose?

Reason for changing antibiotic?

Agent: 4

Agent used:

Name: .....

Single ☐ Combined ☐

Name other agent/s:

- .....

Administration:

Dose: .....

Volume: .....

Frequency: .....

Infusion Time: .....

Duration:

Start Date:

...../...../.....

End Date

...../...../.....

Reason for changing dose?

Reason for changing antibiotic?

Outcomes

Patient Labs:

When antibiotics was started:

- SRCR: .....
- CRP: .....
- WBC: .....

At the end of antibiotics:

- SRCR: .....
- CRP: .....
- WBC: .....

Clinical Cure:

Yes ☐

If yes:

- Clinically stable ☐
- No fever ☐
- White blood cells down ☐
- Discharge from ICU ☐
- No further antibiotics for the same indication within 5 days ☐

No ☐

If no:

Please state:

.....

.....

Additional Information

Appendix 2

Faculty of Science, Engineering and Computing

Kingston University London

Participant Information Sheet

Please read the information provided and don't hesitate to ask questions on anything you do not understand.

- This survey was prepared by a group of researchers and pharmacists from Kingston University and St Georges Hospital.
- The purpose of this survey is to understand the perceptions of intensive care unit nurse's on prolonged and continuous infusions.
- You were selected to participate in this survey as you are a nurse that may prepare and/or administer antibiotics via prolonged/continuous infusion.
- The survey will take around 10 minutes to complete.
- Participation is on a voluntary base. You may decide to not take part if you do not feel comfortable to.
- The information collected as part of the survey will remain strictly confidential, anonymous, and for use within this study only.
- Survey is split into four sections:

  1. Demographics (2 questions)
  2. Subjects self-assessment of knowledge about antibiotic therapy and CI (2 statements & 1 question)
  3. Subjects perception in regard to antibiotic therapy and CI (8 statements & 1 question)
  4. Subjects comfort level in terms of antibiotic therapy and CI (3 Statements)
  5. Subjects experience in terms of CI administration (1 statement & 4 questions)

- If you have any queries and/or comments please contact the researchers via email:

  1. Shereen Nabhani-Gebara: s.nabhani@kingston.ac.uk
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  3. Sarah Fawaz: K1119349@kingston.ac.uk
  4. Anika Hannah: k1419867@kingston.ac.uk

1 Demographics

1. How long have you worked in the ICU?

<1 year	1-3 years	3-5years	5-10 years	>10 years

2. What is your grade?

Band 5	Band 6	Band 7	Band 8

2 Knowledge

3. My general knowledge about antibiotics in the Intensive Care Unit is…

Very Poor	Poor	Acceptable	Good	Very Good

4. My general knowledge about administering antibiotics via prolonged/continuous infusions is…

Very Poor	Poor	Acceptable	Good	Very Good

5. Why do you think prolonged/continuous infusions are used?

_____

_____

_____

_____

_____

3 Perceptions

6. Prolonged/continuous infusions of antibiotics aids in achieving higher clinical cure rate compared with conventional intermittent infusions

Strongly Disagree	Disagree	Not Sure	Agree	Strongly Agree

7. The **preparation** of antibiotics for prolonged/continuous infusions results in an increased workload on nurses compared with conventional intermittent infusions

Strongly Disagree	Disagree	Not Sure	Agree	Strongly Agree

8. The **preparation** of antibiotics via prolonged/continuous infusions is more time consuming compared with conventional intermittent infusions

Strongly Disagree	Disagree	Not Sure	Agree	Strongly Agree

9. Prolonged/continuous infusions are easier to **prepare** compared with conventional intermittent infusions

Strongly Disagree	Disagree	Not Sure	Agree	Strongly Agree

10. The **administration** of antibiotics by prolonged/continuous infusions results in an increased workload on nurses compared with conventional intermittent infusions

Strongly Disagree	Disagree	Not Sure	Agree	Strongly Agree

11. The **administration** of antibiotics via prolonged/continuous infusions is more time consuming compared with conventional intermittent infusions

Strongly Disagree	Disagree	Not Sure	Agree	Strongly Agree

12. Prolonged/continuous infusions are easier to **administer** compared with conventional intermittent infusions

Strongly Disagree	Disagree	Not Sure	Agree	Strongly Agree

13. I think that the **preparation** of continuous infusion antibiotics is more prone to medical errors

Yes	No	Not Sure

If Yes, Why?

Multiple manipulations Calculations Other

_____

4 Comfort

14. I am comfortable discussing antibiotic therapy with other healthcare professionals

Very Uncomfortable	Uncomfortable	Neutral	Comfortable	Very Comfortable

15. I am comfortable discussing laboratory results related to infections with other healthcare professionals

Very Uncomfortable	Uncomfortable	Neutral	Comfortable	Very Comfortable

16. I am comfortable interpreting microbiology results

Very Uncomfortable	Uncomfortable	Neutral	Comfortable	Very Comfortable

5 Experience

17. I routinely conduct visual inspection for of the antibiotics being administered as prolonged/continuous infusions for precipitation throughout the infusion time

Strongly Disagree	Disagree	Not Sure	Agree	Strongly Agree

18. What do you think are the advantages of prolonged/continuous infusions compared with intermittent infusions?

_____

_____

_____

_____

19. What do you think are the disadvantage of prolonged/continuous infusions compared with intermittent infusions?

_____

_____

_____

_____

20. What changes can be made for preparation of prolonged/continuous infusions to improve the process?

_____

_____

_____

_____

21. What changes can be made for administration of prolonged/continuous infusions to improve the process?

_____

_____

_____

_____

Appendix 3

STATEMENT	Frequency	%	Frequency	%	Frequency	%	Frequency	%	Frequency	%	D	S
DEMOGRAPHICS	<1 YEAR		1-3 YEARS		3-5 YEARS		5-10 YEARS		>10 YEARS			
HOW LONG HAVE YOU WORKED IN ICU?	8	15.4	7	13.5	9	17.3	12	23	16	30.8		
KNOWLEDGE	VERY POOR		POOR		ACCEPTABLE		GOOD		VERY GOOD			
MY GENERAL KNOWLEDGE ABOUT ANTIBIOTICS IN THE INTENSIVE CARE UNIT IS…	0	0	0	0	12	23.1	32	61.5	8	15.4	N-D	0.048
MY GENERAL KNOWLEDGE ABOUT ADMINISTERING ANTIBIOTICS VIA PROLONGED/CONTINUOUS INFUSIONS IS…	0	0	2	3.8	8	15.4	31	59.6	11	21.2	N-D	-0.606
PERCEPTIONS	STRONGLY DISAGREE		DISAGREE		UNCERTAIN		AGREE		STRONGLY AGREE			
PROLONGED/CONTINUOUS INFUSIONS OF ANTIBIOTICS AIDS IN ACHIEVING HIGHER CLINICAL CURE RATE COMPARED WITH CONVENTIONAL INTERMITTENT INFUSIONS	1	1.9	0	0	5	9.6	20	38.5	26	50	N-S	-1.636
THE PREPARATION OF ANTIBIOTICS FOR PROLONGED/CONTINUOUS INFUSIONS RESULTS IN AN INCREASED WORKLOAD ON NURSES COMPARED WITH CONVENTIONAL INTERMITTENT INFUSIONS	5	9.6	43	82.7	3	5.8	1	1.9	0	0	P-S	1.072
THE PREPARATION OF ANTIBIOTICS VIA PROLONGED/CONTINUOUS INFUSIONS IS MORE TIME CONSUMING COMPARED WITH CONVENTIONAL INTERMITTENT INFUSIONS	6	11.5	42	80.9	0	0	4	7.7	0	0	P-S	1.698
PROLONGED/CONTINUOUS INFUSIONS ARE EASIER TO PREPARE COMPARED WITH CONVENTIONAL INTERMITTENT INFUSIONS	3	5.8	34	65.4	8	15.4	6	11.5	1	1.9	P-S	1.193
THE ADMINISTRATION OF ANTIBIOTICS BY PROLONGED/CONTINUOUS INFUSIONS RESULTS IN AN INCREASED WORKLOAD ON NURSES COMPARED WITH CONVENTIONAL INTERMITTENT INFUSIONS	3	5.8	40	76.9	0	0	9	17.3	0	0	P-S	1.373
THE ADMINISTRATION OF ANTIBIOTICS VIA PROLONGED/CONTINUOUS INFUSIONS IS MORE TIME CONSUMING COMPARED WITH CONVENTIONAL INTERMITTENT INFUSIONS	4	7.7	32	61.5	1	1.9	15	28.8	0	0	N-D	0.618
PROLONGED/CONTINUOUS INFUSIONS ARE EASIER TO ADMINISTER COMPARED WITH CONVENTIONAL INTERMITTENT INFUSIONS	2	3.8	38	73.1	6	11.5	5	9.6	1	1.9	P-S	1.615
I THINK THAT THE PREPARATION OF CONTINUOUS INFUSION ANTIBIOTICS IS MORE PRONE TO MEDICAL ERRORS			YES		NO		NOT SURE					
			4	7.7	46	85.5	2	3.8				
IF YES, WHY?			MULTIPLE MANIPULATIONS		CALCULATIONS		OTHER					
			1	25%	3	75%	0	0				
COMFORT	VERY UNCOMFORTABLE		UNCOMFORTABLE		NEUTRAL		COMFORTABLE		VERY COMFORTABLE			
I AM COMFORTABLE DISCUSSING ANTIBIOTIC THERAPY WITH OTHER HEALTHCARE PROFESSIONALS	0	0	0	0	10	19.2	32	61.5	10	19.2	N-D	0.000
I AM COMFORTABLE DISCUSSING LABORATORY RESULTS RELATED TO INFECTIONS WITH OTHER HEALTHCARE PROFESSIONALS	0	0	1	1.9	6	11.5	36	69.2	9	17.3	N-D	-0.547
I AM COMFORTABLE INTERPRETING MICROBIOLOGY RESULTS	0	0	4	7.7	8	15.4	30	57.7	10	19.2	N-D	-0.711
EXPERIENCE	STRONGLY DISAGREE		DISAGREE		UNCERTAIN		AGREE		STRONGLY AGREE			
I ROUTINELY CONDUCT VISUAL INSPECTION FOR OF THE ANTIBIOTICS BEING ADMINISTERED AS PROLONGED/CONTINUOUS INFUSIONS FOR PRECIPITATION THROUGHOUT THE INFUSION TIME	0	0	4	7.7	4	7.7	17	32.7	27	51.9	N-D	-0.981

D= distribution; S= skewness; N-D= normal distribution; N-S= negatively skewed; P-S= positively skewed

Appendix 4

Piperacillin-Tazobactam Forced Degradation Study

A.4.1 Introduction to Stress Testing

Stress testing is a term that is commonly used interchangeably with the terms ''accelerated stability'' and ''forced degradation'' (367). Stress testing of pharmaceuticals is used to investigate the degradation pathways of active ingredients and is recognized as an important part of the drug development process (367,368). The ICH guidelines states that stress tests are performed to ''determine the intrinsic stability of the molecule by establishing the degradation pathways in order to identify the likely degradation products and to validate the stability indicating power of the analytical procedure used'' (369). Testing a drug under stress conditions is vital as it provides information that is used to: (1) elucidate the intrinsic stability of the drug substance, (2) predict potential stability problems, (3) develop analytical methods and, (4) identify potential degradation products and pathways (367).

The knowledge gained from stress testing is useful in many areas including:

(1) Analytical method development; it is important to develop a stability indicating analytical method that can detect potential degradation products. Stressing the parent compound under various stress conditions can accelerate the process generating samples containing potential degradation product/s (367). The developed analytical method should be able to separate parent compounds from degradants (368).

(2) Formulation and packaging development; stress tests are performed during the pre-formulation stage to aid in the suitable selection of compounds and excipients (370). Stressing the compound determines it susceptibility to hydrolysis, oxidative, photolytic, and thermal degradation. Information gained is then taken into consideration for the formulation development process as well as defining the appropriate packaging (367).

(3) Shelf-life determination; to determine suitable storage conditions for a drug, information on conditions and environments that induce its degradation is required (367). Studying the stability of a compound under accelerated conditions would show the degradation products observed at the end of its shelf-life (370). Therefore, for accurate shelf life predictions, data from long term stability studies are ideal in most cases (367).

(4) Absorption, distribution, metabolism and excretion studies; these characteristics are extensively and thoroughly studied prior to the marketing of the drug. They are a critical part of any drug

development programme that typically involves the identification of major metabolites. In most cases the degradants detected in stress-testing studies are metabolites; consequently, larger metabolite quantities for characterisation are generated using accelerated conditions (367).

The ability of the proposed method to monitor a change in the chemical properties of the drug over time, almost certainly requires a forced degradation study to be carried out on the drug product/substance (371). Samples prepared under stress conditions are used to demonstrate that the proposed analytical method is 'stability indicating', hence is capable of detecting a loss in active drug concentration and a subsequent increase in degradation product (372). The forced degradation study should therefore assess the stability of the drug when exposed to different pH solutions, in the presence of oxygen and light and at elevated temperatures (371).

The ICH defines stress testing as an investigation of the ''intrinsic stability'' characteristics of the molecule. The concept of intrinsic stability has four main aspects: (1) conditions leading to degradation, (2) rates of degradation, (3) structures of the major degradation pathways and, (4) pathways of degradation [1]. ICH guidelines applicable to forced degradation studies are ICH-Q1A, ICH-Q1B and ICH-Q2B (373). The guidelines state recommendations for the examination of the effects of temperature, oxidation and photolysis. ICH Q1B (374) recommends approaches to assess the photo-stability of drug substances. Stress condition exposure levels are not defined; therefore, the design of a stress test is left down to the applicant; however, scientific justification is needed. ICH Q1A and ICH Q1B state that degradants formed throughout forced degradation studies may not form during stability studies under natural conditions. ICH Q2B gives guidance on how to validate an analytical methodology and recommendation on the use of stressed samples to prove specificity (372).

There are various degradation conditions a compound could be stressed under; these include hydrolytic (acidic/basic), oxidative, photolytic and thermal degradation.

The most common degradation chemical reaction is hydrolysis. Hydrolysis is a chemical process that sources decomposition of molecules by reaction with water (369). Acid/base/neutral stress testing is carried out for drug products/substances in solution at ambient or elevated temperatures and involves catalysis of ionisable functional groups present in the molecule (368,369). HCl and NaOH are employed for generating acidic and basic stress samples. Commonly, the temperature and pH at which a drug solution is stored are major determinants in drugs that are prone to hydrolytic decomposition. Hydrolysis is dependent upon the relative concentration of hydronium and hydroxyl

ions (372). Acid/basic stressing of piperacillin-tazobactam will be performed to force the degradation of the molecule to its primary degradation product (368).

As a mode of decomposition, oxidation is probably second only to hydrolysis. Oxidative degradation is a significant degradation pathway as many drugs undergo oxidation under normal storage conditions by reacting with ground state elemental oxygen (372). Pharmaceutical oxidation could also arise from a variety of mechanisms including: (1) nucleophilic/electrophilic processes, (2) electron transfer process and, (3) hydrogen atom abstraction (367). The most predominantly used oxidation compound is hydrogen peroxide (H_2O_2) as it mimics possible presence of peroxide in excipients (369,375). It can be used in a range of concentrations, 3-30% at a temperature not exceeding 40^{o}C (372).

UV and visible light are the most common electromagnetic radiation sources to which pharmaceutical drugs could be exposed to (368). The rate of photo degradation depends upon the intensity of incident light and quantity of light absorbed by the drug molecule (372). Photolytic studies are performed to generate primary degradants of drugs and to provide knowledge on whether or not a compound is photo labile. Studying the behaviour of piperacillin-tazobactam exposed to light and dark conditions will permit analysis of the effects such conditions have on the efficacy and safety of the product during both handling and administration (376,377).

A rule of thumb: with an increase in temperature, the rate of reaction will increase (372). Data relating to the thermal stability of drugs is usually required to obtain information for handling, shelf-life and usage (378). Thermal stressing of piperacillin-tazobactam involves accelerating degradation by exposing it to high temperatures to induce covalent bond breakage (367). The ICH states that drugs that are in a solid-state should be exposed to both dry and wet heat, whereas drugs in liquid-state can be exposed to dry heat (375). The most widely accepted temperature is 70^{o}C; as temperatures >80^{o}C may not produce a predictive degradation pathway (372).

Several studies that have investigated accelerating piperacillin-tazobactam degradation are described in the literature. Ramalingam and colleagues (379) developed a method for the detection of piperacillin-tazobactam degradants. Piperacillin-tazobactam was stressed using a mild acid and basic solution (0.001M HCl and 0.001M NaOH) to determine the selectivity and specificity of the developed method. Results obtained showed minor degradants for samples stored at low temperatures and more distinct degradants for samples stored on inpatient wards. The developed method was found to be accurate, precise and specific for simultaneous routine analysis of piperacillin and tazobactam in pharmaceutical dosage form (379).

Navle et al (380) developed a method for the determination of piperacillin-tazobactam related substances. Piperacillin-tazobactam was stressed under acidic (2M HCl), basic (0.05M NaOH), oxidative (10% H_2O_2), thermal (105^oC) and photolytic conditions. Results obtained from the forced degradation study confirmed that the proposed method was specific and selective with no co-eluting peaks. Although the method developed separates piperacillin, tazobactam and their degradation products, it has a run time of 45 minutes (380).

Donnelly (321) developed a method for the determination of piperacillin and tazobactam degradation products. Stress conditions considered analysis of solutions in hydrolytic (acidic and basic), oxidative and thermal. Results obtained suggest that in acidic conditions tazobactam had degraded 67% and piperacillin had degraded 56% over 96 hrs. The oxidized sample produced a few minor degradation peaks, however very little change in piperacillin and tazobactam concentration was observed for 96hrs. similar peaks to the oxidized sample were observed for the sample exposed to heat, but in higher concentrations (321).

Forced degradation studies play a crucial role during analytical method development. The developed stability indicating method should accurately measure the changes in active ingredients concentration without interference from other degradation products, impurities and excipients. The exposure of piperacillin-tazobactam to stress conditions will demonstrate the specificity of the developed method with an aim to generate degradation products which are likely to form in realistic storage/administration conditions. The aim of the forced degradation study is to determine whether the developed method is specific enough to distinguish between piperacillin, tazobactam and their degradation products. This study is not designed to establish the qualitative and quantitative limits for change of piperacillin-tazobactam, however, it is intended to demonstrate whether accidental exposure to conditions other than normal ranges makes piperacillin-tazobactam injectable solutions unsafe and to evaluate which specific test parameters will be the best indicators of piperacillin-tazobactam stability.

A.4.2 Materials and Methods

Chemicals and Reagents

Generic brand (Fresenius Kabi) piperacillin-tazobactam vials were obtained from St Georges Hospital, London, UK. HPLC reference standards: (1) piperacillin sodium salt, (2) tazobactam sodium salt and, (3) cephalothin sodium salt were purchased from Sigma Aldrich. Sodium phosphate salt was also purchased from Sigma Aldrich. Reagents used include: (1) HPLC-grade methanol, (2) HPLC-grade acetonitrile, (3) water for injection, (4) phosphoric acid (5) deionized water (6) 1M HCl, (7) 1M NaOH and, (8) hydrogen peroxide, H_20_2.

Instrumentation and Equipment

Quantitative analysis was performed using an Agilent 1260 HPLC system with single wavelength UV detection and Chemstation software.

Calibrated micropipettes (0.5-10µl, 10-100µl and 100-1000µl) were supplied by Eppendorf Ltd.

Chromatographic Conditions

Separation was conducted using a Thermoquest (150×4.6mm) hypersil BDS C-18 column, 5-micron particle size, Part Number 28105-022, Column Number 326334. The mobile phase consisted of H_2O (pH 4), methanol and acetonitrile (H2O:MeOH:ACN; 55:30:15) at flowrate of 1.0ml/min. Analysis was performed at 30^{o}C and detection at 210nm. The injection volume was 10µl with a run time of 4.5 minutes

Stress Condition Method

Acid/Base/Neutral Stress Testing

Acidic solution preparation

The exposure of piperacillin-tazobactam to acidic conditions to force the degradation of drug substances was carried out. Preparation involved weighing 15mg of pharmaceutical formulation piperacillin-tazobactam, into a glass headspace vial. 2ml of the previously prepared 0.1M HCl was added to the vial and further diluted with 13ml of 0.9% sodium chloride solution (15mg in 15ml; 1000PPM)

Basic Solution Preparation

The exposure of piperacillin-tazobactam to basic conditions to force the degradation of drug substances was carried out. Preparation involved weighing 15mg of pharmaceutical formulation piperacillin-tazobactam, into a glass headspace vial. 2ml of the previously prepared 0.1M NaOH was added to the vial and further diluted with 13ml of 0.9% Sodium Chloride (15mg in 15ml; 1000PPM).

Neutral solution preparation

Stress testing under neutral conditions involved reconstituting the drug with water for injection. The preparation of the neutral solution involved weighing 15mg of pharmaceutical formulation piperacillin-tazobactam, into a glass headspace vial. 2ml of water for injection was added to the vial and further diluted with 13ml of 0.9% Sodium Chloride (15mg in 15ml; 1000PPM).

Oxidative solution preparation

In clinical practice piperacillin-tazobactam preparation involves reconstituting 4500mg with 20ml WFI and is then diluted further with 50ml of 0.9% NS (4500mg in 70ml; 64,285PPM). For the oxidative study 450mg of piperacillin tazobactam was weighed and transferred into a vial. 2ml H_2O_2 was added and further diluted with 5ml 0.9% NS (450mg in 7ml; 64,285PPM).

Photolytic solution preparation

Two conditions were considered, light and dark. 900mg of piperacillin-tazobactam was reconstituted with 4ml WFI and further diluted with 10ml 0.9% NS (64,285PPM). 7ml of this stock solution was transferred into a vial which was protected from light using aluminium foil and stored in a dark cupboard. The remaining 7ml was transferred into another vial and left on the bench in natural light.

Thermal solution preparation

For the forced thermal degradation study, 450mg of piperacillin tazobactam was weighed and transferred into vial. 2ml WFI was added and further diluted with 5ml 0.9% NS (450mg in 7ml; 64,285PPM). The vial was then stored in an oven with temperature set at 70^0C.

Physical Testing

Physical Observation

A 4.5g piperacillin-tazobactam (generic brand, Fresenius Kabi) vial (4g piperacillin, 0.5g tazobactam) was prepared under aseptic conditions. The vial was reconstituted with 20ml water for injection and left aside for ~5 minutes until the solution became clear. The reconstituted piperacillin tazobactam solution was then drawn from the vial using a sterile syringe and injected into a 50ml 0.9% NS IV bag. The IV bag was stored at room temperature (24^{o}C) under natural light conditions. Any changes in the physical appearance and compatibility of solution that occurred over 4 weeks were noted. Colour and clarity were monitored at each time point. All samples were checked against: (1) a black background for the observation of particulate matter and, (2) a white background for the observation of colour change.

pH Profile

The pH of samples for all conditions was recorded at every sampling interval using a calibrated pH meter. The pH meter was calibrated at every sampling interval before measurements were obtained, using buffers with known pH of 4.00 and 7.00. The probe was washed with distilled water between measurements of different samples.

Sample preparation for analysis by HPLC

Acid, base, neutral sample preparation

At each of the sampling intervals, 75µl of the prepared acid, base and neutral solutions were pipetted into 2ml HPLC vials. 250µl of internal standard and 675µl of mobile phase were added. Each sample was run in triplicate.

Oxidative, photolytic and thermal sample preparation

At each the sampling intervals, 100µl of each of the solutions was added to 900µl of mobile phase. From these, 10µl was pipetted into another vial and prepared for analysis with 250µl internal standard and 740µl mobile phase.

Sampling intervals

Sampling was carried out over 144 hours. Sampling frequency was 0, 6, 12, 24, 48, 72, 144 hours. For all conditions being examined, the colour and clarity of the solutions were monitored at each time point. All samples were checked against: (1) a black background for the observation of particulate matter and, (2) a white background for the observation of colour change.

A.4.3 Results

Stability indicating capabilities of the method were determined using forced degradation conditions: (1) Hydrolytic (acidic/basic/neutral conditions), (2) Oxidative, (3) Photolytic and (4) Thermal.

Acid, Base and Neutral

Initially, the acidic/basic piperacillin-tazobactam solutions were prepared to the concentration used in practice (64,000PPM) using 1M HCl and NaOH. 2x450mg of piperacillin-tazobactam API was accurately weighed and transferred into a 15ml vials. 5ml of 0.9% NS was added to each of the two vials. 2ml of 1M NaOH was added to one of the vials and 2ml of 1M HCl was added to the other vial. No visible changes were observed in the basic solution; however, the acidic solution precipitated out upon the addition of 2ml 1M HCl (Figure A).

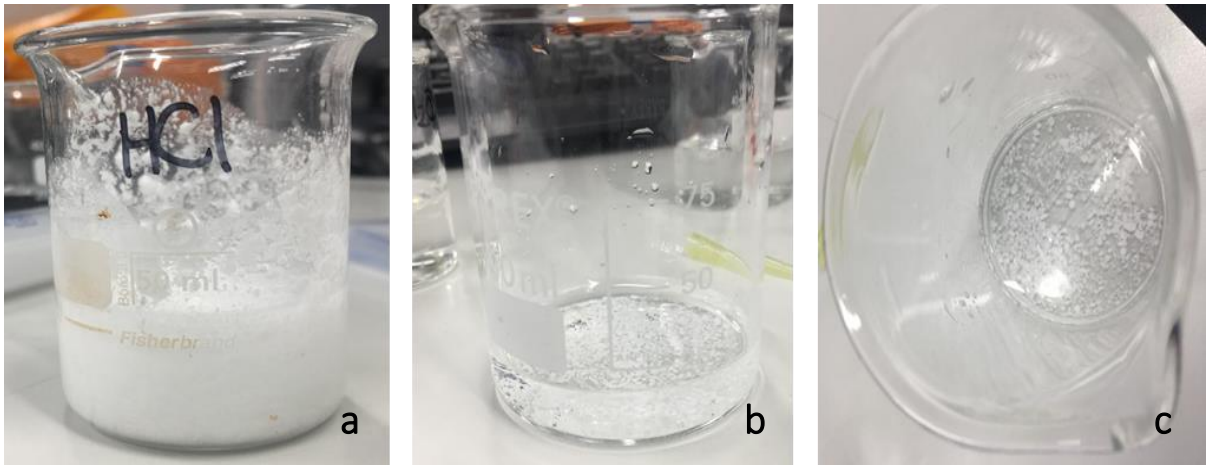

Figure A Showing precipitation occurring with the addition of HCl; a) precipitation with the addition of 1M HCl, b & c) precipitation with the addition of 0.1M HCl.

Figure A Showing precipitation occurring with the addition of HCl; a) precipitation with the addition of 1M HCl, b & c) precipitation with the addition of 0.1M HCl.

Figure A Showing precipitation occurring with the addition of HCl; a) precipitation with the addition of 1M HCl, b & c) precipitation with the addition of 0.1M HCl.

Figure A Showing precipitation occurring with the addition of HCl; a) precipitation with the addition of 1M HCl, b & c) precipitation with the addition of 0.1M HCl.

Consequently, 450mg of piperacillin-tazobactam was transferred to vials and 5ml of 0.9% NS was added to each vial. 2ml of 0.1M NaOH was added to one vial and 2ml of 0.1M HCl was added to the other. The addition of NaOH did not appear to change the physical compatibility of the drug in solution; however, the HCl once again resulted in precipitation. It is believed that lowering the pH

converts the sodium salt of Tazobactam to the penicillanic acid form which is only sparingly soluble in aqueous solution resulting in precipitation (Figure Aa and Ac).

Subsequently, a significantly lower concentration of piperacillin-tazobactam was prepared

A

B

B

B

B

C

C

C

C

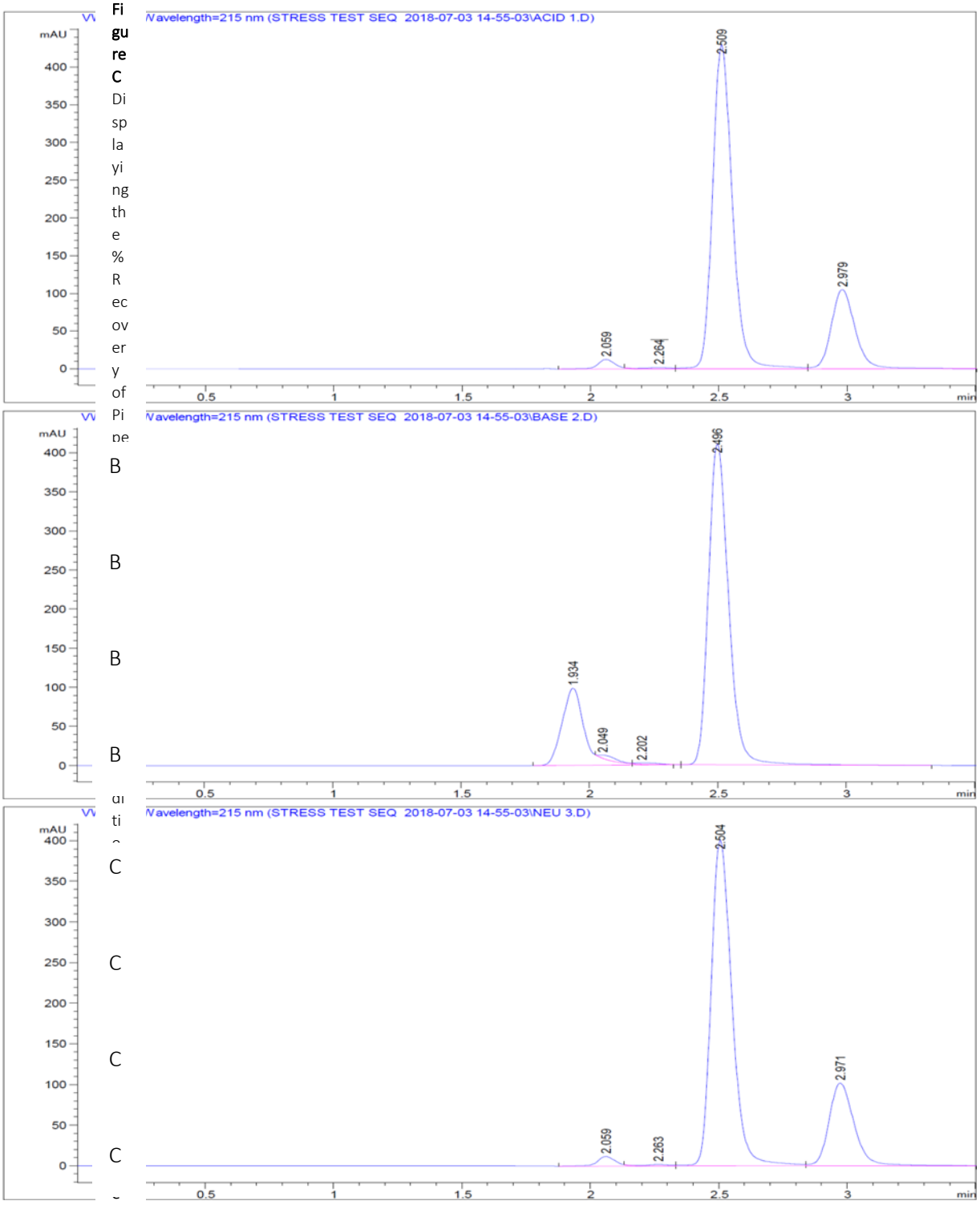

Figure C Displaying the % Recovery of Piperacillin in

(1000PPM) as described in the methods section. The addition of 2ml of 0.1M acidic and basic solutions to the reconstituted drug did not result in precipitation.

Figure B Showing chromatogram obtained for Piperacillin-Tazobactam stressed in acidic conditions at 0 hour; a) Tazobactam TR = 2.059 minutes, Cephalothin TR = 2.509 minutes and Piperacillin TR = 2.979, b) Tazobactam TR = 2.049 minutes and Cephalothin TR = 2.496 minutes and c) Tazobactam TR = 2.059 minutes, Cephalothin TR = 2.504 minutes and Piperacillin TR = 2.971.

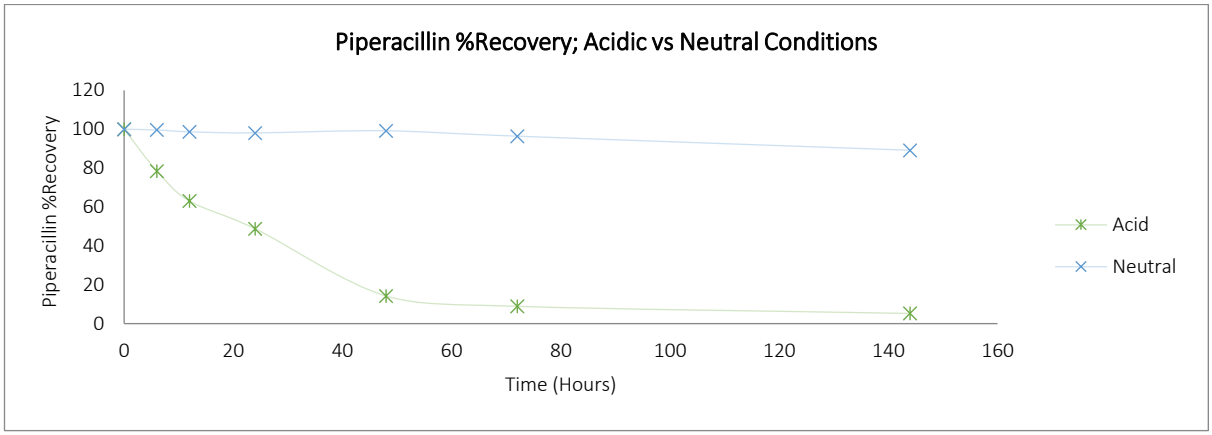

Figure C Displaying the %Recovery of Piperacillin in acidic and neutral conditions

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In basic conditions, at zero hour there was a 100% loss of piperacillin (Figure Bb); Piperacillin degradation product eluted at TR = 1.9 minutes. In acidic conditions, piperacillin degraded at an accelerated rate compared to neutral conditions (Figure C). Tazobactam was stable in all hydrolytic conditions tested.

Oxidative

From the results obtained, it was found that piperacillin degraded significantly by oxidation. The chromatogram (**Figure D**) shows piperacillin degradation products (t_R =2.722 and 1.960 mins). Over 50% loss of the initial piperacillin concentration was observed within 30 minutes of reconstitution (**Figure E**).

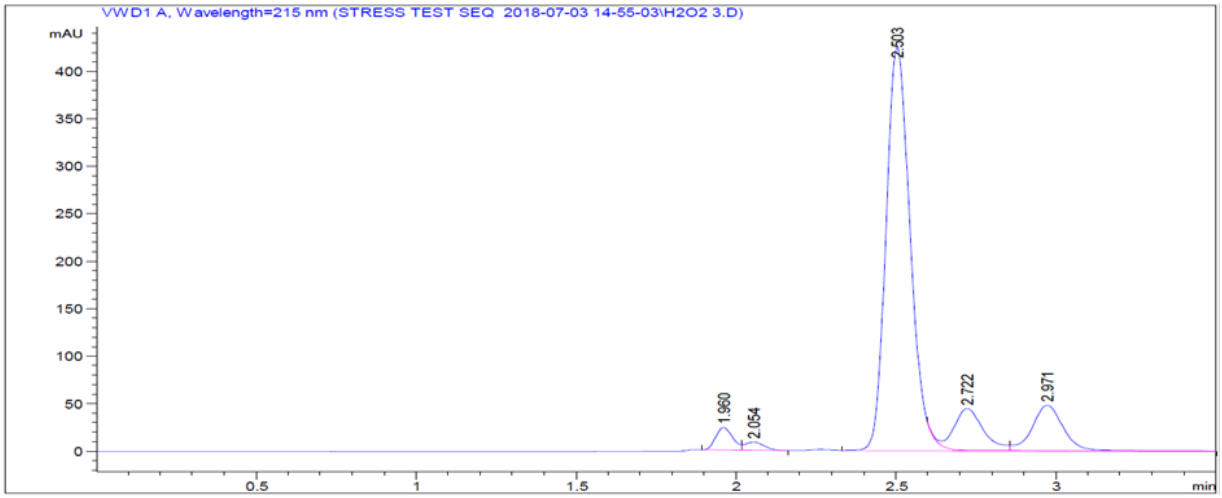

Figure D Showing chromatogram attained for piperacillin-tazobactam when stressed under oxidative conditions at 0.5 hours

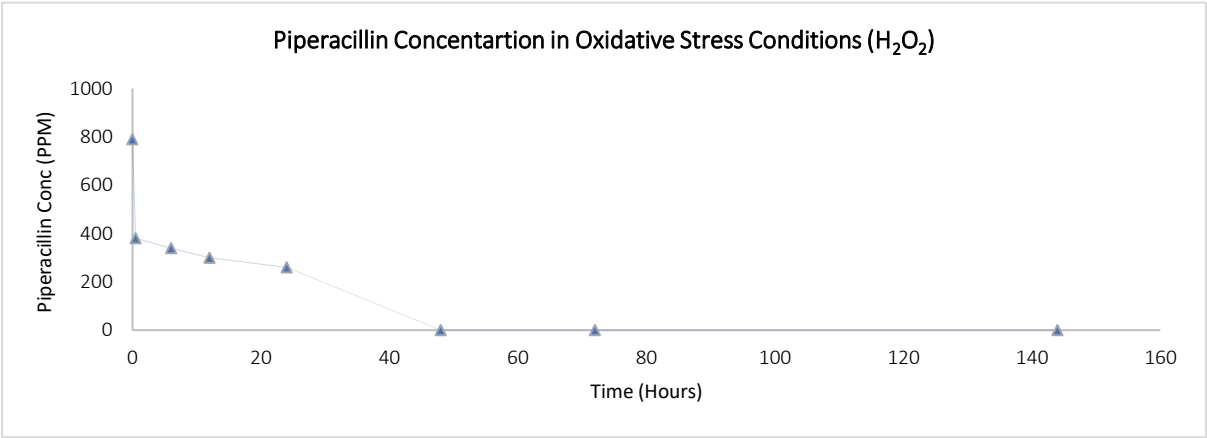

Figure E Showing the decrease of Piperacillin concentration prepared in H_2O

Photolytic

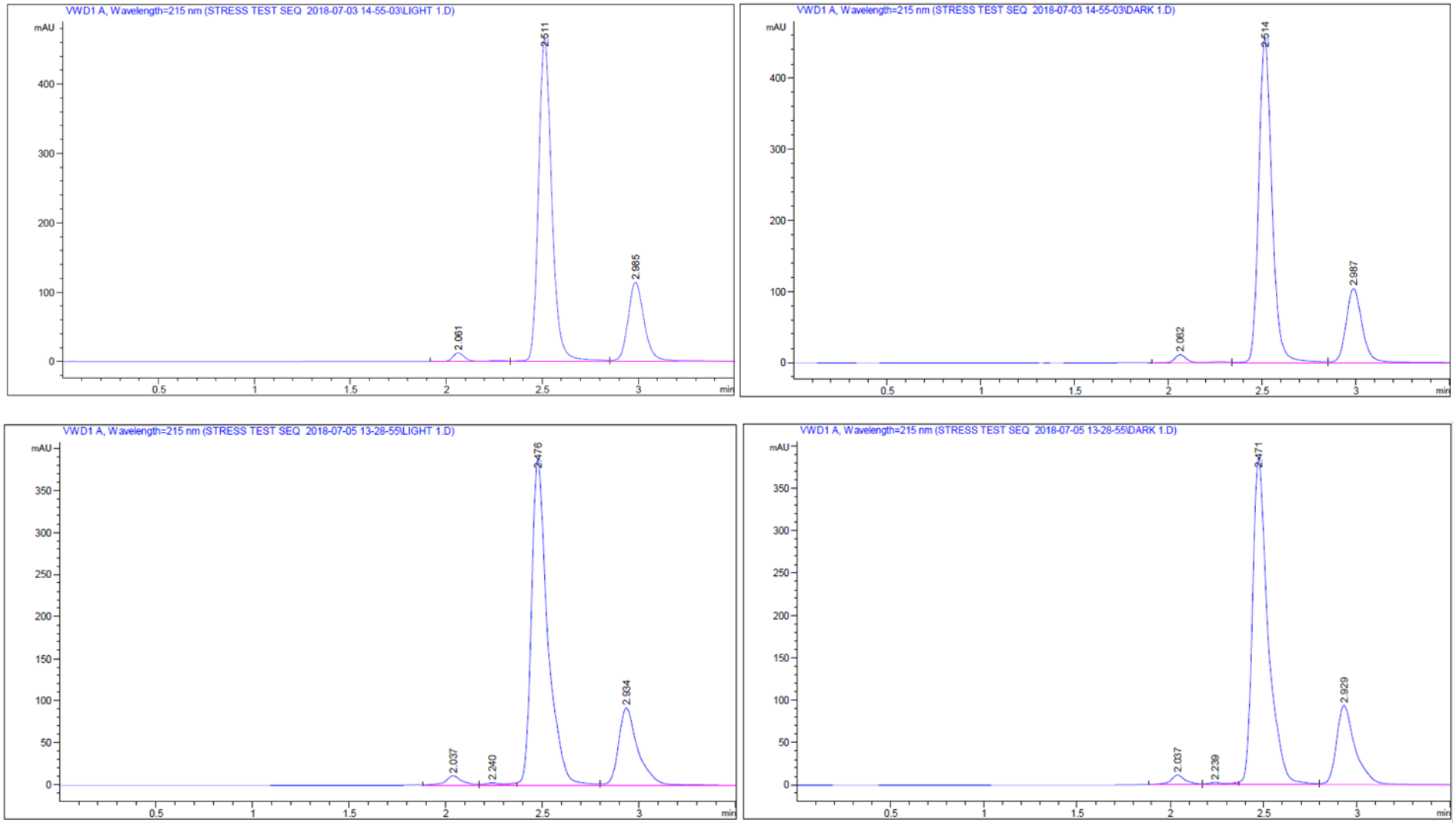

Figure F Showing piperacillin-tazobactam stored in light and dark conditions at 0 hour; a) in light condition stored on bench top and b) in dark condition stored in dark cupboard

Figure G Showing piperacillin-tazobactam stored in light and dark conditions at 72-hour; a) in light condition stored on bench top and b) in dark condition stored in dark cupboard

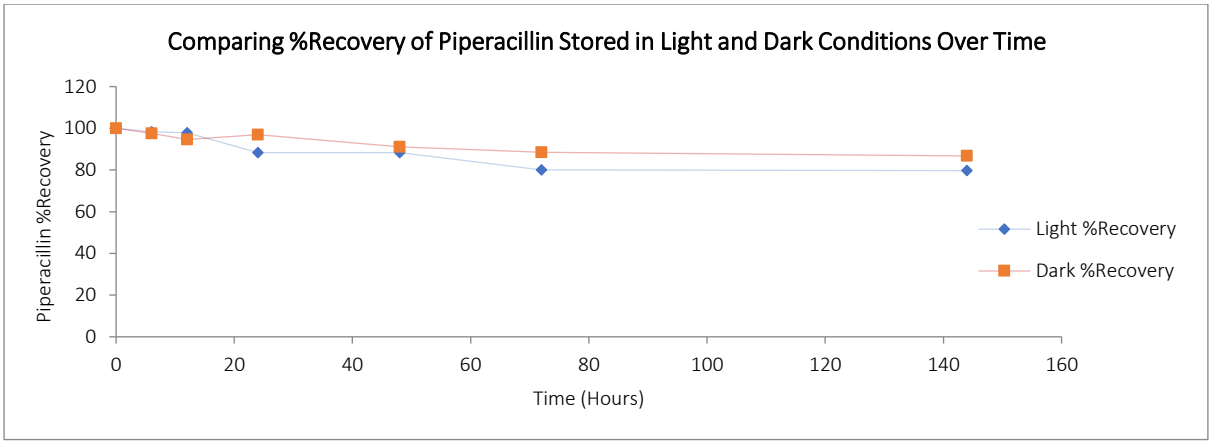

Figure H Comparing the percentage recovery of piperacillin when stored in light and dark conditions

Chromatograms obtained from the HPLC assay (**Figure F** and **Figure G**) showed no changes for both parent compounds in terms of peak shape. The retention times for the parent compounds remained consistent throughout the 144 hours of testing (~2.0 minutes and ~2.9 minutes for tazobactam and piperacillin respectively) without the appearance of a degradation product peak. This indicates that piperacillin and tazobactam are stable in both light and dark conditions for at least 24 hours; this is consistent with information provided by the pharmaceutical manufacturer.

A decrease in peak area was observed when comparing chromatograms obtained at 0-hour and 72-hour, indicating a loss in drug concentration. There was no evidence of a degradation products no other peaks was observed. **Figure H** shows the percentage recovery of piperacillin-tazobactam stored in light and dark conditions over the 72-hour. Piperacillin-Tazobactam maintained ≥90% of its initial concentration for 24 hours and 72 hours when stored in light and dark conditions respectively.

Thermal

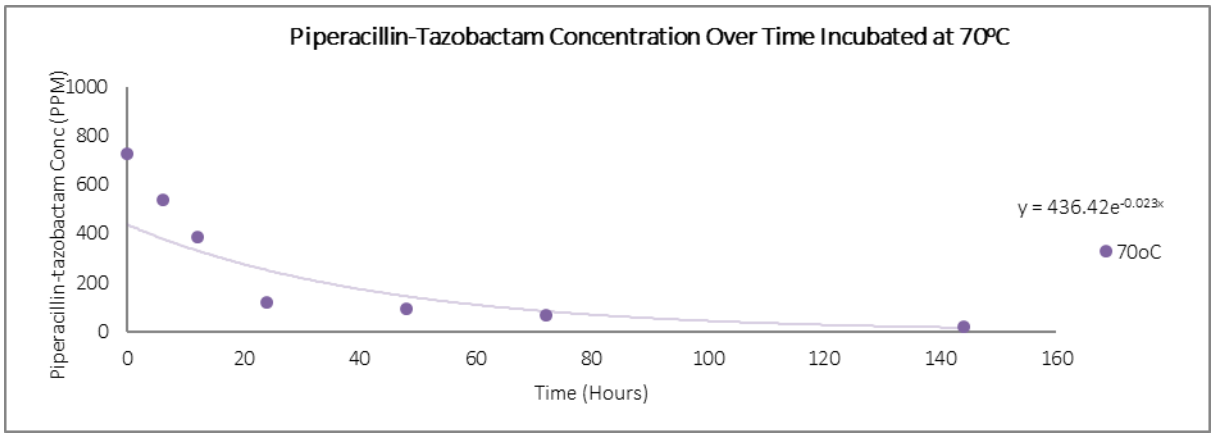

Figure I Showing the decrease in piperacillin-tazobactam concentration when exposed to extreme thermal conditions (70ºC)

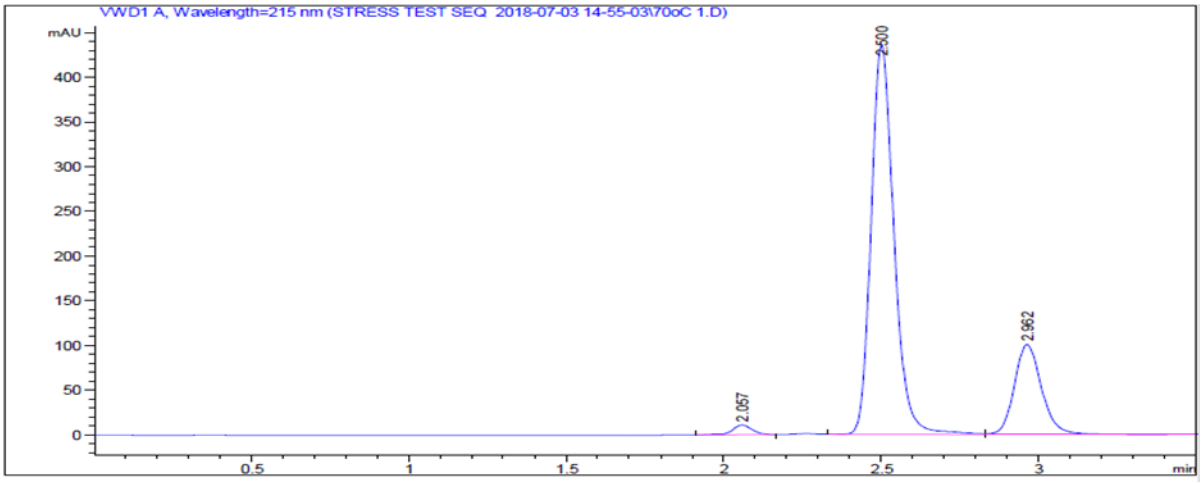

Figure J Showing chromatogram obtained at 0hr for piperacillin tazobactam solution that would be exposed to 70oC

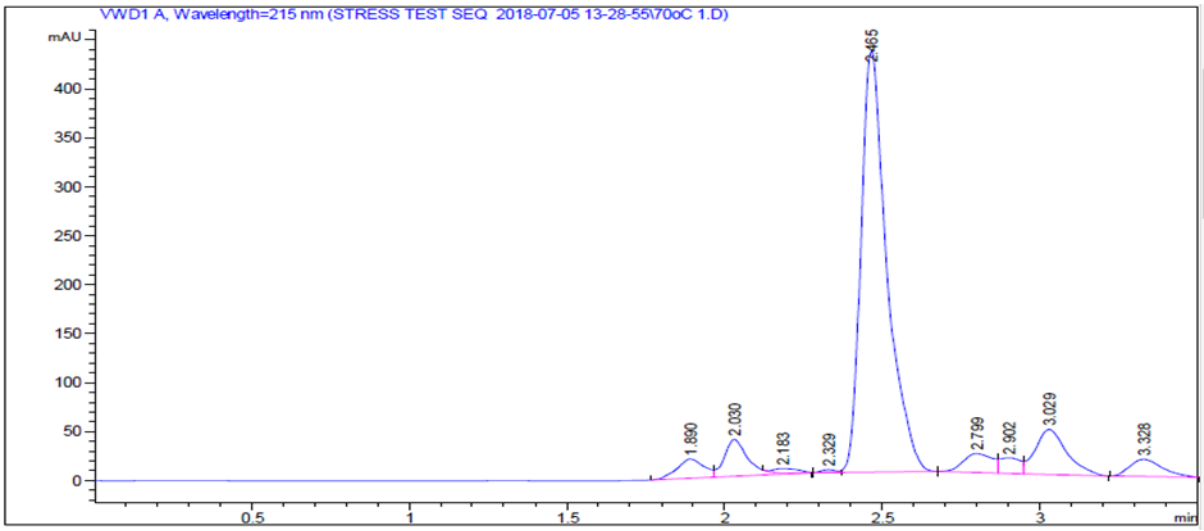

Figure K Showing chromatogram obtained at 72hr for piperacillin tazobactam solution that were exposed to 70oC

A.4.4 Discussion

The forced degradation study was carried out to determine the specificity of the developed stability indicating method. Specificity is a significant factor that determines whether or not an analytical method is stability indicating (381). The ICH Q2B provides guidance on the validation of an analytical method and forced degradation studies performed to prove method specificity (316).

The aim of this study was not to over or under stress piperacillin-tazobactam in solution hence, piperacillin-tazobactam was exposed to stress conditions for 144 hrs to give indicative data of the stability of the molecule.

If too much stress is applied, the parent molecule could possibly lead to the formation of a secondary degradation product; observing unrealistic degradation products that will be unlikely to form in real-world application. This will result in the developed method possibly being unsuitable for the detection of actual degradation products that form during a stability test.

Applying too little stress to the drug substance may not generate sufficient degradation product and some degradation pathways may not be observed; this will therefore not challenge the methods ability to detect and monitor degradation products during stability testing (369).

Acid, Base and Neutral

Hydrolysis reactions are typically acid or base catalysed and are the most common degradation chemical reaction over a range of pH. Hydrolytic study under acidic and basic conditions involves the breakdown of ionisable functional groups present in the molecule. Acidic, basic and neutral conditions were therefore employed to induce all potential hydrolytic reactions (367). Piperacillin is a unique molecule containing an unstable, highly strained and reactive beta-lactam amide bond. The strained bicyclic system should remain in equilibrium with pseudo-piperacillin under natural conditions; hence piperacillin degradation occurs in various environments including: (1) in alkaline or acidic, (2) in the presence of enzyme beta-lactamase or, (3) when treated with weak nucleophiles like water and metal ions (382).

The highly strained ring and its amide bond break open in the presence of acid giving an array of complex products, including penicilloic acid, penicillamine and penilloaldehyde through the highly unstable intermediate. In a strong acidic medium (pH 2 or less), piperacillin-tazobactam undergoes rearrangement through oxazoline formation giving rise to penillic acid (382). Piperacillin concentration was found to rapidly decrease when stressed with an acidic solution. The retention time for the potential degradant is around 2.95 minutes. Around a 21.5% loss of the initial concentration was observed within 6 hours of testing (78.5% of initial concentration remained) and

within 12 hours around a 37% loss of initial concentration was observed (63% of initial concentration remained). Only 5.2% of the initial concentration remained at 144 hours (**Figure C**).

Piperacillin is itself a slightly alkaline antibiotic; however, it has been shown that penicillins are rapidly inactivated in alkaline environments (383). It is known that compounds containing ester functionality, tend to be labile to base hydrolysis (375). In alkaline conditions (pH 7.5-9.0), piperacillin undergoes rapid degradation, where the amide bond opens to give penicilloic acid (382). Piperacillin was found to be extremely labile when stressed with a basic solution. The addition of 0.1M NaOH resulted in total loss of piperacillin (100% loss) (**Figure Bb**). The carboxyl group present on penicilloic acid post bond opening undergoes decarboxylation giving rise to penicilloic acid. The rapid formation of piperacillin degradants (Figure Ob TR = 1.934) leads to total loss of activity (382).

Oxidative

Although acids and bases are the leading catalysts that control pharmaceuticals hydrolytic behaviour, they are not the primary factors in oxidation. One of the most common mechanisms of drug degradation is through oxidative reactions. Two lead molecules that most frequently affect the stability of drug substances are water and molecular oxygen (dioxygen). Three major oxidative pathways for drug degradation exist; (1) radical initiated oxidation, (2) peroxided mediated oxidation and, (3) electron transfer mediated oxidation. The oxidation of piperacillin-tazobactam was caused by the exposure to peroxide H_2O_2 (367). Pharmaceuticals functional groups that are susceptible to oxidation by H_2O_2 include heteroatom (N-oxides and sulfones), benzylic sites, aldehydes, and ketones. Amines, sulphides and phenols are susceptible to electron transfer oxidation to give N-oxides.

When exposing piperacillin-tazobactam to oxidative conditions the temperature at which the solution was stored at was maintained >30^{o}C due to the weak peroxide bond in H_2O_2; the O-O bond cleave at elevated temperatures forming hydroxyl radicals (HO-OH→2HO·). Hydroxyl radicals are much harsher oxidative reagents that would aggressively oxidize piperacillin and tazobactam by unrealistic or non-predictive pathways. Piperacillin-tazobactam was therefore stored under controlled laboratory temperature (24^{o}C). No physical changes in piperacillin-tazobactam solutions were observed with the addition of H_2O_2. The solution remained clear and no cloudiness or precipitation occurred. Throughout the duration of testing no change in colour was observed, this was confirmed using white and black backgrounds.

Since oxidation is second only to hydrolysis as a mode of decomposition, it is essential that the proposed analytical methodology be evaluated for its specificity to piperacillin and tazobactam

oxidative degradants. Formulation and administration approaches utilized to control drug degradation are dependent on the mechanism of oxidation. From results obtained, piperacillin-tazobactam was found to be unstable to oxidative degradation. Under H_2O_2 stress conditions, the desirable level of degradation (10%), was achieved within 30 minutes of experimental initiation. The reaction in 30% H_2O_2 at room temperature occurred rapidly with a loss of 51.88% drug concentration within half an hour (**Figure E**) giving rise to the formation of two degradation products observed at 1.960 and 2.722 minutes (**Figure D**). The resolution in the presence of oxidative degradation product was satisfactory. Within 48 hours a 100% loss in piperacillin-tazobactam concentration was observed.

Photolytic

Understanding the need for photo-stability testing to support the administration of photosensitive pharmaceuticals is an area that is significantly underdeveloped. These studies are important as the exposure to a variety of photochemical conditions could adversely impact the efficiency and safety of the product, both during handling and during administration (376). In some cases, even when the API is known to be photo-stable, it doesn't necessarily mean that the formulation as a whole will be. The literature suggests that degradation in injectable formulation could be promoted by photosensitivity of excipients (384) and diluents.

Photolytic degradation is known to result from exposure of the pharmaceutical compound to ultraviolet or visible light in the wavelength range of 300-800nm (367). A number of presentations and administration routes exist when administration of pharmaceuticals is via injection, each with implications for the possible extent and duration of exposure to photolytic conditions. The risk of exposure to photochemical conditions is substantially lower for a bolus injection reconstituted at the patient's bedside than a large volume injection administered via a slow infusion using a narrow bore, high surface area line over a 12-24hr infusion. These pharmaceuticals that are administered in large volumes require significant manual manipulations and preparation prior to administration (376).

The variability of: (1) clinical practice, (2) light exposure conditions and, (3) administration conditions makes it impossible to cover every possible condition/situation the drug could/will be exposed to (376). Therefore, the photo stability of both piperacillin and tazobactam in solution was studied under two conditions: (1) artificial light (laboratory light) to mimic lighting in hospitals and (2) dark (container covered with light impermeable covering; aluminium foil). Photo-degradation rates are directly dependant on the on the amount of incident radiation the piperacillin-tazobactam solution is exposed to as well as the amount of radiation the solutions absorbed (367).

The photo-stability of piperacillin tazobactam was evaluated to demonstrate that light exposure does not result in unacceptable change. This study allows the analysis of the effects of light on the spectral behaviour of piperacillin tazobactam, so that precautions are taken during the development of the stability indicating analytical method. To minimize the effect of temperature changes during exposure to light and dark, temperature control was considered; the piperacillin tazobactam solutions were placed in the selected storage place in the laboratory (on the bench-side and in dark cupboard) under controlled laboratory temperature (24^{o}C).

Photochemical reactions involve electronically excited states which are formed through absorption of visible light by the molecules, thus generating primary degradants of drug substances. Light stress conditions can induce photo oxidation by free radical mechanism as drugs with functional groups likely to be photosensitive include N-oxide, alkenes, aryl chlorides, weak C-H and O-H bonds, sulphides and polyenes.

The solutions exposed to both light and dark conditions were analysed for any changes in physical properties, such as appearance, clarity, colour of solution and for assay and degradants. Throughout the 144-hour sampling interval, no changes in the drugs physical compatibility were observed for both solutions stored in light and dark conditions. Piperacillin-tazobactam solutions stored in light conditions did not physically display any loss of quality upon the exposure of light.

Chromatograms obtained from the HPLC assay (Figure F and Figure G) showed no changes for both parent compounds in terms of peak shape. The retention times for the parent compounds remained consistent throughout the 144 hours of testing (~2.0 minutes and ~2.9 minutes for tazobactam and piperacillin respectively) without the appearance of a degradation product peak. This indicates that piperacillin and tazobactam are stable in both light and dark conditions for at least 24 hours; this is consistent with information provided by the pharmaceutical manufacture(320)

A decrease in peak area was observed when comparing chromatograms obtained at 0-hour and 72-hour, indicating a loss in drug concentration. Figure H shows the percentage recovery of piperacillin-tazobactam stored in light and dark conditions over the 72 hours. Piperacillin-Tazobactam maintained ≥90% of its initial concentration for 24 hours and 72 hours when stored in light and dark conditions respectively.

Piperacillin-tazobactam would not be considered photosensitive as: (1) it maintained its physical compatibility, (2) there was no sign of a degradation product, and, (3) it maintained 90% of initial concentration for 24 hrs and over for both conditions. Results obtained from this forced degradation study indicate that piperacillin is stable for longer (~3 times longer) when stored in dark conditions

compared to when exposed to light. In the presence of light, strained rings often cleave as photo-reactivity is a frequent occurrence in four membered rings (367).

Thermal

Degradation caused by exposure to temperatures high enough to induce covalent bond breakage is known as thermolytic degradation (367). Thermolytic degradation involves different reactions such as hydrolysis, decarboxylation, isomerization, rearrangement and polymerization (385). The literature reports that cleavage of the beta-lactam amide bond occurs in water when it is subjected to elevated temperatures, however this cleavage is slower than that of beta-lactamase inhibitors (382).

Typically, the rate of reaction increases with an increase in temperature; hence at higher temperatures, drugs are prone to accelerated degradation. As a rule of thumb in most biological and chemical reactions, the reaction rate doubles when the temperature increases every 10^{o}C (367). Kulkarni and Alsante indicated in their study that storing a drug at 30^{o}C for one year is equivalent to incubating the drug at 70^{o}C for three weeks (368). Thermal degradation studies are normally carried out between 40^{o}C and 80^{o}C. The most widely utilized temperature is 70^{o}C as incubating at a temperature higher than 70^{o}C may not produce predictive degradation pathways (385).

The solution exposed to elevated temperature was analysed for any changes in physical properties. Upon preparation, the solution to be stressed was clear and colourless with no signs of cloudiness or precipitation. A slight change in colour (tinged yellow) was observed after 72 hours. The longer the sample was incubated at 70^{o}C the more distinct the colour change became. The visual colour change observed is not reported in the literature, however it is likely due to the degradation process and the formation of degradation product/s. It is known that the beta-lactam ring is unstable and will undergo acid-catalysed hydrolysis, breaking the four membered ring; the rate of this reaction is significantly increased hence could potentially be the cause of the change in the solutions colour. A pungent odour was also noticed once the colour change became apparent. The longer the solution was incubated at 70^{o}C the and the darker the solution became the stronger the pungent odour.

At 72 hours the heat degraded sample produced some degradation peaks at retention times 1.890, 2.183, 2.799 and 3.029 minutes. As a sample degrades, the concentration decreases; in **Figure J** and **Figure K** the chromatograms display a loss in piperacillin concentration (T_R= ~2.9 minutes), whereas the peak area at ~2.0 minutes (T_R of tazobactam) increased, suggesting potential co-elution with a degradant

A.4.5 Conclusion

Forced degradation studies are key for the development of a stability indicating method as they provide knowledge about the degradation chemistry of drug substances/products. This forced degradation study involved subjecting piperacillin-tazobactam to various stress conditions to determine the extent of degradation based on a decrease in the parent component response. The developed stability indicating HPLC method is specific to reveal degradation products and separate them from the parent components. Results obtained suggest that piperacillin-tazobactam stability is dependent on the pH of the solution because it was observed to be extremely labile in acidic and basic solutions. The rate at which piperacillin-tazobactam degraded increased with the increase in temperature. The proposed RP-HPLC method is highly reliable to correlate potency, resistance and storage conditions, indicating and educating healthcare professionals in the safe use and storage of piperacillin tazobactam.

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