Antimicrobial therapy in critically ill patients:
Improving clinical outcomes using a translational pharmacological approach

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Abstract
The University of Manchester
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PhD
Antimicrobial therapy in critically ill patients: improving clinical outcomes using a translational pharmacological approach
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Pulmonary infections in critically ill patients are common, frequently lethal and treatment may be complicated by bacterial resistance. Piperacillin-tazobactam (PTZ) is a broad-spectrum β-lactam antibiotic, frequently used for pulmonary infections. Lung antibiotic concentration reflects target site concentrations in patients with pneumonia. Critically ill patient's exhibit marked pharmacokinetic (PK) variability. PTZ exposures resulting in maximal bacterial killing and prevention of emergence of drug resistance are not known. Administration of PTZ by extended infusions (EI) or using Bayesian dosage optimisation, instead of a fixed bolus regimen, may improve clinical outcomes.

Experimental work was conducted in an in vitro hollow fibre infection model (HFIM) using two densities of Pseudomonas aeruginosa. Experimental data was described by a mathematical model allowing identification of PTZ exposures associated with bacterial killing and suppression of the emergence of resistance. The population PK of PTZ in the plasma and lung of 17 critically ill patients was estimated. Monte Carlo simulation was used to explore the proportion of patients that achieve the plasma and lung PTZ exposures associated with bacterial killing and resistance suppression and to determine the effect of administration schedule. Finally, the population PK of PTZ in the plasma of 146 critically ill patients was estimated and used to construct computer software that can individualise PTZ dosing. Precision of the dosing software was assessed in 8 additional individuals.

At low bacterial density a trough piperacillin:MIC ratio of 3.4 for bolus and 10.4 for EI regimens were able to suppress the emergence of resistance. At higher bacterial density all regimens were associated with growth of a resistant sub-population. Pulmonary piperacillin and tazobactam concentrations were unpredictable and negatively correlated to pulmonary permeability. Simulations revealed that EI, compared with bolus dosing, of PTZ is associated with a higher likelihood of bacteria killing. Similar probability of developing resistance was predicted with PTZ administration by EI and by bolus administration. Performance of the dose optimisation software was satisfactory.

Current PTZ regimens are insufficient to treat pneumonia in ≈14% of critically ill patients. Delivery of PTZ by EI may be a more effective method of administration for some patients with nosocomial infections. Individualised PTZ regimens, delivering a target piperacillin concentration, identified in a HFIM, are achievable and should improved clinical outcomes. Patients with a high bacterial burden may required alternative therapeutic strategies to maximize bacterial killing and prevent antimicrobial resistance.
LAY ABSTRACT

Pneumonia is a frequently fatal disease affecting patients in intensive care units. These patients are commonly treated with piperacillin-tazobactam, a broad spectrum antibiotic that is extensively used in the NHS. Currently all critically ill patients (except those in renal failure) are administered the same dose of piperacillin-tazobactam. However this results in considerable variability in antibiotic concentrations in the blood and lungs. Patients with low concentrations may be at increased risk of not responding to piperacillin-tazobactam or developing resistance to antibiotics. High levels of drugs increase the risk of side-effects. The first part of this study used a laboratory infection model to identify the amount of the drug required to kill bacteria and prevent development of antibiotic resistance. The second part of this study described the concentrations of antibiotic in the blood and lungs of patients in the intensive care unit. Mathematical modelling was used to show that changing from current bolus regimens (were the drug is administered over 30 minutes every 8 hours) to delivering piperacillin-tazobactam by infusion (were the drug is administered over 4 to 8 hours every 8 hours) may cure more patients but does not stop emergence of resistance. Finally computer software was developed which could be used to identify the optimal dose for individual patients associated with clinical cure and suppression of emergence of resistance. Computer simulation demonstrated the reliability of the dosing software.
DECLARATION

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DEDICATION

To Clare, Mia and Eleanor.

Thank you for your support and patience.
ABBREVIATIONS

AIC  Akaike information criterion
AKI  Acute kidney injury
ARC  Augmented renal clearance
ARDS Acute respiratory distress syndrome
AUC  Area under the concentration time curve
BAL  Bronchoalveolar lavage
BMI  Body mass index
Ca   Calcium
CDC  Centre for Disease Control
CFU  Colony forming units
CI   Clearance
CLSI Clinical Laboratory Sciences Institute
Cmax Maximum concentration
Cmin Minimum concentration
COPD Chronic obstructive pulmonary disease
CPIS Clinical pulmonary infection score
CVVH Continuous veno-venous haemofiltration
°C  Degree centigrade
ELF  Epithelial lining fluid
ESBL Extended spectrum β-lactamase
ETA  Endotracheal aspirate
EUCAST European Committee on Antimicrobial Susceptibility Testing
f   Free (unbound)
FiO₂ Fraction of inspired oxygen
T >MIC Fraction of the dosing interval the drug concentration is above the minimum inhibitory concentration
g   grams
H₂O Water
HAP Hospital associated pneumonia
HCAI Healthcare associate infection
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>HELICS</td>
<td>Hospitals in Europe Link for Infection Control through Surveillance</td>
</tr>
<tr>
<td>HFIM</td>
<td>Hollow fibre infection model</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>Hr</td>
<td>Hour</td>
</tr>
<tr>
<td>ICU</td>
<td>Intensive care unit</td>
</tr>
<tr>
<td>Il</td>
<td>interleukin</td>
</tr>
<tr>
<td>IVAC</td>
<td>Infection-related Ventilator-Associated Complication</td>
</tr>
<tr>
<td>kDa</td>
<td>KiloDalton</td>
</tr>
<tr>
<td>kPa</td>
<td>KiloPascal</td>
</tr>
<tr>
<td>L</td>
<td>Litre</td>
</tr>
<tr>
<td>LC/MS/MS</td>
<td>Liquid chromatography tandem mass spectroscopy</td>
</tr>
<tr>
<td>MH</td>
<td>Mueller-Hinton</td>
</tr>
<tr>
<td>MHRA</td>
<td>Medicine and Medicines and Healthcare Products Regulatory Agency</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration</td>
</tr>
<tr>
<td>μm</td>
<td>micrometers</td>
</tr>
<tr>
<td>mL</td>
<td>millilitre</td>
</tr>
<tr>
<td>mm</td>
<td>millimetres</td>
</tr>
<tr>
<td>MRSA</td>
<td>Methicillin-resistant Staphylococcus aureus</td>
</tr>
<tr>
<td>NBL</td>
<td>Non-directed bronchial lavage</td>
</tr>
<tr>
<td>NNIS</td>
<td>National Nosocomial Infections Surveillance</td>
</tr>
<tr>
<td>NPAG</td>
<td>Non-parametric adaptive grid</td>
</tr>
<tr>
<td>OAT</td>
<td>Organic anion transporter</td>
</tr>
<tr>
<td>PBP</td>
<td>Penicillin binding protein</td>
</tr>
<tr>
<td>PD</td>
<td>Pharmacodynamics</td>
</tr>
<tr>
<td>PEEP</td>
<td>Positive end expiratory pressure</td>
</tr>
<tr>
<td>PiO₂</td>
<td>Partial pressure of inspired oxygen</td>
</tr>
<tr>
<td>PK</td>
<td>Pharmacokinetics</td>
</tr>
<tr>
<td>PTA</td>
<td>Probability of target attainment</td>
</tr>
<tr>
<td>PTZ</td>
<td>Piperacillin-tazobactam</td>
</tr>
<tr>
<td>RRT</td>
<td>Renal replacement therapy</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>SOFA</td>
<td>Sequential organ failure assessment</td>
</tr>
<tr>
<td>sTREM-1</td>
<td>Soluble triggering receptor expressed on myeloid cells-1</td>
</tr>
<tr>
<td>TDM</td>
<td>Therapeutic drug monitoring</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra-violet</td>
</tr>
<tr>
<td>VAP</td>
<td>Ventilator associated pneumonia</td>
</tr>
<tr>
<td>Vd</td>
<td>Volume of distribution</td>
</tr>
<tr>
<td>VRE</td>
<td>Vancomycin resistance enterococci</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

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Finally I would like to thank my advisor Professor Ashley Woodcock for his insight and guidance throughout this PhD and the rest of my career.
INTRODUCTION

1. Overview

Infections in critically ill patients are common. At any one time half of all critically ill patients are considered infected while approximately 70% of patients are being treated with antibiotics\(^1\). Despite improvements in the management of infection and sepsis, mortality remains high\(^2\). The mortality of critically ill patients with infection is double that of non-infected patients\(^1\).

The respiratory tract is the most common site of infection in critically ill patients\(^1\). Community acquired pneumonia accounts for 5.9% of patients admitted to United Kingdom (UK) intensive care units (ICU)\(^3\). The respiratory tract accounts for 45.3% of all healthcare associated infections in (HCAI) UK critically ill patients while nosocomial pneumonia occurs in 25.5% of critically ill patients in the United States of America (USA)\(^4,5\).

Piperacillin-tazobactam (PTZ) is a common choice for the management of infection in critically ill patients\(^6\). Approximately a third of all English critically ill patients with respiratory HCAI are treated with PTZ which is more than any other antibiotic\(^4\). Current guidelines recommend PTZ as a first-line choice for the management of critically ill patients with hospital-acquired and ventilator-associated pneumonia\(^7,8\).

Marked physiological changes are seen in critically ill patients as a result of alterations in cardiac output, tissue perfusion, end-organ dysfunction, capillary leakage, hypoalbuminaemia and underlying co-morbidities\(^9\). These physiological changes can alter the volume of distribution and clearance of antimicrobial agents, such as PTZ\(^10,11\). This PK variability results in a wide spectrum of observed drug concentration in the plasma and tissue of critically ill patients\(^12\). Low antimicrobial agent concentrations
may be associated with therapeutic failure and emergence of antimicrobial resistance while high antimicrobial agent concentrations increase the risk of drug toxicity\textsuperscript{13}.

Pulmonary infections in critically ill patients are caused by a wide range of organisms, including difficult-to-treat organisms such as \textit{P. aeruginosa}\textsuperscript{14}. \textit{P. aeruginosa} is isolated from as many as 25\% of critically ill patients with respiratory tract infections\textsuperscript{1,4}. The identification of \textit{P. aeruginosa} in a critically ill patient has been shown to be associated with an increase in ICU mortality\textsuperscript{15}. Treatment of patients with \textit{P. aeruginosa} may be complicated by antimicrobial resistance\textsuperscript{16}. A 20\% increase in mortality is observed in critically ill patients with resistant strains of \textit{P. aeruginosa}\textsuperscript{16}.

2. \textbf{Ventilator associated pneumonia}

The clinical classification of pneumonia depends on whether the pulmonary infection developed in an inpatient or outpatient setting\textsuperscript{17}. Community acquired pneumonia occurs in outpatients\textsuperscript{17,18}. Nosocomial pneumonia includes hospital acquired pneumonia, ventilator associated pneumonia (VAP) and healthcare associated pneumonia\textsuperscript{8}. Hospital acquired pneumonia is a respiratory infection occurring at least 48 hours after hospital admission\textsuperscript{7}. VAP is a severe respiratory infection occurring at least 48 hours after the initiation of mechanical ventilation via endotracheal tracheal intubation\textsuperscript{8}. Finally healthcare associated pneumonia is a respiratory infection occurring in community nursing home patients and is caused by a spectrum of pathogens more commonly encountered in hospitals rather than the community\textsuperscript{14}.
2.1. Diagnosis of VAP

Conceptually, VAP is the presence intra-alveolar neutrophils, resulting in diffuse consolidation of bronchioles and adjacent alveoli, caused by one or more infectious pathogens not present at the time of intubation. The presence of infectious pathogen in the alveolus results in an inflammatory response causing inflammatory cells to enter the lung but also manifest systemically with changes to body temperature and white cell count in the blood. Histopathology, with detection of alveolar pathogen and neutrophils, is used as the “gold standard” for validation of diagnostic scoring systems and potential biomarkers\(^\text{19}\).

Histopathology is not suitable as a routine diagnostic tool due to the risk associated with biopsy. Identification of patients with VAP is commonly based on evaluation of clinical, radiographic and microbiological features. Typical clinical criteria for commencing antibiotics are the presence of new or progressive radiographic infiltrates on chest radiograph associated with: either a core body temperature of \(>38.5^\circ\text{C}\) or \(<36.5^\circ\text{C}\), white cell count \(>1.0\times10^9/\text{L}\) or \(<15\times10^9/\text{L}\) or purulent tracheal secretions\(^\text{20}\). The diagnostic accuracy of diagnosing VAP using clinical criteria is poor. Only 42% of patients, who have been ventilated for more than 48 hours and develop fever with pulmonary infiltrates on plain chest X-ray, have developed a VAP\(^\text{21}\). Adult respiratory distress syndrome, ventilator-associated tracheobronchitis, congestive cardiac failure, trauma, surgery, deep vein thrombosis, pancreatitis, pulmonary embolism, pulmonary infarction, atelectasis, sinusitis, catheter-related blood stream infection and urinary tract infection either alone or in combination have been shown to masquerade as VAP\(^\text{20,21}\).
2.1.1. **Scoring systems**

A number of scoring systems have been developed to aid diagnosis of VAP. Scoring systems are employed: (a) to aid clinical management of critically ill patients, (b) for surveillance of VAP so that the incidence may be compared between populations, (c) as a quality assurance tool, particularly in the USA and (d) as part of diagnostic and treatment clinical trials. Scoring systems include the Clinical Pulmonary Infection Score (CPIS)\textsuperscript{22,23}, Hospitals in Europe Link for Infection Control through Surveillance (HELICS) criteria\textsuperscript{24} and the Centre for Disease Control’s National Nosocomial Infections Surveillance (CDC NNIS) system\textsuperscript{25}. The brevity of the CPIS makes it clinically useful at the bedside. The HELICS criteria and the CDC NNIS are designed to be used as surveillance tools and are difficult to implement in the clinical arena. The CPIS and HELICS criteria have been used as inclusion criteria and outcome measures in clinical trials.

2.1.1.1. **Clinical Pulmonary Infection Score**

CPIS has been developed to improve the utility of clinical diagnosis for patients with VAP (Table 1)\textsuperscript{22,23}. Using only the clinical component of the CPIS has been shown not to improve the accuracy of clinical diagnosis\textsuperscript{26}. However using a CPIS score >6, which includes a microbiological element, has been shown to have a sensitivity of 95% and specificity of 85% in a post-mortem study comparing diagnosis of VAP with histological diagnosis\textsuperscript{27}. A meta-analysis of 23 clinical studies using CPIS > 6 to diagnose VAP found the score to have a sensitivity of between 22 to 93% and a specificity of 45 to 100\%\textsuperscript{28}.
### Table 1. Clinical Pulmonary Infection Score.

<table>
<thead>
<tr>
<th>Score</th>
<th>Temp (°C)</th>
<th>Blood leukocytes (mm$^3$)</th>
<th>Airway secretions</th>
<th>PiO$_2$/FiO$_2$ (kPa)</th>
<th>Chest radiograph</th>
<th>Microbiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&gt;36.0 to &lt;38.4</td>
<td>&gt;4000 To &lt;11000</td>
<td>No secretions</td>
<td>&gt;33 or ARDS</td>
<td>No infiltrate</td>
<td>No growth</td>
</tr>
<tr>
<td>1</td>
<td>&gt;38.5 to &lt;38.9</td>
<td>&lt;4000 Or &gt;11000</td>
<td>Non-purulent</td>
<td>&lt;33 without ARDS</td>
<td>Patchy, diffuse infiltrate</td>
<td>Moderate or heavy growth</td>
</tr>
<tr>
<td>2</td>
<td>&lt;36.0 or &gt;39.0</td>
<td>Purulent</td>
<td></td>
<td></td>
<td>Localised infiltrate</td>
<td></td>
</tr>
</tbody>
</table>
2.1.1.2. Hospitals in Europe Link for Infection Control through Surveillance criteria

The HELICS criteria is outlined in Table 2\textsuperscript{24}. Similar to CPIS, HELICS combines a radiological, clinical and microbiological assessment of patients with suspected VAP.

There are no studies comparing the HELICS criteria with pathological studies to establish the sensitivity and specificity of the HELICS criteria. A significant criticism of the HELICS criteria is the subjective nature of the clinical component.

2.1.1.3. Centre for Disease Control’s National Nosocomial Infections Surveillance system

The CDC NNIS system has recently been updated with previous versions of the system criticised for being too subjective\textsuperscript{25}. The current version of the CDC NNIS system does not include a radiological assessment. Comparison of the previous versions of the CDC NNIS system and the CPIS shows poor concordance\textsuperscript{29}.
Table 2. Hospitals in Europe Link for Infection Control through Surveillance criteria.

<table>
<thead>
<tr>
<th>Radiology</th>
<th>Two or more serial chest X-rays or CT-scans with a suggestive image of pneumonia for patients with underlying cardiac or pulmonary disease. In patients without underlying cardiac or pulmonary disease one definitive chest X-ray or CT-scan is sufficient.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>and at least one of the following</td>
</tr>
<tr>
<td>Symptoms</td>
<td>• Fever &gt; 38 °C with no other cause</td>
</tr>
<tr>
<td></td>
<td>• Leukopenia (&lt;4,000 WBC/mm3) or leukocytosis (≥12,000 WBC/mm3)</td>
</tr>
<tr>
<td></td>
<td>and at least one of the following</td>
</tr>
<tr>
<td></td>
<td>• New onset of purulent sputum, or change in character of sputum</td>
</tr>
<tr>
<td></td>
<td>• Cough or dyspnoea or tachypnoea</td>
</tr>
<tr>
<td></td>
<td>• Suggestive auscultation</td>
</tr>
<tr>
<td></td>
<td>• Worsening gas exchange</td>
</tr>
<tr>
<td>Microbiology</td>
<td>and according to the used diagnostic method</td>
</tr>
<tr>
<td>Bacteriologic diagnostic performed by:</td>
<td>• Positive quantitative culture from minimally contaminated LRTI specimen</td>
</tr>
<tr>
<td></td>
<td>• Bronchoalveolar lavage with a threshold of &gt;10^4 CFU/ml or ≥ 5% of BAL obtained cells contain intracellular bacteria on direct microscopic exam</td>
</tr>
<tr>
<td></td>
<td>• Protected brush with a threshold of &gt;10^3 CFU/ml</td>
</tr>
<tr>
<td></td>
<td>• Distal protected aspirate with a threshold of &gt;10^3 CFU/ml</td>
</tr>
<tr>
<td></td>
<td>• Positive quantitative culture from possibly contaminated LRT specimen</td>
</tr>
<tr>
<td></td>
<td>• Quantitative culture of LRT specimen with a threshold of 10^6 CFU/ml</td>
</tr>
<tr>
<td>Alternative microbiology methods:</td>
<td>• Positive blood culture not related to another source of infection</td>
</tr>
<tr>
<td></td>
<td>• Positive growth in culture of pleural fluid</td>
</tr>
<tr>
<td></td>
<td>• Pleural or pulmonary abscess with positive needle aspiration</td>
</tr>
<tr>
<td></td>
<td>• Histological pulmonary exam shows evidence of pneumonia</td>
</tr>
<tr>
<td></td>
<td>• Positive exams for pneumonia with virus or particular germs</td>
</tr>
<tr>
<td></td>
<td>• Positive detection of viral antigen or antibody from respiratory secretions</td>
</tr>
<tr>
<td></td>
<td>• Positive direct exam or positive culture from bronchial secretions or tissue</td>
</tr>
<tr>
<td></td>
<td>• Sero-conversion</td>
</tr>
<tr>
<td></td>
<td>• Detection of antigens in urine</td>
</tr>
</tbody>
</table>
### Table 3. Centre for Disease Control’s National Nosocomial Infections Surveillance system.

<table>
<thead>
<tr>
<th><strong>Patient</strong></th>
<th><strong>Centre for Disease Control’s National Nosocomial Infections Surveillance system.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline Condition</strong></td>
<td>Patient has a baseline period of stability or improvement on the ventilator, defined by ≥ 2 calendar days of stable or decreasing FiO2 or PEEP.</td>
</tr>
</tbody>
</table>
| **Stable or Improvement** | After a period of stability or improvement on the ventilator, the patient has at least one of the following indicators of worsening oxygenation:  
1) Increase in FiO2 for ≥ 2 calendar days.  
2) Increase in PEEP for ≥ 2 calendar days |
| **Ventilator-Associated Condition** | On or after calendar day 3 of mechanical ventilation and within 2 calendar days before or after the onset of worsening oxygenation, the patient meets both of the following criteria:  
1) Temperature > 38°C or < 36°C OR white blood cell count ≥ 12,000 cells/mm³ or ≤ 4,000 cells/mm³  
2) A new antimicrobial agent(s) is started, and is continued for ≥ 4 calendar days. |
| **Infection-related Ventilator-Associated Complication** | Possible Ventilator-Associated Pneumonia: On or after calendar day 3 of mechanical ventilation and within 2 calendar days before or after the onset of worsening oxygenation, ONE of the following criteria is met:  
1) Purulent respiratory secretions  
2) Positive culture (qualitative, semi-quantitative or quantitative) of sputum, endotracheal aspirate, bronchoalveolar lavage, lung tissue, or protected specimen brushing  
Probable Ventilator-Associated Pneumonia: On or after calendar day 3 of mechanical ventilation and within 2 calendar days before or after the onset of worsening oxygenation, ONE of the following criteria is met:  
1) Purulent respiratory secretions AND one of the a positive semi-quantitative culture of endotraheal aspirate, bronchoalveolar lavage, lung tissue or protected specimen brush  
2) Either positive pleurad fluid culture, positive lung histopathology, positive diagnostic test for *Legionella* spp. or positive diagnostic test on respiratory secretions for influenza virus, respiratory syncytial virus, adenovirus, parainfluenza virus |

#### 2.1.2. Microbiological diagnosis

Microbiological diagnosis of VAP is complicated by early colonisation of the upper respiratory tract and endotracheal tube. Contamination of samples with pathogen from the upper airways reduces the utility of microbiological sampling such as endotracheal aspiration (ETA). The specificity of endotracheal aspirates has been shown to be as low as 27%. In order to
improve the value of airway microbiological sampling and reduce the impact of upper airway colonisation, both quantitative culture and protect airway sampling has been suggested. Protected airway sampling techniques include conventional bronchoalveolar lavage (BAL), protected specimen brushes and non-directed bronchial lavage. Comparison of microbiological techniques, with histological diagnosis as a gold standard, reveals them to all have diagnostic yields with sensitivity and specificity ranging from 43% to 83% and 67% to 91% respectively \(^{28}\). Comparison of two of the most commonly used techniques, BAL with quantitative culture and ETA with non-quantitative culture, suggests the two techniques result in similar clinical outcomes and antibiotic use \(^{32}\).

2.1.3. **Biomarkers**

A number of biomarkers have been suggested to aid diagnosis of VAP. To date these have not been validated and are not currently utilised within any of the diagnostic scoring systems. The most promising markers are interleukin (IL)-1\(\beta\), IL-8, procalcitonin and soluble triggering receptor expressed on myeloid cells-1 (sTREM-1). Identification of IL-1 and IL-8 in BAL fluid has been shown to distinguish patients with VAP from those who do not have VAP \(^{33}\). Procalcitonin has been shown to be significantly increased in the serum of patients with VAP compared with patients without VAP \(^{34}\). Soluble TREM-1 has been shown to be raised in the BAL of patients with VAP \(^{35}\). The implementation of these tests has been limited by the significant rate of false positives. Further studies are ongoing.

2.2. **Epidemiology of VAP**

Respiratory infections are the most common cause of infection in patients in the ICU accounting for 64% of all infections \(^{1}\). The overall incidence of VAP varies regionally but is between 9 and 22.8% \(^{14,36-38}\). The risk of
acquiring VAP is highest in the first week and subsequently drops as ICU stay is prolonged\textsuperscript{37}. As a result of acquiring VAP, a patients ICU stay is extended from 6.0 to approximately 8.4 days\textsuperscript{39,40}.

2.3. **Mortality, morbidity and associated healthcare costs**

The crude mortality of patient with VAP is approximately 30\%\textsuperscript{14}. The excess mortality attributed to VAP, which takes into account the mortality of the underlying diagnosis, is estimated to be 13\%\textsuperscript{41}. Although in specific subgroups the excess mortality may be as high as 69\%\textsuperscript{41–43}. The odds ratio for mortality for patients who acquire VAP, compared to those patients without VAP, is 1.3-2.0\textsuperscript{39,44} Overall the healthcare cost for a critically ill patient with VAP is approximately £8,000-£24,000 more than the healthcare cost of patients without VAP\textsuperscript{39,40}. This increase in cost is largely due to increasing length of stay on the ICU\textsuperscript{39,40}.

2.4. **Risk factors for developing VAP**

A number of factors have been associated with an increased risk of development of VAP\textsuperscript{20,37,45,46}. These are summarised in Table 4.
Table 4. Table of risk factors for developing VAP.

<table>
<thead>
<tr>
<th>Host factors</th>
<th>Intervention factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>• ARDS</td>
<td>• Bronchoscopy</td>
</tr>
<tr>
<td>• COPD</td>
<td>• Duration of hospital stay</td>
</tr>
<tr>
<td>• Gastric colonization</td>
<td>• Duration of ICU stay</td>
</tr>
<tr>
<td>• Hypoalbuminaemia</td>
<td>• Emergency intubation</td>
</tr>
<tr>
<td>• Impaired consciousness</td>
<td>• Frequent ventilator circuit changes</td>
</tr>
<tr>
<td>• Immunosuppression</td>
<td>• Multiple venous line insertions</td>
</tr>
<tr>
<td>• Large-volume gastric aspiration</td>
<td>• Sedatives</td>
</tr>
<tr>
<td>• Old age (≥60 years)</td>
<td>• Nasogastric tube</td>
</tr>
<tr>
<td>• Organ failure</td>
<td>• PEEP</td>
</tr>
<tr>
<td>• Oro-pharyngeal colonization</td>
<td>• Prior antibiotics</td>
</tr>
<tr>
<td>• Post-surgical</td>
<td>• Re-intubation</td>
</tr>
<tr>
<td>• Presence of co-morbidities</td>
<td>• Stress ulcer prophylaxis</td>
</tr>
<tr>
<td>• Severity of underlying illness</td>
<td>• Supine head position</td>
</tr>
<tr>
<td>• Sinusitis</td>
<td>• Transport out of the ICU</td>
</tr>
<tr>
<td>• Thermal injury (Burns)</td>
<td></td>
</tr>
<tr>
<td>• Trauma</td>
<td></td>
</tr>
</tbody>
</table>

2.5. **Pathogenesis of VAP**

The respiratory tract is protected from infection by a variety of defence mechanisms which include: (a) filtration and humidification of air in the upper airways; (b) anatomic barriers, such as the glottis and larynx; (c) cough reflexes; (d) tracheobronchial secretions; (e) mucociliary epithelium; (f) cell-mediated (involving both alveolar macrophages and neutrophils) and (g) humoral immunity. Following placement of the endotracheal tube there is localised injury to the mucosa of the respiratory tract near the cuff and mucociliary clearance is dramatically impaired. The upper airway, glottis and larynx are bypassed by the endotracheal tube. Colonisation of the oropharynx occurs with pooling of secretions around the cuff of the endotracheal tube. Organisms enter the lung following micro-aspiration from the oropharynx into the proximal airway. Pneumonia develops as a result of a defect in the remaining host defences, invasion by a particularly virulent pathogen or inoculum of organism with sufficient density to overwhelm the remaining host defence.
2.6. **Aetiology of VAP**

*Staphylococcus aureus* and *P. aeruginosa* are the two most common causes of VAP\(^{14,48}\). *Klebsiella, Enterobacter, Acinetobacter* species and *Escherichia coli* are each responsible for between approximately 5-15% of cases\(^{49,50}\). Most bacteria typically produce VAP after approximately 2 days of intubation while *Stenotrophomonas maltophilia* and *P. aeruginosa* are typically isolated from patients with VAP that develops after 6 days\(^{51}\). There is marked geographical variation in the spectrum of organisms responsible for VAP with *Klebsiella* or *Acinetobacter* species being the most frequent cause VAP in some Asian countries\(^{48,52}\). The spectrum of organisms differs from that found to be responsible for either community or hospital-acquired pneumonia with *Streptococcus pneumonia* and *Haemophilus influenza* being much less common in VAP\(^{14,48}\).

<table>
<thead>
<tr>
<th>Causative organism</th>
<th>Percentage of VAP cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SENTRY(^{46})</td>
</tr>
<tr>
<td></td>
<td>USA only</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> (of which MRSA)</td>
<td>31.9</td>
</tr>
<tr>
<td><em>Pseudomonas</em> species</td>
<td>21.4</td>
</tr>
<tr>
<td><em>Klebsiella</em> species</td>
<td>6.6</td>
</tr>
<tr>
<td><em>Enterobacter</em> species</td>
<td>8.8</td>
</tr>
<tr>
<td><em>Acinetobacter</em> species</td>
<td>5.3</td>
</tr>
<tr>
<td><em>Streptococcus pneumonia</em>/<em>Haemophilus influenzae</em></td>
<td>6.6</td>
</tr>
</tbody>
</table>

Antimicrobial resistance is more commonly seen in Gram negative organisms causing VAP compare to those responsible for hospital acquired pneumonia (HAP)\(^{48}\). Resistance to commonly used antibiotics (PTZ, meropenem and ceftazidime) is found in approximately half of all *S. aureus*,
a third of all *P. aeruginosa* and *Klebsiella* species and 80% of *Acinetobacter* species causing VAP\(^4^8\). Rates of susceptibility are reducing over time as illustrated by a ≥5% drop in susceptibility in *Klebsiella* species to new antibiotics such levofloxacin, cefepime, ceftazidime, meropenem, doripenem and tigecycline in the last ten years\(^4^8\).

2.6.1. *Pseudomonas aeruginosa*

*P. aeruginosa* is a common cause of nosocomial infection including VAP and, for this reason was the organism used in the in-vitro PK-PD study\(^1^4,4^8,5^3\). *P. aeruginosa* is a ubiquitous motile aerobic Gram-negative rod which is an important pathogen of patients with Cystic Fibrosis, the critically ill and immunocompromised hosts, especially following bone marrow or lung transplantation\(^5^4-5^6\). Choice of antimicrobial agent is crucial with mortality reducing from 30.7% to 17.8% with appropriate initial treatment of Pseudomonas bacteraemia\(^5^7\). Mortality in patients with Pseudomonas pneumonia is similar when patients are treated with either appropriate monotherapy or combination therapy\(^5^8\). Combination therapy improves the chances of using appropriate monotherapy before antibacterial susceptibility is known. Over the last 20 years *P. aeruginosa* shows increasing rates of antimicrobial resistance\(^5^9\). Rates of resistance are highest in organisms isolated from patients in the ICU\(^6^0\). Pre-clinical data shows that combination therapy with two appropriate antibiotics is superior at suppressing the emergence of resistance when compared with monotherapy\(^6^1\).

*Pseudomonas aeruginosa* demonstrates a number of intrinsic resistance mechanisms which impact on the efficacy of a wide range of structurally unrelated antimicrobial agents\(^6^2\). Three key mechanisms predominate: (a) productions of β-lactamases; (b) up-regulation of efflux pumps which move
intra-cellular molecules into the extra-cellular space and (c) loss of outer membrane proteins with associated changes to membrane permeability. Other mechanisms include mutational gyrases and other deactivating enzymes. Additionally *P. aeruginosa* may acquire resistance both through genetic transfer and spontaneous mutation. Multiple resistance mechanisms may be present within a single multi-drug resistant strain.

2.7. **Pharmacological management of VAP**

Critically ill patients diagnosed with VAP are typically treated with one or two empirical broad-spectrum antimicrobial agents due to the time required to culture and identify causative organisms. The choice of antimicrobial agents takes into account local patterns of causative organisms and resistance and may be followed by conversion to a narrower spectrum agent following microbiological culture results. Delaying instigation of treatment in patients with infection is associated with a worse clinical outcome making the correct choice of antibiotic imperative. Due to the high incidence of difficult-to-treat organisms, such as *P. aeruginosa*, and organisms harbouring resistance mechanisms, current guidelines favour antimicrobial agents with activity against these organisms. Early-onset VAP, where the risk of a patient acquiring *P. aeruginosa* or a resistant organism is low, may be treated with an agent that lacks anti-pseudomonal activity. The recommended antimicrobial agents to treat VAP are shown in Table 6.

All antimicrobial agents used for the treatment of patients with VAP have been investigated in non-inferiority studies. As a result no agent has shown superior outcomes for patients with VAP. The only exception to this is the superiority of linezolid over vancomycin for the treatment of VAP caused by MRSA.
A number of strategies have been suggested to improve outcome from VAP. The combination of antimicrobial agents does not appear to improve clinical outcome with meropenem or meropenem and ciprofloxacin resulting in the same 28 day mortality\textsuperscript{70}. However, this study did have a relatively low number of drug-resistant organisms and was not powered adequately to identify whether suppression in emergence of antimicrobial resistance was possible. Meta-analysis of all empirical treatment trials for VAP suggests the mortality and treatment failure rate is the same whether one or more drugs are used\textsuperscript{70}. Delivery of β-lactam antibiotics by infusion may improve their efficacy in patients with VAP and is being adopted by some centres\textsuperscript{71,72}. 
Table 6. Table showing the current guidelines for empirical treatment of VAP.

<table>
<thead>
<tr>
<th>Society</th>
<th>Early-onset VAP</th>
<th>Late-onset VAP or high-risk of resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>British Society of Antimicrobial Chemotherapy⁷</td>
<td>- Amoxicillin/clavulanate</td>
<td>- Cefotaxime</td>
</tr>
<tr>
<td></td>
<td>- Cefuroxime</td>
<td>- Ceftriazone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Ciprofloxacin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Meropenem</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Piperacillin/tazobactam</td>
</tr>
<tr>
<td>American Thoracic Society &amp; Infectious Diseases Society of America⁸</td>
<td>- Ampicillin/sulbactam</td>
<td>- Cefepime</td>
</tr>
<tr>
<td></td>
<td>- Ceftriazone</td>
<td>- Ceftazidime</td>
</tr>
<tr>
<td></td>
<td>- Ciprofloxacin</td>
<td>- Imipenem/cilastatin</td>
</tr>
<tr>
<td></td>
<td>- Ertapenem</td>
<td>- Meropenem</td>
</tr>
<tr>
<td></td>
<td>- Levofloxacin</td>
<td>- Piperacillin/tazobactam plus</td>
</tr>
<tr>
<td></td>
<td>- Moxifloxacin</td>
<td>- Amikacin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Ciprofloxacin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Gentamicin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Levofloxacin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Tobramycin</td>
</tr>
<tr>
<td>European Respiratory Society &amp; European Society of Clinical Microbiology &amp; Infectious Diseases &amp; European Society of Intensive Care Medicine⁶⁷</td>
<td>- Amoxicillin/clavulanate</td>
<td>- Cefazidime</td>
</tr>
<tr>
<td></td>
<td>- Ampicillin/sulbactam</td>
<td>- Imipenem/cilastatin</td>
</tr>
<tr>
<td></td>
<td>- Cefotaxime</td>
<td>- Meropenem</td>
</tr>
<tr>
<td></td>
<td>- Ceftriazone</td>
<td>- Piperacillin/tazobactam plus</td>
</tr>
<tr>
<td></td>
<td>- Cefuroxime</td>
<td>- Ciprofloxacin</td>
</tr>
<tr>
<td></td>
<td>- Levofloxacin</td>
<td>- Gentamicin</td>
</tr>
<tr>
<td></td>
<td>- Moxifloxacin</td>
<td>- Levofloxacin</td>
</tr>
</tbody>
</table>

Shorter courses of antibiotics are generally as effective as longer courses with no increase in mortality observed between 8-day and 15-day treatment courses⁷³. However a higher recurrence rate was observed in the shorter course group. The use of procalcitonin to guide length of treatment has been shown to reduce the overall length of time patients with VAP receive antimicrobial therapy⁷⁴,⁷⁵.
In patients with VAP both an inappropriate choice of antimicrobial agent or delayed administration of treatment is associated with a worse clinical outcome\textsuperscript{76–78}. Failure of treatment occurs in 20 to 40% of patients treated for VAP\textsuperscript{67,79}. This is due to a combination of mis-diagnosis of VAP, incorrect choice of antimicrobial agent and inadequate dosing due to marked PK variability, seen in critically ill patients.

2.8. Prevention of VAP

A number of strategies have been trialled to prevent the occurrence of VAP. Placing patients in the semi-recumbent position reduces the incidence of VAP, potentially by reducing aspiration of gastric contents\textsuperscript{80}. The use of either selective oral (with 2% chlorhexidine) and digestive tract decontamination (with antibiotics) may also reduce the incidence of VAP\textsuperscript{81}. A number of alterations to the ventilator circuit and endotracheal tube also reduce the rate of VAP. These include the use of heat and moisture exchange filters, rather than heat humidifiers, and subglottic drainage\textsuperscript{82,83}.

A growing number of prevention strategies has led to the development of care bundles for the prevention of VAP which includes: (a) no changes to ventilator circuit tubing; (b) strict hand hygiene; (c) staff education and training; (d) sedation holds and weaning protocol and (e) improved oral care\textsuperscript{84}. Implementation of VAP prevention protocols has reduced the incidence of VAP\textsuperscript{85–88}. 
3. Pharmacokinetic and pharmacodynamic principles: Focus on critically ill patients

3.1. Pharmacokinetics: An overview

Pharmacokinetics (PK) describes the changes with time of a drug’s concentration within the body and includes both plasma and tissue concentrations. The PK of any drug is determined by its absorption, distribution, metabolism and excretion. Tissue concentrations of an antimicrobial agent may reflect the target site concentration at the site of a specific infection (e.g. intra-pulmonary concentration in patients with pneumonia). The volume of distribution (Vd) of a drug, clearance (Cl) and tissue distribution are all crucial to identifying whether the administered dose of an antimicrobial agent will result in an effective concentration at the site of infection. The Vd is the apparent volume (typically in litres) in the patient which relates dose with the observed plasma drug concentration. Clearance is the volume of plasma that is completely cleared of a drug per unit time (typically in litres/hour).

A drug’s PK can be described using a mathematical model fitted to observed drug concentrations from a patient or cohort of patients. The simplest model is a “one-compartment” model which includes two parameters: Vd and Cl. To adequately describe the observed data it may be necessary to increasing the complexity of the mathematical models by adding further compartments. For each additional compartment rate constants are required to describe the flow of drug between compartments. Population PK models are derived from a cohort of patients and estimate the mean, median and standard deviation for each of the parameters (Vd, Cl) allowing assessment of the inter-individual variability.
3.2. **Pharmacokinetic considerations in critically ill patients**

Critically ill patients demonstrate marked PK variability of antimicrobial agents often resulting from physiological derangement such as end-organ dysfunction, capillary leakage, hypoalbuminaemia and alterations in cardiac output and tissue perfusion. The variability in drug exposure resulting from currently marketed antimicrobial agent regimens means that critically ill patients may receive sub-optimal regimens.

### 3.2.1. **Volume of distribution**

Changes in the Vd of antimicrobial agents results from critical illness-related pathophysiology and consequent medical interventions such as large volume fluid resuscitation. Critically ill patients demonstrate a Vd for hydrophilic agents such as β-lactams, aminoglycosides, glycopeptides, linezolid and colistin, which may be double the Vd observed in non-critically ill patients. These hydrophilic compounds typically exhibit a low Vd but administration of standard doses to critically ill patients results in lower concentrations than in non critically ill individuals. Lipophilic agents, such as fluoroquinolones and macrolides, have a larger Vd which is less affected by capillary leak and extra-vascular fluid observed with hydrophilic compounds.

Hypoalbuminemia increases the Vd of both aminoglycosides and highly protein bound β-lactams such as flucloxacillin and ceftriaxone. A reduction in albumin concentration results in an increase in the unbound fraction of antimicrobial agent which in turn increases the amount of drug available for both distribution and elimination.

Extra-corporeal circuits, such as extra-corporeal membrane oxygenation and renal replacement therapy, alter the Vd of some antimicrobial agents.
This may be due to drug adsorption to circuit materials. This has been shown with gentamicin and voriconazole, which bind avidly to extra-corporeal circuits, resulting in a reduction in plasma antimicrobial agent concentrations. The plasma concentrations of other antimicrobial agents, such as caspofungin, are unaffected by the presence of an extra-corporeal circuits\textsuperscript{112–115}. 

3.2.2. Clearance

Clearance of antimicrobial agents may be due to metabolism and/or excretion. CI is altered in critically ill patients primarily due to changes in function of the eliminating organ (e.g. kidneys, liver, biliary tract)\textsuperscript{94,103–107,116}. Many commonly used antimicrobial agents are dependent on renal clearance mechanisms with changes in renal function affecting concentrations of antimicrobial agents\textsuperscript{117–122}. Acute kidney injury (AKI) is a rapid, progressive loss of renal function which results in elevation of serum creatinine concentrations, reduction in urinary creatinine clearance and a reduction in urine output\textsuperscript{123}. Renally-cleared antimicrobial agent concentrations are typically higher in patients with AKI necessitating a decrease in dose. Dose reduction will maintain drug exposures within the therapeutic window and reduce the risk of toxicity.

Low antimicrobial agent concentrations have been demonstrated in patients with sepsis due to increased glomerular filtration. This phenomenon, named “Augmented Renal Clearance” (ARC), occurs as a result of increased renal perfusion due to increased cardiac output and reduced systemic vascular resistance associated with a systemic inflammatory response\textsuperscript{124}. The clearance of some antimicrobial agents has been shown to triple. Approximately 80% of patients with ARC will not achieve
therapeutic concentrations of antimicrobial agents$^{13,124}$. ARC occurs most commonly in younger males with sepsis, burns, trauma or pancreatitis$^{125}$.

Hypoalbumenia, which causes an increase in the unbound plasma fraction of a drug, may result in supra-normal clearance of highly protein bound agents, such as flucloxacillin and ceftriaxone$^{109,126}$.

Patients with severe AKI are frequently managed with renal replacement therapy (RRT). RRT, including continuous veno-venous haemofiltration and intermittent haemodialysis, is an effective clearance mechanism for many hydrophilic antimicrobial agents$^{127–132}$. Dose reduction in patients requiring RRT leads to up to 50% of patients not achieving therapeutic concentrations of antimicrobial agents such as β-lactam antibiotics, vancomycin and ciprofloxacin$^{133,134}$. There is a lack of studies comparing the effects of different modes and settings of RRT on drug clearance. Elimination of lipophilic drugs and highly protein bound agents by RRT is poor$^{135}$.

Hepatic dysfunction in critically ill patients results in a reduction in the metabolism and clearance of antimicrobial agents whose Cl is dependent on metabolism by the liver or those agents that undergo trans-intestinal clearance$^{136,137}$. Antimicrobial agents whose elimination is primary hepatic and whose clearance is reduced in hepatic dysfunction include ciprofloxacin, moxifloxacin and ceftriaxone$^{138–140}$.

3.2.3. Target site (particularly pulmonary) concentration

Blood sampling provides easy access to a key physiological compartment. Plasma drug concentrations are the most frequently measured PK observation. Infection frequently affects tissue compartments making plasma antimicrobial agent concentration a surrogate for drug
concentration at the site of infection\textsuperscript{141,142}. Diffusion of an antimicrobial agent into a tissue is dependent on the concentration gradient across a biological membrane, the surface area of the membrane and a diffusion coefficient\textsuperscript{143}. The diffusion coefficient is primarily determined by physicochemical characteristics of the drug (e.g. the degree of hydrophilicity and lipophilicity) and the degree of protein binding\textsuperscript{109,144}. Inflammation and micro-vascular dysfunction associated with infection can affect the diffusion characteristics of a tissue and influence antimicrobial agent penetration\textsuperscript{145}.

Tissue penetration studies in critically ill patients, where antimicrobial agents are measured within the tissue compartment, suggest that tissue penetration of some antimicrobial agents may be impaired\textsuperscript{105,145,146}. Tissue antimicrobial agent concentrations may be as low as one-tenth the observed concentration in plasma\textsuperscript{105,145,146}. Altered tissue penetration in critically ill patients makes extrapolation of studies performed in healthy volunteers unreliable.

In patients with pulmonary infection the epithelial lining fluid (ELF) represents an accessible compartment from which clinically relevant drug concentrations may be measured\textsuperscript{147,148}. Pulmonary penetration may be expressed as a ratio of ELF to unbound plasma concentration or exposure\textsuperscript{147}. The ratio of exposure is preferable to concentration due to the influence of hysteresis\textsuperscript{141}. Hydrophilic compounds, such as aminoglycosides, β-lactams and glycopeptides, typically produce a ratio of ELF to unbound plasma drug concentrations of \(\leq 1\)\textsuperscript{149–151}. For lipophilic compounds, such as linezolid, macrolides and fluoroquinolones, the ratio of ELF to plasma free drug concentration is typically greater than one\textsuperscript{147,152}. The impact of impaired pulmonary penetration is not well defined but higher
than currently administered doses may be required to optimise ELF exposures, especially for hydrophilic compounds\textsuperscript{153,154}.

3.3. **Pharmacodynamics: An overview**

Pharmacodynamics (PD) describes the relationship between a drug and its effect. Clinically relevant PD study endpoints for antimicrobial agents include clinical or microbiological cure, emergence of antimicrobial resistance and drug-induced toxicity. In contrast, pre-clinical PD studies link antimicrobial agent exposure with bacterial killing, inhibition of bacterial growth and emergence of antimicrobial resistance. Establishing the PD relationship in both the pre-clinical and clinical setting allows estimation of an antimicrobial agents’ exposure that is required for maximal antimicrobial effect\textsuperscript{155}. Mathematical modelling may be used to describe the exposure-response relationship of antimicrobial agents.

3.3.1. *The minimum inhibitory concentration*

The MIC is the lowest concentration of an antimicrobial agent capable of inhibiting the visible growth of a organism after overnight incubation\textsuperscript{156}. The MIC, an assessment of the susceptibility of an organism to an antimicrobial agent, is frequently used as a component of a mathematical PD target.

3.3.2. *Antimicrobial effect*

Three different measures of drug exposure are used to link the exposure of an antimicrobial agent with bacterial killing\textsuperscript{88,155,157}. Firstly, the fraction of the dosing interval that the concentration of unbound (free) drug is greater than MIC ($f_{T>MIC}$); secondly, the ratio of the area under the unbound (free) drug concentration time curve to the MIC ($f_{AUC/MIC}$) and finally the ratio of the peak unbound (free) drug concentration during a dosing interval to the MIC ($f_{C_{max}/MIC}$).
A summary of PK-PD indices for various antimicrobial agents is shown in Table 7. Pre-clinical studies of β-lactams suggest an $fT_{\text{MIC}}$ of 40-50% and 60-70% is required for *Enterobacteriaceae* and *Staphylococcus aureus* respectively. A $fT_{\text{MIC}}$ of 90-100% may be required for Gram-negative bacilli such as *P. aeruginosa*. For aminoglycosides a ratio of peak concentration to MIC of ≥10 is associated with near maximal bacterial killing. A ratio of $\text{AUC}_{(24\text{hours})}/\text{MIC}$ of at least 400 mg/L is required for vancomycin. For linezolid and the fluoroquinolones, a ratio of $\text{AUC}_{(24\text{hours})}/\text{MIC}$ of 80 and 125, respectively, has been proposed.

### 3.3.3. Suppression of emergence of antimicrobial resistance

Antimicrobial exposures required to suppress emergence of antimicrobial resistance may be different from exposures required to bacterial killing. For fluoroquinolones, the AUC/MIC ratio has been shown to link drug exposure
with suppression of resistance\textsuperscript{169}. For aminoglycosides and carbapenems the ratio of peak (Cmax/MIC) or trough (Cmin/MIC) concentrations, respectively, relate drug concentration with suppression of resistance\textsuperscript{170,171}. Emergence of resistance follows an inverted “U” shaped curve with least resistance emerging at very low and very high drug exposures\textsuperscript{169}. As with bacterial killing, mathematical models may be developed to link drug exposure with emergence of resistant bacteria\textsuperscript{172}.

3.3.4. \textit{Toxicity}

Concentration or exposure-dependent toxicity has been described for a limited number of antimicrobial agents. For gentamicin, the probability of nephrotoxicity is related to the area-under-the-concentration time curve\textsuperscript{173}. The risk of rhabdomyolysis, defined by a rise in creatine phosphokinase, has been related to daptomycin trough concentration\textsuperscript{174}. Trough voriconazole concentrations have been shown to relate to the probability of neurotoxicity\textsuperscript{175}. No exposure-toxicity relationships exist for β-lactam antibiotics. Adverse events, specifically seizures, are more common in patients administered high doses or with renal impairment suggesting high exposures increase the risk of adverse events\textsuperscript{176}.

3.3.5. \textit{Pharmacodynamic considerations in critically ill patients}

The susceptibility of organisms causing infections in critically ill patients may be lower (i.e. the MIC may be higher) than observed in other patient cohorts\textsuperscript{177,178}.
3.4. **Optimal dosing in critically ill patients using PK-PD concepts**

Delivery of an antimicrobial agent at an exposure associated with near-maximal antimicrobial effect should be associated with optimal patient outcomes. Three approaches to modifying current antimicrobial regimens have been suggested to exploit knowledge gained from PK-PD studies: (a) alteration of length of administration from bolus dosing to extended or continuous infusion, (b) changing the dosing interval and (c) dose optimisation following therapeutic drug monitoring (TDM). PK-PD indices and target concentrations for a selection of antimicrobial agents, which may be used for dosage optimisation, are shown in Table 7.

<table>
<thead>
<tr>
<th>Class of drug</th>
<th>Optimal PK-PD index</th>
<th>PK-PD Target Description</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminoglycosides</td>
<td>fCmax/MIC, fAUC24/MIC</td>
<td>Cmax/MIC 8-30 fAUC24/MIC 70-100</td>
<td>164,179</td>
</tr>
<tr>
<td>Carapenems</td>
<td>fT&gt;MIC</td>
<td>40% - 75% fT&gt;MIC</td>
<td>158,159,180,181</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>fT&gt;MIC</td>
<td>60-100% fT&gt;MIC 95% fT&gt;3.3xMIC</td>
<td>120,160,182,185</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>fAUC24/MIC, fCmax/MIC</td>
<td>fAUC24/MIC &gt;30-250 Cmax/MIC ≥8</td>
<td>168,186–188</td>
</tr>
<tr>
<td>Linezolid</td>
<td>fT&gt;MIC</td>
<td>fAUC24/MIC ≥85 85% fT&gt;MIC</td>
<td>167,189</td>
</tr>
<tr>
<td>Penicillins</td>
<td>fT&gt;MIC</td>
<td>40-50% fT&gt;MIC</td>
<td>72,163,190,191</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>fAUC24/MIC</td>
<td>fAUC24/MIC 86-460</td>
<td>165,192,193</td>
</tr>
</tbody>
</table>

[MIC – minimum inhibitory concentration; AUC0-24/MIC – ratio of area under the concentration time curve during a 24 hour period to MIC; Cmax/MIC – ratio of maximum concentration of antibiotic in a dosing interval to MIC; T>MIC – percentage of dosing interval that the antibiotic concentration is maintained above the MIC; Cmin – minimum concentration of antibiotic in a dosing interval; f – free fraction of drug not bound to plasma proteins].

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3.4.1. Administration of antimicrobial agents by extended infusion

Delivering of an antimicrobial agent by either prolonged infusion (an infusion lasting 40-50% of the dosing interval) or continuous infusion leads to a reduced peak concentration but sustains a higher concentration for a greater proportion of the dosing interval. Compared with bolus dosing, extended infusions will increase the fraction of the dosing interval the concentration of an antimicrobial agent is above the MIC\textsuperscript{72}. Use of extended infusion is most commonly proposed for optimising β-lactam antibiotics where the PK-PD index is fT\textgreater MIC.

Pre-clinical in vivo data supporting the use of extended infusions for administering β-lactam antibiotics is relatively sparse. Delivery of ceftazidime by infusion to leukopenic rats is associated with greater bacterial killing compared with bolus administration\textsuperscript{194–196}. The most compelling evidence for delivering β-lactam antibiotics by extended infusion comes from the results of Monte Carlo simulation\textsuperscript{197}. Simulation consistently shows a greater number of critically ill patients achieve 50% fT\textgreater MIC when β-lactam antibiotics are delivered by infusion compared with bolus regimens\textsuperscript{105,198–202}. A small number of retrospective clinical studies have compared administration of β-lactam antibiotics by infusion or bolus regimen\textsuperscript{72,158–161}. Most studies concluded that delivering β-lactam antibiotics by extended infusion prolongs the time the β-lactam concentration is above the MIC. However not all studies observed improved antibacterial efficacy\textsuperscript{72,158–161}. Many of these trials have been included in meta-analyses which provide conflicting results. The most recent meta-analysis demonstrated that administration of carbapenems or PTZ (but not cephalosporins) by infusion, rather than bolus administration,
is associated with a lower mortality\textsuperscript{203}. In contrast, two previous meta-
analyses have shown no advantage to using infusions over bolus 
administration\textsuperscript{204,205}. A recent randomised controlled trial, not included in 
the meta-analyses, reported a significantly higher clinical cure rate following 
administration of β-lactam antibiotics by infusion\textsuperscript{71}.

Studies investigating administration of vancomycin by continuous infusion 
have also produced conflicting results\textsuperscript{206–208}. Current guidelines for use of 
vancomycin do not support the use of continuous infusions\textsuperscript{209}.

3.4.2. Alternative dosing intervals

For aminoglycosides the degree of bacterial killing has been shown to be 
related to peak drug concentration. By altering aminoglycoside regimens 
from three times daily to once daily dosing the efficacy of these compounds 
was improved without an increase in drug-related toxicity\textsuperscript{210}. Once daily 
administration of aminoglycosides is now considered the standard of 
care\textsuperscript{211}.

3.4.3. Dose optimisation

Dose optimisation (commonly known as therapeutic drug monitoring 
(TDM)) involves the measurement of plasma concentrations of 
antimicrobial agents and dose adaptation (either dose increase or 
reduction) by comparing the observed concentration with a therapeutic (PK-
PD) target. Dose optimisation may be guided by clinical experience, 
nomogram, non-linear regression or by Bayesian techniques. Traditionally 
dose optimisation is reserved for drugs with: (a) a narrow therapeutic 
range; (b) potential for drug-induced toxicity; (c) lack of clinical parameters 
to adjust the dose; (d) well-defined exposure-response relationship and (e) 
unpredictable PK\textsuperscript{212}. In critically ill patients, PK variability increases the
likelihood of sub-therapeutic concentrations of antimicrobial agents. Dose optimisation may be able to ensure all patients achieve optimal PK-PD targets. However few studies have assessed the impact of dose optimisation in critically ill patients\textsuperscript{96}. Dose optimisation is increasingly utilised for aminoglycosides where dose optimisation has been shown to reduce toxicity, length of hospital stay and mortality in critically ill patients\textsuperscript{213,214}. Dose optimisation has been applied to a number of other antimicrobial agents including β-lactams, vancomycin, teicoplanin and linezolid\textsuperscript{166,190,209,215–220}. The majority of these dose optimisation studies have primarily focused on PK outcome measures (i.e. achieving predefined drug concentrations) and few studies have included an assessment of clinical outcomes. Prospective randomised controlled trials are required to evaluate the potential advantage of dose optimisation.

3.4.3.1. Dosing nomograms

Dosing nomograms allow comparison of a measured concentration of an antimicrobial agent at a specific time point, in relation to an administered dose, via a graph that defines the therapeutic range of concentrations at that stated time point. The dosage or dosing interval of an antimicrobial agent can be adjusted with the aim of ensuring the next measured concentration of the antimicrobial agent is within the therapeutic range. Nomograms are simple to use and have been implemented widely for aminoglycosides and vancomycin to reduce the risk of drug-induced toxicity. However nomograms are not informed by PK-PD data specifically from critically ill patients and rely on clinic experience to make dosage adjustments\textsuperscript{221,222}. 
3.4.3.2. Non-linear regression based dose adaptation

Non-linear regression analysis calculates basic PK parameters (AUC, Cl, $C_{\text{max}}$ and $C_{\text{min}}$) from a series of concentrations of an antimicrobial agent, collected during a single dosing interval. Dosage adjustment may be undertaken to achieve the required PK-PD target.

3.4.3.3. Bayesian dose estimation and optimisation

Bayesian dose optimisation combines patient specific data, in the form of observed concentrations of an antimicrobial agent, and a population PK model, from a relevant critically ill population. This allows computer software to accurately determine a patient's actual PK. Knowledge of a patients PK parameters allows the software to estimate the optimal dose to achieve a pre-defined exposure$^{223}$. 
4. **Piperacillin and tazobactam**

Piperacillin-tazobactam (PTZ) is a combination of a β-lactam/β-lactamase inhibitor which was first licensed in the UK in 1992 (MHRA; Personal correspondence). The clinical formulation comprises a formulation of the sodium salts in an 8:1 ratio of piperacillin to tazobactam.

4.1. **Structure**

Piperacillin is a semi-synthetic aminobenzyl-penicillin derivative with the chemical name (2S,5R,6R)-6-[[2R)-2-[(4-ethyl-2,3-dioxopiperazine-1-carbonyl)amino]-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid. Piperacillin has an acid dissociation constant (pKa) of 4.41 and solubility in water of 714 g/L.

![Chemical structure of Piperacillin.](image)
Tazobactam is a triazolymethyl-penicillanic-acid sulfone. Tazobactam has the chemical name (2S,3S,5R)-3-methyl-7-oxo-3-(1H-1,2,3-triazol-1-ylmethyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide\textsuperscript{224}. Tazobactam has an acid dissociation constant (pKa) of 2.1 and solubility in water of >500 g/L\textsuperscript{225}.

![Chemical structure of Tazobactam.](image)

**Figure 3. Chemical structure of Tazobactam.**

4.2. *Mechanism of action*

Bacterial cell walls are constructed of cross-linked peptidoglycan polymers. Cross-linking of peptidoglycans is performed by transpeptidases, known as penicillin-binding protein (PBP). This cross-linking is crucial for bacterial cell wall structural integrity\textsuperscript{226}. Without cell wall stability, bacteria undergo lose of shape, osmotic rupture, lysis and ultimately cell death. As with other β-lactam antibiotics, piperacillin results in irreversible inhibition of PBPs. Piperacillin, compared with other penicillin, has particular affinity for PBP-3 which increases its activity against Gram-negative bacteria.

Tazobactam inactivates Ambler class A β-lactamases by initially forming a non-covalent then a covalently bound complex\textsuperscript{227}. This complex shields piperacillin from the hydrolytic activity of the β-lactamases.
4.3. Pharmacokinetics of piperacillin and tazobactam

4.3.1. Basic pharmacokinetics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Piperacillin</th>
<th>Tazobactam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral bioavailability (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Molecular weight (g/mol)</td>
<td>517.55</td>
<td>300.29</td>
<td></td>
</tr>
<tr>
<td>Plasma protein binding (%)</td>
<td>20 - 30</td>
<td>20 - 23</td>
<td></td>
</tr>
<tr>
<td>pKₐ</td>
<td>4.41</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>Solubility (g/L)</td>
<td>714</td>
<td>&gt;500</td>
<td></td>
</tr>
</tbody>
</table>

PTZ is the only penicillin based antimicrobial agent licensed in critically ill patients. The Vd of piperacillin is significantly larger in critically ill patients, most of whom have sepsis, when compared to healthy volunteers\textsuperscript{104,200}. In contrast, the clearance of piperacillin is significantly reduced when compared to healthy volunteers\textsuperscript{104,200,228}. A small study of 9 patients showed piperacillin is efficiently cleared by continuous veno-venous haemofiltration (CVVH) with no reduction of dose required\textsuperscript{229}. This study suggests that tazobactam is cleared less effectively than piperacillin and may accumulate in patients on CVVH.

Population PK studies in both healthy volunteers and patient groups demonstrate piperacillin may have linear, non-linear or a combination of clearance mechanisms\textsuperscript{120,146,228,230–232}. Differences in study design may explain some of the disparities between studies. Most of the studies that demonstrate piperacillin exhibits linear PK were performed on low or standard dosages, i.e. 2 to 4 grams, and only studied the first administered dose. Non-linear clearance requires further investigation if dose escalation above the current dosages is required.
The majority of studies investigating PTZ PK concentrate on piperacillin. Tazobactam PK is less well understood. However, tazobactam may also exhibit non-linear PK\textsuperscript{233}.

4.3.2.  \textit{Bioavailability}

Both piperacillin and tazobactam require parenteral administration as neither are absorbed from the gastro-intestinal tract. Peak plasma piperacillin and tazobactam concentrations are achieved immediately following intravenous infusion\textsuperscript{234}.

4.3.3.  \textit{Tissue (including pulmonary) penetration}

Following administration, piperacillin and tazobactam are widely distributed in tissue and body fluids. Measurement of drug concentrations in ELF is clinically relevant for patients with pneumonia\textsuperscript{147,148}. Healthy volunteer data suggests piperacillin ELF exposure is \(\approx 25\%\) of plasma exposure and tazobactam ELF exposure is \(\approx 50\%\) of the plasma exposure\textsuperscript{235}. There is only very limited data regarding ELF penetration of piperacillin and tazobactam in critically ill patients but two recent studies suggest that piperacillin and tazobactam ELF concentrations are \(\approx 50\%\) and 65-90\% of their respective paired plasma concentrations\textsuperscript{150,151}.

4.3.4.  \textit{Protein binding}

Piperacillin and tazobactam are 20-30\% and 20-23\% bound to plasma proteins, respectively\textsuperscript{225}. The protein binding of piperacillin and tazobactam is unaffected by the presence of the other agent\textsuperscript{225}. 
4.3.5. **Metabolism**

Metabolism plays only a minor role in the clearance of both piperacillin and tazobactam. Piperacillin is metabolised to a less active N-desethyld metabolite by cleavage of its β-lactam ring. Likewise, the β-lactam ring within tazobactam is opened to form a single inactive metabolite (M1). The N-desethyl derivative represents 6-9% of the total piperacillin dose while the M1 metabolite is 27% of the tazobactam dose.

4.3.6. **Elimination**

Both piperacillin and tazobactam are eliminated by the kidney via glomerular filtration and tubular secretion. Following administration, ≈50% piperacillin and ≈60% tazobactam are excreted in the urine over 24 hours. Administration of piperacillin reduces the excretion of tazobactam by ≈10-20% while the presence of tazobactam has no effect on elimination of piperacillin. The interaction between piperacillin and tazobactam is thought to occur in the kidney where there is competitive inhibition of active tazobactam clearance by piperacillin. Piperacillin and tazobactam have been shown to be substrates for the organic anion transporter (OAT)-1 within the proximal renal tubule. Piperacillin, tazobactam, and N-desethyl-piperacillin are also secreted into the bile.

4.4. **Administration**

PTZ is administered intravenously usually as a bolus dosage. The dosage may be delivered over five minutes but is more typically delivered over 30 minutes. A dose of PTZ 4g/0.5g is normally delivered every six or eight hours. Increasingly PTZ is administered as a prolonged or continuous infusion over half or the entire dosing interval as discussed in section 3.4.1.
4.4.1. **Impaired renal function and renal replacement therapy**

The half-lives of both piperacillin and tazobactam are increased by a decrease in creatinine clearance. For a creatinine clearance of below 20 ml/min there is a two-fold and four-fold decrease in the clearance of piperacillin and tazobactam respectively\textsuperscript{225}. Both piperacillin and tazobactam are removed by methods of RRT with variation in clearance rates observed between different methods of RRT and different filter membrane materials\textsuperscript{240}.

4.4.2. **Impaired liver function**

The half-lives of both piperacillin and tazobactam are increased by approximately 25% and 18%, respectively, in patients with hepatic cirrhosis when compared to healthy subjects. Dosage adjustment in patients with hepatic disease is not required.

4.5. **Spectrum of antimicrobial activity**

PTZ has a broad spectrum of activity against both Gram-positive and Gram-negative bacteria\textsuperscript{241}. Methicillin-sensitive strains of *S. aureus* and *S. epidermidis*, including most β-lactamase producing strains, are susceptible to PTZ\textsuperscript{242}. Methicillin-resistant strains of *S. aureus* and *S. epidermidis* have a reduced susceptibility to PTZ\textsuperscript{242}. The majority of strains of streptococci and enterococci are susceptible to PTZ. Vancomycin-resistant enterococci (VRE) are an exception and exhibit reduced susceptibility\textsuperscript{241}.

The majority of Gram-negative bacteria, including most Enterobacteriaceae, *P. aeruginosa* and anaerobes, such as *Bacteroides fragilis*, demonstrate *in vitro* susceptibility to PTZ\textsuperscript{243}. Class A β-lactamase producing organisms such as *H. influenzae* and *Moraxella catarrhalis* are inhibited by tazobactam therefore maintaining the activity of piperacillin.
against these organisms\textsuperscript{234}. However, extended-spectrum \(\beta\)-lactamases (ESBL)-producing strains of \textit{Klebsiella pneumoniae}, \textit{E.coli} and \textit{Proteus} spp. demonstrate reduced susceptibility to PTZ\textsuperscript{244}. PTZ is generally ineffective against \textit{Acinetobacter baumannii} and \textit{S. maltophilia} due to non-class A \(\beta\)-lactamases and other resistance mechanisms\textsuperscript{234}.

4.5.1. \textit{Resistance to piperacillin-tazobactam}

Resistance to antibiotics may be due to reduction in permeability of the outer bacteria membrane affecting diffusion of the drug; inactivation of the drug by enzymes; or reduction of the antibiotic’s affinity for its target\textsuperscript{245}. Manifestations of all these mechanisms occur in clinically relevant scenarios. Alterations in membrane permeability arise due to loss of outer membrane porins and expression of efflux pumps. Production of \(\beta\)-lactamases such as metallo-\(\beta\)-lactamases and ESBL deactivate piperacillin. These enzymes are not inhibited by tazobactam\textsuperscript{241}. Finally, the presence of PBPs for which piperacillin has a low affinity reduces the efficacy of PTZ. Examples include \textit{E. faecium} with PBP-5, in methicillin-resistant \textit{S. aureus} with PBP-2a and even with \textit{P. aeruginosa} with piperacillin-induced mutation of PBP-3\textsuperscript{246}. Antimicrobial resistance of \textit{P. aeruginosa}, specifically related to PTZ, is discussed in greater detail in section 2.6.1.

4.6. \textbf{Pharmacodynamics of piperacillin and tazobactam}

4.6.1. \textit{Pharmacodynamics of piperacillin}

The PD index that best links piperacillin exposure with the observed antibacterial effect is the fraction of the dosing interval that free drug concentrations are above the MIC\textsuperscript{157}. Near-maximal effect is observed when free concentrations exceed the MIC for at least 50\% of the dosing interval (50\% \(fT_{\text{MIC}}\))\textsuperscript{89}. 

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4.6.2. *Pharmacodynamics of tazobactam*

The PD index for tazobactam and other β-lactamase inhibitors is not well defined. Both the AUC and the fraction of the dosing interval that the β-lactamase inhibitor concentration is above a threshold have been shown to be important\textsuperscript{247–249}. Proposed threshold concentrations are either 4 mg/L or related to the β-lactam MIC of the organism\textsuperscript{249,250}.

4.7. *Toxicity*

PTZ is well tolerated with adverse events rarely leading to discontinuation of the drug\textsuperscript{234}. Adverse events are usually detected in <2% of patients but in specific patient cohorts toxicity has been reported in as many as ≈50% of patients\textsuperscript{234}. The most commonly reported adverse reactions, with a frequency of between 1-10% are diarrhoea, nausea, vomiting and rash\textsuperscript{251}. Reported adverse events and their frequency are shown in Table 9.

<table>
<thead>
<tr>
<th>Table 9. Adverse events reported with piperacillin-tazobactam.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Haematological disorders/investigations</strong></td>
</tr>
<tr>
<td><strong>Uncommon</strong></td>
</tr>
<tr>
<td></td>
</tr>
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<td></td>
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<tr>
<td><strong>Rare</strong></td>
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<tr>
<td><strong>Very rare</strong></td>
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</table>

**Nervous system disorders**

<table>
<thead>
<tr>
<th><strong>Uncommon</strong></th>
<th>Headache</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rare</strong></td>
<td>Muscle weakness</td>
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<td></td>
<td>Hallucination</td>
</tr>
<tr>
<td><strong>Convulsion</strong></td>
<td><strong>Dry mouth</strong></td>
</tr>
<tr>
<td>---------------</td>
<td>--------------</td>
</tr>
<tr>
<td><strong>Gastrointestinal disorders</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Common</strong></td>
<td>Diarrhoea</td>
</tr>
<tr>
<td></td>
<td>Nausea</td>
</tr>
<tr>
<td></td>
<td>Vomiting</td>
</tr>
<tr>
<td><strong>Uncommon</strong></td>
<td>Constipation</td>
</tr>
<tr>
<td></td>
<td>Dyspepsia</td>
</tr>
<tr>
<td></td>
<td>Jaundice</td>
</tr>
<tr>
<td></td>
<td>Stomatitis</td>
</tr>
<tr>
<td><strong>Rare</strong></td>
<td>Abdominal pain</td>
</tr>
<tr>
<td></td>
<td>Pseudomembranous colitis</td>
</tr>
<tr>
<td></td>
<td>Hepatitis</td>
</tr>
<tr>
<td><strong>Renal and urinary disorders</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Uncommon</strong></td>
<td>Blood creatinine increased</td>
</tr>
<tr>
<td><strong>Rare</strong></td>
<td>Interstitial nephritis</td>
</tr>
<tr>
<td></td>
<td>Renal failure</td>
</tr>
<tr>
<td><strong>Very rare</strong></td>
<td>Blood urea nitrogen increased</td>
</tr>
<tr>
<td><strong>Skin and subcutaneous tissue disorders</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Common</strong></td>
<td>Rash</td>
</tr>
<tr>
<td><strong>Uncommon</strong></td>
<td>Pruritus</td>
</tr>
<tr>
<td></td>
<td>Urticaria</td>
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<tr>
<td></td>
<td>Erythema</td>
</tr>
<tr>
<td><strong>Rare</strong></td>
<td>Bullous dermatitis</td>
</tr>
<tr>
<td></td>
<td>Erythema multiforme</td>
</tr>
<tr>
<td></td>
<td>Increased sweating</td>
</tr>
<tr>
<td></td>
<td>Eczema</td>
</tr>
<tr>
<td></td>
<td>Exanthema</td>
</tr>
<tr>
<td><strong>Very rare</strong></td>
<td>Stevens-Johnson Syndrome</td>
</tr>
<tr>
<td></td>
<td>Toxic epidermal necrolysis</td>
</tr>
<tr>
<td><strong>Musculoskeletal and connective tissue disorders</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Rare</strong></td>
<td>Arthralgia</td>
</tr>
<tr>
<td></td>
<td>Myalgia</td>
</tr>
<tr>
<td><strong>Metabolism and nutrition system disorders</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Very rare</strong></td>
<td>Hypoalbuminaemia</td>
</tr>
<tr>
<td></td>
<td>Hypoglycaemia</td>
</tr>
<tr>
<td></td>
<td>Hypoproteinaemia</td>
</tr>
<tr>
<td></td>
<td>Hypokalaemia</td>
</tr>
<tr>
<td><strong>Infections</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Uncommon</strong></td>
<td>Candida superinfection</td>
</tr>
<tr>
<td><strong>Cardiovascular disorders</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Uncommon</strong></td>
<td>Hypotension</td>
</tr>
<tr>
<td></td>
<td>Phlebitis</td>
</tr>
<tr>
<td></td>
<td>Thrombophlebitis</td>
</tr>
<tr>
<td><strong>Rare</strong></td>
<td>Flushing</td>
</tr>
<tr>
<td><strong>General disorders and administrative site conditions</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Uncommon</strong></td>
<td>Fever</td>
</tr>
<tr>
<td></td>
<td>Injection site reaction</td>
</tr>
<tr>
<td>Rare</td>
<td>Rigors</td>
</tr>
<tr>
<td>--------------</td>
<td>---------</td>
</tr>
<tr>
<td></td>
<td>Tiredness</td>
</tr>
<tr>
<td></td>
<td>Oedema</td>
</tr>
</tbody>
</table>

**Immune system disorders**

<table>
<thead>
<tr>
<th>Uncommon</th>
<th>Hypersensitivity reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rare</td>
<td>Anaphylactic/anaphylactoid reaction (including shock)</td>
</tr>
</tbody>
</table>

**Hepato-biliary disorders**

<table>
<thead>
<tr>
<th>Uncommon</th>
<th>Alanine aminotransferase increased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rare</td>
<td>Aspartate aminotransferase increased</td>
</tr>
<tr>
<td></td>
<td>Bilirubin increased</td>
</tr>
<tr>
<td></td>
<td>Blood alkaline phosphatase increased</td>
</tr>
<tr>
<td></td>
<td>Gamma-glutamyltransferase increased</td>
</tr>
<tr>
<td></td>
<td>Hepatitis</td>
</tr>
</tbody>
</table>

[very common ≥1/10, common ≥1/100 and <1/10, uncommon ≥ 1/1000 and <1/100, rare, ≥1/10 000 and <1/1000 and very rare, <1/10 000. Table based on summary of product characteristics.]
5. **List of manuscripts and author contribution**

Below is an outline of the individual contributions of authors to published manuscripts. Each of the published manuscripts are available in the appendices.


*T. W. Felton*: Literature review, mathematical modelling and simulation, preparation of the manuscript.

*W. W. Hope*: Supervision of research, input on manuscript.

*B. M. Lomaestro*: Provided data for analysis, input on manuscript.

*J. M. Butterfield*: Provided data for analysis, input on manuscript.

*A. L. Kwa*: Provided data for analysis, input on manuscript.

*G. L. Drusano*: Provided data for analysis, expert opinion on PK modelling, input on manuscript.

*T. P. Lodise*: Provided data for analysis, expert opinion on PK modelling, input on manuscript.

T. W. Felton: Literature review, study design, conduct of in vitro modelling, bacterial quantification, mathematical modelling and simulation, preparation of the manuscript.

J. Goodwin: Assistance with in vitro modelling, bioanalysis

L O’Connor: Assistance with conduct of in vitro modelling, bacterial quantification

A. Sharp: Assistance with conduct of in vitro modelling, bacterial quantification

L. Gregson: Assistance with conduct of in vitro modelling, bacterial quantification

J. Livermore: Assistance with conduct of in vitro modelling, bacterial quantification

W. W. Hope: Supervision of research, input on manuscript.

Chapter 3: Pulmonary penetration of piperacillin and tazobactam in critically ill patients. Clinical Pharmacology and Therapeutics. Accepted for publication 4th June 2014.

T. W. Felton: Literature review, study design, regulatory approvals, conduct of clinical trial including recruitment and consent, mathematical modelling and simulation, preparation of the manuscript.

K. McCalman: Conduct of clinical trial.

I. Malagon: Expert opinion in cardiothoracic intensive care.

B. Isalska: Expert opinion in clinical microbiology.
S. Whalley: Bioanalysis

J. Goodwin: Bioanalysis

A. M. Bentley: Expert opinion in intensive care, input on manuscript.

W. W. Hope: Supervision of research, input on manuscript.

Chapter 4: Individualization of Dosing of Piperacillin for Critically Ill Patients: Dosing Software to Optimize Antimicrobial Therapy. Antimicrobial Agents and Chemotherapy. Accepted for publication 30th April 2014.

T. W. Felton: Literature review, study design, mathematical modelling and simulation, preparation of the manuscript.

J. A. Roberts: Provided data for analysis, input on manuscript.

T. P. Lodise: Provided data for analysis, input on manuscript.


E. Boselli: Provided data for analysis, input on manuscript.

M. N. Neely: Expert opinion on mathematical modelling, input on manuscript.

W. W. Hope: Supervision of research, input on manuscript.
GENERAL HYPOTHESIS AND AIMS

6. Hypothesis

The overriding hypothesis of this research is that mathematical modelling techniques can be used to identify drug exposure targets that are associated with effective antimicrobial therapy. Furthermore, modelling techniques can be used to optimise outcomes for individual critically ill patients.

7. Aims

1. Identify the magnitude of drug exposure for piperacillin that results in maximal bacterial killing and minimises the development of drug resistance in an *in vitro* hollow fibre infection model with *Pseudomonas aeruginosa*.

2. Describe the population PK of piperacillin and tazobactam in plasma and lung of critically ill patients.

3. Construct computer software to individualise piperacillin-tazobactam dosing for critically ill patients.
8. **Hollow fibre infection model**

The hollow-fibre cartridge consists of an outer plastic casing which contains fine semi-permeable capillaries (Figure 4). The cartridge has an internal service area of ≥3000cm$^2$, extra-capillary volume of 20 mL and allows 50% of molecules weighing 20 kDa and 5% of molecules weighing 100 kDa to diffuse across the capillary wall.

![Image of hollow fibre cartridge](image)

**Figure 4. Cross section of a hollow fibre cartridge.**

The hollow fibre infection model (HFIM) circuit is illustrated in Figure 5. Outflow from the central compartment was connected, via a pump, to the hollow fibre cartridge, which then returned to the central compartment. Fresh media was pumped from a reservoir into the central compartment. A bacterial suspension was prepared following overnight culture, adjusted to the required inoculum and injected into the HFIM. Drug was added to the central compartment utilising a programmable syringe driver. Waste was removed, by pump, from the central compartment. Repeated aspiration of
a small volume from the HFIM and quantitative culture allowed enumeration of bacteria. Drug concentrations were quantified from samples from the central compartment.

**Figure 5. Schematic illustration of the hollow fibre infection model.**

9. **Clinical trial design**

The PROPEL (Plasma and intrapulmonary population pharmacokinetics of piperacillin/tazobactam in critically ill patients) study was conducted to investigate the plasma and intra-pulmonary PK of piperacillin and tazobactam in critically ill patients. The clinical trial protocol, case reporting
form, information sheets and consent forms for the clinical trial are included as appendices.

9.1. **Design overview**

An open-label PK study, without a control group, was designed with the aim of investigating the plasma and lung concentrations of piperacillin and tazobactam in critically ill patients. Patients were included either when PTZ was initiated or when PTZ had previously been commenced by the intensive care medical team. Patients included prior to initiation of PTZ had first-dose followed by steady-state PK assessed. Patients already established on PTZ had only steady-state PK assessed. All patients were given the standard dose of PTZ as per hospital guidelines. Patients were followed up until discharge from University Hospital of South Manchester (UHSM) or thirty-five days from the commencing the study drug. A total of 40 patients were initially required for the study. The number of patients required was reduced to 20 patients approximately six months into the conduct of the trial due to difficulties in recruiting the initial estimate of 40 patients in the stated timeframe.

The trial was designed to assess PTZ PK following both the first administered dose and at steady state. Five episodes of sample collection occurred during the clinical trial. Patients could be enrolled for all five episodes or just for episode 2. Patients enrolled solely for episode 2 had only steady-state PK samples collected.

9.1.1. **Study episodes**

9.1.1.1. Episode 1

Prior to antibiotic administration a venous blood sample and non-direct bronchial lavage (NBL) sample were collected. The aim of this episode was
to identify the infective organism in the patient’s lung and to assess the plasma pharmacokinetics following the first dose of PTZ. Serial blood sampling was performed with plasma samples collected at six time points during the first dosing interval. The exact times the samples were collected was identified from the population pharmacokinetics analysis and D-optimal design performed in Chapter 1 of this thesis. Prior to administration of PTZ all women of child-bearing age underwent pregnancy testing, if this has not already been performed by the clinical care team. Anonymised data describing the patients’ demographic, underlying clinical condition, previous antibiotic use, serum creatinine and selected scoring systems was collected.

9.1.2. **Episode 2**

The second episode assessed the PK of PTZ during the maintenance phase of treatment. A pre-dose (trough) plasma samples was collected followed by five further samples during the dosing interval. The exact times the samples were collected was identified from the population pharmacokinetics analysis and D-optimal design performed in Chapter 1. NBL samples were collected twice during the dosing interval. A paired plasma urea sample was collected with each NBL to allow calculation of the dilution factor introduced by the lavage fluid.

9.1.2.1. **Episode 3**

Following five days of PTZ a plasma and NBL sample was collected. If the patient had been extubated a sputum sample for microbiological culture was collected.
9.1.2.2. Episode 4

Upon extubation the endotracheal tube was collected, labelled and frozen. The sample will be analysed by microbiological culture and identification of pathogen DNA. During the conduct of the trial the investigators were unable to collect any endotracheal tubes from patients.

9.1.2.3. Episode 5

Fourteen to seventeen days after starting PTZ a single blood sample will be collected to determine the concentration of the piperacillin-albumin adduct. This data is not included in this thesis.

9.2. Enrolment criteria

Initially subjects were included in the study if they met the following inclusion criteria:

1. Subject is an adult aged 18 to 75

2. Subject requires piperacillin/tazobactam as directed by clinical Intensive Care Unit medical staff for suspected pulmonary infection

3. Subject has been intubated and mechanically ventilated for greater than 48 hours

The inclusion criteria were changed following a major amendment to the clinical trial authorisation to try and increase patient recruitment. Following the amendment the subjects were included in the study if they met the following inclusion criteria:

1. Subject is an adult aged 18 to 85
2. Subject requires or has been commenced on PTZ as directed by clinical ICU medical staff

9.3. **Exclusion criteria**

Subjects were excluded from the study if they met any of the exclusion criteria:

1. Subject is known to be intolerant of β-lactams antibiotics

2. Subject has an infection with a PTZ resistant organism (i.e. *P. aeruginosa* with MIC ≥16 mg/L, other organism with MIC ≥ 8mg/L) or organisms with inherent resistance (e.g. *Stenotrophomonas*)

3. Subject is immunocompromised (neutropenia, patients with human immunodeficiency virus)

4. Subject is unlikely to survive longer than 48 hours

5. Subject is pregnant or breast-feeding or plans to become pregnant during the course of the study

6. Subject is enrolled in another Clinical Trial of an Investigational Medical Product.

9.4. **Location**

The study was initially only conducted within the Adult ICU at UHSM. A substantial amendment to the clinical trial authorisation, approximately one year into the trial, was made to allow recruitment from the Cardiothoracic ICU also at UHSM.
9.5. **Consent**

The Principal Investigator was responsible for ensuring that informed consent was given by each patient or their legal representative. Informed consent was sought from a Personal Legal Representative or Professional Legal Representative for patients who lacked capacity to give informed consent, due to alteration in their conscious level caused by critical illness and sedation. Treatment of infection (including VAP) in critically ill patients is a medical emergency and delay in the administration of antibiotics is associated with a significant increase in mortality\(^{43,253}\). Therefore patients treated with PTZ had samples collected without prospective consent (as laid out in The Medicines for Human Use (Clinical Trials) Amendment (No.2) Regulations 2006). In these cases consent was sought retrospectively.

9.6. **Regulatory requirements**

The trial was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and outlined by the Medicines and For Human Use (Clinical Trials) Regulations 2004. Regulatory approval was given by the South Manchester Research Ethics Committee and Clinical Trial Authorisation was obtained from the Medicines and Healthcare products Regulatory Agency prior to the start of the trial. The trial was registered on the UK National Institute for Health Research Clinical Research Portfolio.

9.7. **Ethical issues**

A number of ethical issues were anticipated during development of the clinical trial protocol.
9.7.1. **Informed patient consent**

Gaining consent from critically ill patients was not possible due to lack of mental capacity caused by a combination of illness and sedation. The process of taking consent from patients who did have capacity was retained within the trial design but was not used. In all cases consent was sought from the patients’ representatives. Retrospective consent was sought from all patients who recovered capacity.

9.7.2. **Vulnerable population.**

The study design meant that it would be impossible to perform this study in another patient group. The interests of the trial patients were protected at all times. The clinical team was able to alter the patients’ management at any time.

9.7.3. **Blood volumes**

A maximum of 82mls of blood was collected over a two week period. The required volume of blood was carefully calculated in order to minimise the required volume.

9.7.4. **Non-directed bronchial lavage**

For each patient a maximum of four NBL samples could be collected during the PROPEL trial. The complication rate from NBL was expected to be low with complications only reported in \( \approx 1.5\% \) of patients\(^{23,254} \). Critically ill patients are sedated during the procedure and were not expected to find the procedure distressing. The most commonly reported complication following NBL is reduction in patients’ oxygen saturations. Oxygen saturations return to baseline oxygen levels within 3 hours (mean time to recovery 45 minutes). To reduce the risk of complication, all trial patients
were administered supplemental oxygen prior to NBL being performed. Additionally NBL samples could not be collected on patients fulfilling any of the four safety criteria detailed below:

1. Requiring FiO\textsubscript{2} > 80%
2. Requiring PEEP > 12 cmH\textsubscript{2}O
3. Severe bronchospasm
4. Uncontrolled intracranial pressure

9.7.5. **Sample storage and transfer**

All patient samples were coded with a unique study number and stored in a secure room in a locked freezer with limited access. Records were kept of the samples obtained and tracking logs were completed for the transfer of samples between the UHSM and university laboratories.

9.7.6. **Data storage and transfer**

All study participants were allocated a unique identifying code. Data was stored in a password protected database. Only the research team had access to the database.

10. **Mathematical modelling**

10.1. **Population modelling**

Data from the PK and PD studies were analysed using a population methodology using the non-parametric adaptive grid program Pmetrics in the R statistical environment (previously a standalone software called NPAG)\textsuperscript{255,256}. Each population analysis utilised a structural mathematical model which comprised a series of inhomogeneous ordinary differential
equations. All data were weighted by the inverse of the estimated assay variance. A polynomial describing the variance was determined for each specific dataset (i.e. \( SD = C_0 + C_1 Y + C_2 Y^2 + C_3 Y^3 \)). In the fitting process, each concentration was weighted by its Fisher information, which is the inverse of the standard deviation squared, or the variance. Additionally, we chose the option in Pmetrics to multiply the variance by gamma (\( \gamma \)), which is an adaptive scalar that captures additional noise such as errors in timing of samples or doses.

Pmetrics uses a non-parametric adaptive grid algorithm to find the maximally likely distribution of each of the pharmacokinetic parameter (e.g. clearance) values for a given mathematical model. This grid can be considered as a multi-dimensional search space, with each axis corresponding to a parameter in the model plus an additional axis for probability. Initially the space is filled with a uniform distribution of support points, each of which comprises a set of values for the model parameters, and the associated probability of that support point, based on its ability to describe the observed data. As this is an iterative process, involving many cycles, the grid is “adapted” at each cycle of an inner loop by discarding points with very low probability and augmenting the space with daughter points around the survivors. When the inner loop change in log-likelihood is \( \leq 0.0001 \), the grid is augmented with further random points and the process begins again as the outer loop, to minimise the chance of finding parameter value distributions which are not truly maximally likely. When no improvement in the log-likelihood \( >0.01 \) can be achieved between outer loops, the algorithm has converged. This method is “non-parametric” as it does not assume that the distributions of the parameter values is Gaussian (in contrast with other methods of population analysis). The final “grid”, therefore, contains a discreet number of support points, which can at most
equal the number of subjects, and these are encoded in a “density” file. This density file is an output of the Pmetrics analysis.

For an individual patient without data, i.e. prior to any measured piperacillin concentrations, his/her parameter value joint distribution is the same as for the population. However, if observed data are available, the population distribution (i.e. the Bayesian prior) may be updated to a new distribution (i.e. the Bayesian posterior) that better predicts the individual’s observations. The support points do not move, but their relative probabilities change, based on ability to predict the patient’s observed concentrations for his/her dosing history.

For each model the means, medians, and standard deviations of the population parameters were estimated. The fit of each of the structural models to the data was further assessed by: (i) the log-likelihood value; (ii) the coefficient of determination ($r^2$) from a linear regression of the observed-predicted plots both before and after the Bayesian step and (iii) the Akaike information criterion (AIC). Differences between the models were also assessed statistically by calculating the difference in log-likelihood values and comparing this value to a $\chi^2$ distribution with the degrees of freedom equal to the difference in the number of parameters between each model.

10.2. Monte Carlo simulation

Monte Carlo simulation was performed using the ADAPT 5 program\textsuperscript{257,258}. Monte Carlo simulation was used to explore the influence of pharmacokinetic variability on the pharmacodynamic endpoints (e.g. $T_{\text{MIC}}$). ADAPT 5 uses Monte Carlo parametric expectation maximization as a method of simulation. Alternative methods of Monte Carlo simulation are
implemented in other software packages including a non-parametric simulator which is now available within the Pmetrics package\textsuperscript{255}.

In order to perform Monte Carlo simulation within ADAPT 5, the structural mathematical model (a series of ordinary differential equations), a mean estimate of each of the model parameters and the variance of these parameters must be known. Typically 5,000-subject simulations are performed. A diagonal covariance matrix may be entered which involves the variance of each parameter vector. Alternatively the full covariance matrix may be entered which also describes any relationship between the parameters. Either normal or log-normal parameter distributions for each of the parameters may be assumed. The ability to recapitulate the original parameter values and their dispersions was used to select which parameter distribution was selected.

Monte Carlo simulation was primarily utilised to estimate the probability of target attainment for each of the population models. A binary outcome related to the time the drug concentration was above a threshold was estimated for each of the 5,000 subjects. A typical threshold was the minimum inhibitory concentration (MIC). For a series of MICs the fractions of the 5,000 subjects who achieve a 50\% T_{\text{\textgreater} \text{MIC}} could then be estimated and displayed graphically with MIC on the X-axis and probability on the Y-axis.

10.3. **D-Optimal design**

The multiple model file, which contains information on the support points from the population analysis and is an output of NPAG/Pmetrics was used. Each of the parameter vectors were utilized in a D-optimal design analysis using the ADAPT 5 program\textsuperscript{257}. The optimal sampling points were then weighted by their probability and presented in a histogram, as previously described by Tam and Drusano\textsuperscript{259}. Summation of the D-optimal design
analysis for each support point allowed delineation of six optimal sampling times for the population. Each of the sampling times optimally identified a parameter within the structural model.

10.4. **Dose optimisation software**

The dose optimisation software, “BestDose”, was used to estimate each individual patient’s PK and the optimal individual dosages for each patient. The software required four specific components: (a) a structural mathematical model; (b) the density file, which is an output from a population analysis and serves as the Bayesian prior; (c) a patient “past” file that contains the observed drug concentrations and details of the administered regimen, (d) a patient “future” file which contains the target drug concentrations deemed to be appropriate for that patient and initial estimates of the required drug dosages and frequency of administration. The dose optimisation software then calculated the drug dose that minimized the expected weighted squared error (over the Bayesian posterior distribution) between the predicted and user-specified target drug concentrations in the future data file.

11. **Drug quantification**

The bio-analytical methods established for quantification of piperacillin and tazobactam using high-performance liquid chromatography (HPLC) and the liquid chromatography–tandem mass spectrometry (LC/MS/MS) were performed by Joanne Goodwin.

11.1. **High-performance liquid chromatography**

Drug concentrations from *in vitro* samples were measured using validated HPLC methods\textsuperscript{260,261}. The extracted sample was injected into an HPLC column. A standard curve encompassing each drug in the relevant matrix
was constructed. The internal standard was penicillin G. The mobile phase was adjusted by a gradient-controlled pump. The drug was detected using a ultra-violet (UV) detector. The intra- and inter-assay coefficient of variation and limit of detection was determined for each drug.

11.2. **Liquid chromatography–tandem mass spectrometry**

Drug concentrations from clinical samples were measured using a validated LC/MS/MS method. The extracted sample was injected into a liquid chromatography column. A standard curve encompassing for each drug in the relevant matrix was constructed. The internal standard was caffeine in water. The intra- and inter-assay coefficient of variation and limit of detection was determined for each drug.
CHAPTER 1: POPULATION

PHARMACOKINETICS OF EXTENDED INFUSION

PIPERACILLIN-TAZOBACTAM IN HOSPITALIZED PATIENTS WITH NOSOCOMIAL INFECTIONS

12. Abstract

While extended infusions of piperacillin-tazobactam (PTZ) are increasingly used in practice, its effect on the pharmacokinetic profile of PTZ has not been widely assessed. To assess its effect on the pharmacokinetic profile of PTZ, seven serum samples were collected from 11 hospitalized patients who received PTZ 3.375 grams intravenously over 4 hours every 8 hours. Population pharmacokinetic models were fit to the PK data utilizing first-order, Michaelis-Menten (MM), and parallel first-order/MM clearance. A population PK model with first-order clearance was fit to the tazobactam PK data. Monte Carlo simulations (MCS) were used to determine the most effective administration schedule to ensure free piperacillin concentrations were above the minimum inhibitory concentration (MIC) for at least 50% of the dosing interval (50% fT>MIC) and to quantify the extent of the non-linear clearance. The model incorporating parallel linear/MM clearance best fitted the piperacillin PK data. The MCSs demonstrated that approximately 50% of the administered piperacillin is cleared by the non-linear clearance mechanism. The results of the MCSs also revealed that more intensive PTZ extended infusion dosing schemes (3.375-4.5 grams intravenously (3 hour infusion) every 6 hours) than those commonly used in clinical practice were needed to maximize 50% fT>MIC for MICs ≥ 8 mg/L. This study suggests that extended infusion of PTZ is the most effective method of administration for patients with nosocomial infections. Due to
the hyper-clearance nature of the hospitalized patient populations studied, more intensive PTZ dosing regimens may be needed to maximize $fT>MIC$ in certain hospitalized populations.
13. Introduction

Piperacillin-tazobactam (PTZ) is a combination of an extended-spectrum β-lactam antibiotic and a β-lactamase inhibitor and is frequently used for nosocomial infections\(^{262,263}\). For-β-lactam antibiotics, the PD index that best links drug exposure with the observed antibacterial effect is the fraction of the dosing interval that free drug concentrations are above the MIC\(^{157}\). Near-maximal effect is generally observed when free concentrations exceed the MIC for at least 50% of the dosing interval (\(fT_{>\text{MIC}} 50\%\))\(^89\). Although PTZ is frequently administered as a rapid infusion, extended infusions of PTZ are increasingly used in clinical practice because it facilitates extension of \(fT_{>\text{MIC}}\).

Although more commonplace, the impact of prolonging the infusion time of PTZ on its PK profile has not been widely assessed. Here we describe the population PK for both piperacillin and tazobactam among hospitalized patients receiving an extended infusion regimen. The goal was to identify the model that best explained the observed clearance of both piperacillin and tazobactam. When modelling piperacillin, it is important to consider linear, Michalis-Menten (MM), and parallel first order/MM models. Piperacillin is cleared via a combination of renal tubular secretion and glomerular filtration\(^{225}\). While glomerular filtration is a linear process, tubular secretion, via an anion transporter system (Organic Anion Transporting Polypeptide 1), is non-linear (i.e. the transporter activity is saturable and has a maximum rate). Understanding the optimal clearance mechanism of piperacillin has important implications for clinical practice. If piperacillin is found to have MM or parallel first order/MM clearance, then increasing the piperacillin dosage may lead to a disproportionate increase in plasma exposure and may potentially result in better probability of target attainment or, perhaps, toxicity\(^{191,264}\).
Monte Carlo simulation was also used to explore the potential clinical consequences of inherent PK variability in hospitalized patients. In particular, simulation studies were used to explore the potential PD benefits of using an extended infusion, as suggested by Lodise et al.\textsuperscript{196}, compared to the licensed schedule of administration over 30 minutes.\textsuperscript{234} Alternative PTZ regimens were also explored in an effort to maximize the probability of target attainment (PTA) against a range of MIC values at the higher end of the CLSI susceptibility range for non-fermentative Gram-negative pathogens. Finally, D-optimal design was used to identify the most informative sampling times to generate robust population PK parameter estimates for future studies.\textsuperscript{259}
14. Material and methods

14.1. Pharmacokinetic study

Patients who received extended infusion PTZ for suspected or documented nosocomial infections at the Albany Medical Centre Hospital (Albany, New York) were eligible for enrolment. This study received approval from the Albany Medical Centre Hospital institutional review board. Only patients that resided in the hospital for at least 48 hours prior to starting PTZ were considered. As standard hospital protocol, patients were administered piperacillin 3 grams in combination with tazobactam 0.375 grams intravenously over a 4 hour infusion period every 8 hours. Written informed consent was obtained from all patients participating in the study. Demographic data (included age, sex, race, height and weight of the patient), cause of admission to hospital and underlying renal function were recorded.

For each patient, the length of infusion and infusion start and stop times were recorded for 24 hours prior to the dose being studied. Seven blood samples were collected after the third, but before the eleventh dose (i.e. at steady state). The times for the collection of samples for this study, 2, 4, 4.5, 5, 5.5, 6.5 and 7 hours after the initiation of the infusion, were developed using D-optimal design and based on a previous population PK model\(^1\). The exact time of each 3-5 mL sample acquisition was recorded. All blood samples were allowed to clot for 15 minutes at room temperature and then centrifuged at 2400 rpm for 10 minutes. Sera from each sample was separated into three vials and stored at -80°C.
14.2. Piperacillin and tazobactam assay

Piperacillin and tazobactam concentrations were measured using a previously validated high performance liquid chromatography (HPLC) method. Briefly, drug concentrations were measured using a gradient-controlled pump (Model 626; Waters, Milford, Mass., USA), an autosampler (WISP 717 plus; Waters) and a UV detector (Model sm 4000, LDC Analytical, Riviera Beach, Fla., USA). A reverse-phase HPLC column (Bondpak C 18 Guard-pak precolumn; Phenomenex prodigy, 10 μm, 4.6 mm x 250 mm) was used. The injection volume was 5 μL. A standard curve encompassing 2-100 mg/L and 1 – 50 mg/L for piperacillin and tazobactam respectively was constructed from stock solutions. The internal standard was penicillin G. The mobile phase was A: 90% HPLC grade acetonitrile and 10% sodium phosphate buffer (0.01 M, pH = 2.7 v/v), B: 100% HPLC methanol, C: 3% HPLC grade acetonitrile and 97% sodium phosphate buffer (0.01 M, pH = 2.7, v/v), and D: 100% water. The gradient-controlled pump was programmed as follows: 5% of A and 95% of C at 0–10 min, 45% of A and 55% of C at 10–18 min, 5% of A and 95% of C at 18–22 min. The mobile phase flow rate was 1.2 mL/min. The wavelength of the UV detector was programmed as 218 nm at 0–10 min, 254 nm at 10–20 min, and 218 nm at 20–22 min. The intra and inter-assay coefficient of variation was <5.8% for both compounds. The lower limit of quantification was 1mg/L and 2mg/L for tazobactam and piperacillin, respectively.

14.3. Population pharmacokinetic modelling

All data were analyzed using a population PK methodology. The nonparametric adaptive grid (NPAG) program Pmetrics within the R statistical environment was used. The PK data were weighted by the inverse of the estimated assay variance for both piperacillin and
tazobactam. A polynomial describing the assay variance, derived from regression of the measured drug concentrations and observed assay variances, was determined. An adaptive scalar (γ) was used which multiplies the polynomial described above and was determined with each cycle to obtain the best approximation to the homoscedastic assumption. The mean, median and standard deviations of the population parameters were estimated. Bayesian estimates for the parameters (using the “population of one” utility in NPAG) for each patient were also obtained. Scatter plots of observed-versus-predicted piperacillin concentrations were examined for individual patients and for the population as a whole. The fit of each of the structural models to the data was assessed in the following way: (a) the log-likelihood value; (b) the coefficients of determination ($r^2$) from regression of the observed-predicted plots before and after the Bayesian step and (c) the Akaike information criterion (AIC)\textsuperscript{265}. Statistically significant differences between models were determined by assessing twice the difference in log likelihood values against a $\chi^2$ distribution, with the appropriate number of degrees of freedom (i.e. difference in parameter number for the respective models).

For piperacillin, three two-compartment structural mathematical models were evaluated: (a) elimination as a first-order process; (b) a MM process alone; and (c) a MM process with parallel first-order elimination. The reason for the model choices is based on the known drug handling of piperacillin. The drug is known to be cleared by glomerular filtration, which is a linear process. It is also known to be tubularly secreted, which is an inherently MM process. The question being examined is to determine the amount of drug clearance due to the MM process relative to the linear process. If the amount of MM clearance is low relative to the linear process
is low, the linear system should suffice. If the amount of MM drug clearance is higher, then the MM or parallel first order/MM model would be needed.

The ordinary differential equations for these models were as follows:

**Equation 1.1a (Linear):**
\[
\frac{dX_1}{dt} = R(1) - \left( \frac{Cl}{V_c} + k_{cp} \right) \times X_1 + k_{pc} \times X_2
\]

**Equation 1.1b (MM):**
\[
\frac{dX_1}{dt} = R(1) - \left( \frac{V_{max}}{\left( (k_m \times V_c) + X_1 \right)} + k_{cp} \right) \times X_1 + k_{pc} \times X_2
\]

**Equation 1.1c (MM/Linear):**
\[
\frac{dX_1}{dt} = R(1) - \left( \frac{V_{max}}{\left( (k_m \times V_c) + X_1 \right)} \right) \times X_1 + (\frac{Cl}{V_c}) + k_{cp} \times X_2
\]

**Equation 1.2:**
\[
\frac{dX_1}{dt} = k_{cp} \times X_1 - k_{pc} \times X_2
\]

Where: \(X_1\), and \(X_2\) is the amount of piperacillin (in milligrams) in the central compartment and peripheral compartment, respectively. \(R(1)\) represents the infusion of drug, \(CL\) (litres per hour) is the clearance and \(V_c\) is the volume of the central compartment (litres). \(V_{max}\) is the maximum rate of enzyme activity (milligrams per hour) and \(Km\) is the concentration of piperacillin where enzyme activity is half-maximal (milligrams per liter). \(K_{cp}\) and \(K_{pc}\) are the first-order inter-compartmental rate constants.

For tazobactam only a first-order elimination model was assessed. The ordinary differential equations for this model were as follows:

**Equation 1.3:**
\[
\frac{dX_1}{dt} = R(1) - \left( \frac{Cl}{V_c} + k_{cp} \right) \times X_1 + k_{pc} \times X_2
\]
Equation 1.4:

\[
\frac{dX_2}{dt} = k_{cp} \times X_1 - k_{pc} \times X_2
\]

Where: \(X_1\), and \(X_2\) is the amount of tazobactam (in milligrams) in the central compartment and peripheral compartment, respectively. \(R(1)\) represents the infusion of drug, \(CL\) (litres per hour) is the clearance and \(V_c\) is the volume of the central compartment (litres). \(K_{cp}\) and \(K_{pc}\) are the first-order inter-compartmental rate constants.

### 14.4. Monte Carlo simulation

Each Monte Carlo simulation was performed using a 5,000-subject simulation. The mean parameter vector and the full covariance matrix from the population PK analysis was embedded in subroutine PRIOR of the ADAPT 5 program\textsuperscript{257,258}. Both normal and log-normal parameter distributions were explored in the simulations and distinguished on the ability to recapitulate the original parameter values and their dispersions.

As an additional method for assessing the overall fit of the models to the PK data, 5,000 subject simulations of 3 grams of piperacillin administered over 4 hours every 8 hours was performed using each of the three mathematical models: (a) a first-order process; (b) an MM process alone; and (c) an MM process with parallel first-order elimination. For each mathematical model, the median, 5\textsuperscript{th} and 95\textsuperscript{th} percentile concentrations for the population were identified for both piperacillin at the beginning and end of drug administration and every 15 minutes throughout the ninth dosing interval (steady state). The observed patient data was simultaneously plotted with the simulated concentration-time curves. The fidelity by which
the concentration-time curves mirrored the raw data was assessed by visual inspection.

To compare the amount of piperacillin removed, over 24 hours, by the clearance terms in the parallel linear/MM, 5,000-subject simulations were performed using 3 doses of 4g of piperacillin administered over either 30 minutes or 4 hours every 8 hours for both models. The amount of piperacillin cleared by each of the clearance mechanisms was assessed using the differential equations below. Equations 1.5 and 1.6 were used to determine the amount of drug cleared by the MM and linear clearance mechanisms respectively. Additionally, for the parallel linear/MM model, the time the piperacillin concentration was above the Michaelis-Menten constant, during the third dosing interval, was determined.

Equation 1.5:
\[
\frac{dX_3}{dt} = \frac{V_{max}}{(k_m \times V_c + X_1)} \times X_1
\]

Equation 1.6:
\[
\frac{dX_4}{dt} = \left(\frac{CL}{V_c}\right) \times X_4
\]

Finally, for the overall best fitting model, 5,000-subject simulations were performed using 3 or 4 grams of piperacillin administered over 30 minutes or 4 hours every eight hours and 3 or 4 grams of piperacillin administered over 30 minutes or 3 hours every 6 hours. For each regimen the fraction of simulated subjects who achieved the PD target of 50% \(f_{T>\text{MIC}}\) for a range of MIC values from 0.5-128 mg/L was determined.

14.5. Optimal sampling schedule

The optimal sampling times, following the first dose and at steady state (9th dose), for an infusion of 4 grams of piperacillin and 0.5 grams of
tazobactam administered over 30 minutes or 4 hours every eight hours were investigated. The multiple model file from the output of NPAG was used. The parallel linear/MM PK model was used. Each of the parameter vectors were utilized in a D-optimal design analysis using the ADAPT 5 program$^{257}$. The optimal sampling points were then weighted by their probability and presented in a histogram, as previously described by Tam and Drusano$^{259}$. Summation of the D-optimal design analysis for each support point allowed delineation of six optimal sampling times for the population. Each of the sampling times optimally identifies a parameter within the structural model. This process was performed for both the first dose and a steady-state sampling schedule for both piperacillin and tazobactam.
15. Results

15.1. Pharmacokinetic study

Between February and July 2005, 11 patients were enrolled with a mean age of 44.7 ± 12.5 years. Mean height and weight was 1.90 ± 0.23 meters and 78.0 ± 22.1 kg, respectively. All patients were caucasian and 7 (64%) were male. Receipt of PTZ during ICU stay occurred in 7 (58.33%) of patients. Mean baseline creatinine clearance was 122 ± 35 ml/min. The demographics and clinical characteristics are displayed in Table 10. A total of 71 plasma concentrations were obtained after multiple dosing from 11 individuals.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean (SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>44.7 ± 12.5</td>
<td>20 - 58</td>
</tr>
<tr>
<td>Sex - Male (%)</td>
<td>7 (64%)</td>
<td></td>
</tr>
<tr>
<td>Bodyweight (kg)</td>
<td>78.0 (22.1)</td>
<td>38.1 - 122.5</td>
</tr>
<tr>
<td>Height (meters)</td>
<td>1.90 (0.23)</td>
<td>1.50 – 2.15</td>
</tr>
<tr>
<td>Race</td>
<td>All white</td>
<td></td>
</tr>
<tr>
<td>ICU admission (%)</td>
<td>7 (64%)</td>
<td></td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>122.0 (35.0)</td>
<td>77.4-169.1</td>
</tr>
</tbody>
</table>

[SD is the standard deviation.]

15.2. Population pharmacokinetic modelling

The means, medians and standard deviation of the population parameter estimates for the three models are shown in Table 11. The goodness-of-fit for each of the four models to the data was comparable. The log-likelihood values, AIC and outputs from the regression of observed versus predicted values after the Bayesian step, including the coefficients of determination ($r^2$), are shown in Table 12.
Table 11. Piperacillin population pharmacokinetic parameter estimates obtained by Pmetrics.

<table>
<thead>
<tr>
<th>Model and parameter</th>
<th>Vmax (mg/hr)</th>
<th>Km (mg/L)</th>
<th>Vc (L)</th>
<th>Kcp (h⁻¹)</th>
<th>Kpc (h⁻¹)</th>
<th>Cl (L/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piperacillin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>Mean</td>
<td>18.90</td>
<td>2.62</td>
<td>28.38</td>
<td>17.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>9.19</td>
<td>3.91</td>
<td>17.41</td>
<td>6.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>16.41</td>
<td>0.57</td>
<td>39.62</td>
<td>15.14</td>
<td></td>
</tr>
<tr>
<td>Michaelis-Menten</td>
<td>Mean</td>
<td>1634.68</td>
<td>78.57</td>
<td>13.10</td>
<td>20.89</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>597.33</td>
<td>52.51</td>
<td>7.13</td>
<td>14.72</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>1863.70</td>
<td>89.69</td>
<td>14.73</td>
<td>30.09</td>
<td></td>
</tr>
<tr>
<td>Parallel First-Order/MM</td>
<td>Mean</td>
<td>898.91</td>
<td>90.13</td>
<td>13.67</td>
<td>20.95</td>
<td>6.62</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>402.61</td>
<td>74.14</td>
<td>7.20</td>
<td>16.91</td>
<td>3.81</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>808.31</td>
<td>77.43</td>
<td>15.78</td>
<td>28.58</td>
<td>6.89</td>
</tr>
<tr>
<td>Tazobactam</td>
<td>Mean</td>
<td>21.13</td>
<td>5.96</td>
<td>29.10</td>
<td>15.16</td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>SD</td>
<td>10.75</td>
<td>11.77</td>
<td>17.14</td>
<td>4.63</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>20.31</td>
<td>0.37</td>
<td>39.69</td>
<td>13.68</td>
<td></td>
</tr>
</tbody>
</table>

[SD is the standard deviation; Vmax is the maximum elimination rate; Km is the Michaelis-Menten constant; Vc is the apparent volume of distribution of the central compartment; Kcp and Kpc are first order inter-compartmental transfer rate constant; Cl is clearance; MM is Michaelis-Menten.]
Table 12. Evaluation of the predictive performance of piperacillin and tazobactam population models.

<table>
<thead>
<tr>
<th>Model type</th>
<th>Log Likelihood</th>
<th>Linear regression of observed-predicted for each patient</th>
<th>AIC</th>
<th>Probability (compared with Linear)</th>
<th>Probability (compared with MM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intercept</td>
<td>Slope</td>
<td>r²</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Piperacillin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>-169.41</td>
<td>0.653</td>
<td>1.010</td>
<td>0.918</td>
<td>184.41</td>
</tr>
<tr>
<td>Michaelis-Menten</td>
<td>-156.86</td>
<td>0.945</td>
<td>0.992</td>
<td>0.943</td>
<td>177.86</td>
</tr>
<tr>
<td>Parallel First-Order/ MM</td>
<td>-154.54</td>
<td>0.822</td>
<td>1.000</td>
<td>0.939</td>
<td>182.54</td>
</tr>
<tr>
<td><strong>Tazobactam</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>-51.78</td>
<td>0.009</td>
<td>1.030</td>
<td>0.908</td>
<td>66.78</td>
</tr>
</tbody>
</table>

[r² is the coefficient of determination for the best-fit linear regression for the predicted-observed plot after the Maximal A-posteriori Probability (MAP) Bayesian step. AIC is the Akaike information criterion. MM is Michaelis-Menten.]
For the linear model fitted to the piperacillin data, a linear regression of the observed-predicted plot for each patient after the Bayesian step revealed:

\[
\text{observed} = 1.01 \times \text{predicted} + 0.653; \quad r^2 = 0.918.
\]

For the MM model:

\[
\text{observed} = 0.992 \times \text{predicted} + 0.945; \quad r^2 = 0.94.
\]

For the parallel first order/MM model, the regression line was:

\[
\text{observed} = 1.000 \times \text{predicted} + 0.822; \quad r^2 = 0.939.
\]

The log-likelihood values were -169.41, -156.86 and -154.54 for the linear, MM and parallel first-order/MM models respectively. Evaluation of the log-likelihood values against a \( \chi^2 \) distribution, with the appropriate number of degrees of freedom, revealed \( p < 0.005 \) for the MM and parallel first-order/MM models compared to the linear model (i.e. differences in the log likelihood values were statistically significant despite the larger number of parameters). The Akaike information criterion was 184.41, 177.86 and 182.54 for the linear, MM and parallel first-order/MM models respectively.

For tazobactam, linear regression of the observed-predicted plot for each patient after the Bayesian step revealed best fit regression line of observed

\[
\text{observed} = 1.03 \times \text{predicted} - 0.009; \quad r^2 = 0.908.
\]

The log-likelihood and AIC were -51.78 and 66.78, respectively. The population estimates for tazobactam clearance and volume of distribution were 15.16 L/hr and 21.13 L, respectively.

15.3. **Monte Carlo simulation**

15.3.1. **Observed versus simulated concentration-time profiles**

The three mathematical models: (a) first-order process; (b) a MM process alone; and (c) a MM process with parallel first-order elimination were compared using a 5,000 subject simulation of 3 grams of piperacillin administered over 4 hours every 8 hours. The mean population parameter
values and their SD for piperacillin, using a log-normal distribution, were readily recapitulated. Figure 6 shows the median, 5\(^{th}\) and 95\(^{th}\) percentile piperacillin concentrations for the population generated by each model alongside the observed patient data. Visual inspection shows the parallel linear/MM model most accurately represented the observed data.

15.3.2. **Comparison of amount of piperacillin removed by the clearance terms in the parallel linear/MM model**

Following administration of 3 doses of 4 grams of piperacillin over 4 hours every 8 hours, a median (% total, +/- 1 SD; range) of 6.13g (53.25%; +/- 2.24g; 0.35-11.52g) was cleared by the MM mechanism and a median (% total, +/-1 SD; range) of 5.39g (46.75%; +/- 2.17g; 0.35-11.52g) cleared by the linear mechanism for the parallel linear/MM model. Following administration of 3 doses of 4 grams of piperacillin over 30 minutes every 8 hours a median (%, +/- 1 SD; range) of 5.33g (45.75%; +/- 2.10g; 0.33-11.23g) was cleared by the MM mechanism and a median (%, +/-1 SD; range) of 6.31g (54.25%; +/- 2.14g; 0.41-11.64g) cleared by the linear mechanism. The mean (+/- SD) portion of the third dosing interval the total piperacillin concentration was greater that the Michaelis-Menten constant was 18.84% (+/-24.43%) for the third dose of 4 grams of piperacillin administered over 4 hours and 22.05% (+/- 16.67%) for 4 grams of piperacillin administered over 30 minutes. However, 2674 and 625 patients administered 4 grams of piperacillin over 4 hours and 30 minutes respectively, never had a piperacillin concentration above the Michaelis-Menten constant.
Figure 6. Comparison of the three mathematical models evaluated for piperacillin.
[The lines represent the median, 5th and 95th percentile concentration of unbound piperacillin concentration at steady-state for piperacillin 4g administered over 4 hours. Open circles represent observed piperacillin plasma concentrations. A - linear model; B - Michaelis-Menten model and C - Parallel First-Order/ Michaelis-Menten model.]
15.3.3. Probability of target attainment analyses

Although there were no significant differences in log-likelihood values and AIC between the MM and parallel linear/MM models, the parallel linear/MM models were used for the PTA analyses based on the results of the observed vs. simulated concentration-time profile plots. Also, this model is best supported by the known physiology. The results of the PTA analyses for piperacillin are displayed in Figure 7. Infusion of 4 grams of piperacillin, over 3 or 4 hours every 6 or 8 hours, results in a PTA of over 98% for organisms with MICs ≤8mg/L. For pathogens with an MIC of 16 mg/L the PTA was 94% when piperacillin was administered over 3 hours, 6 hourly and 82% when piperacillin was administered over 4 hours every 8 hours. For MICs > 16mg/L the PTA rapidly plummeted to zero for both regimens. Administration of 4 grams of piperacillin over 30 minutes, 6 or 8 hourly, results in a PTA of 95% and 87% for an MIC of 0.5mg/L. A gradual decline in target attainment was then observed as the MIC increased with a PTA of 81% and 61% for the 6 and 8 hourly regimens, respectively, for an MIC of 8mg/L. Administering 3 grams of piperacillin, over 3 or 4 hours every 6 or 8 hours, results in a PTA of over 98% for organisms with MICs ≤ 8 mg/L. For organisms with an MIC of 16mg/L the PTAs were 81% and 59% for piperacillin 3 grams administered over 3 or 4 hours every 6 or 8 hours, respectively, and 51% and 27% for piperacillin 3 grams administered over 30 minutes every 6 or 8 hours, respectively.
Figure 7. The results of the Monte Carlo simulation with the fractional target attainments.

15.4. Optimal sampling schedule for future studies

Summation of the D-optimal design analysis for each support point, following weighting by their probability, allowed identification of 6 sample time points (Figure 8). For piperacillin 4g/tazobactam 0.5g administered over 30 minutes every 8 hours the sampling time points should be 1.0,
3.25, 4.5, 5.25, 7.25 and 8 hours after initiation of the first dose and 0.25, 1.75, 4.0, 4.75, 6.0 and 7.75 hours after initiation of the dose at steady state (Table 13). For piperacillin 4g/tazobactam 0.5g administered over 4 hours every 8 hours the sampling time points should be 0.25, 1.0, 2.25, 3.25, 5.50 and 7.25 hours after initiation of the first dose and 0.25, 0.75, 2.0, 3.75, 4.5 and 6.25 hours after initiation of the dose at steady state.
Figure 8. Histograms showing the results of the D-optimal design analysis.
[A – Piperacillin 4g administered over 30 minutes; B – Piperacillin 4g administered over 4 hours; C – Tazobactam 0.5g administered over 30 minutes and D – Tazobactam 0.375g administered over 4 hours. The timings identified are during both the first-dose and the steady-state interval.]
Table 13. Table showing the optimal timings after the first and a steady-state dose identified by D-optimal design.

<table>
<thead>
<tr>
<th>Drug regimen</th>
<th>First dose (hours post-dose)</th>
<th>Steady state (hours post-dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piperacillin 4g administered over 4 hours</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tazobactam 500mg administered over 4 hours</td>
<td>0.25 0.75 2.25 4.00 4.75 6.50</td>
<td>0.25 0.75 1.75 2.75 3.50 4.75</td>
</tr>
<tr>
<td>Piperacillin 4g/Tazobactam 500mg administered over 4 hours</td>
<td>0.25 1.00 2.25 3.25 5.50 7.25</td>
<td>0.25 1.00 2.50 5.00 5.75 8.00</td>
</tr>
<tr>
<td>Piperacillin 4g administered over 30 minutes</td>
<td>1.00 4.25 5.75 6.25 7.25 8.00</td>
<td>0.25 1.00 4.25 5.00 6.00 7.75</td>
</tr>
<tr>
<td>Tazobactam 500mg administered over 30 minutes</td>
<td>0.75 2.25 3.50 4.25 7.00 8.00</td>
<td>0.50 2.25 3.75 4.25 6.25 8.00</td>
</tr>
<tr>
<td>Piperacillin 4g/ Tazobactam 500mg administered over 30 minutes</td>
<td>1.00 3.25 4.5 5.25 7.25 8.00</td>
<td>0.25 1.75 4.00 4.75 6.00 7.75</td>
</tr>
</tbody>
</table>
16. Discussion

Analysis of this cohort of hospitalized patients receiving extended infusions of PTZ found that use of a population model for piperacillin with a parallel linear/MM clearance term best described the observed data. After the Bayesian step, the predictive performances of the MM and parallel linear/MM model were statistically superior to the linear model. Although there were no significant differences in log-likelihood values and AIC between the MM and parallel linear/MM models the results of the observed vs. simulated concentration-time profiles plots (Figure 6) clearly demonstrated the parallel linear/MM model best fit the data. For these reasons the parallel linear/MM model was used for the PTA simulations and the D-optimal design. The superiority of a non-linear clearance structural model for piperacillin is consistent with the finding of several previous studies and contradictory to others. While there is support in the literature for both clearance structures, the parallel model linear/MM is most physiologically plausible as piperacillin is cleared via both linear glomerular filtration and non-linear tubular secretion. Quantification of the non-linear clearance demonstrated that on average just under half of the administered dose is cleared by the MM mechanism with both extended and intermittent dosing. Furthermore, the results of the simulations indicated that, on average, concentrations exceeded the Km for about 20% of the dosing interval with both infusion methodologies. While these findings do not represent a major concern with the PTZ dosing schemes currently used in clinical practice, the presence of a saturable clearance mechanism for piperacillin will be an important consideration if more intensive PTZ dosing schemes (>16 grams of piperacillin/day) are contemplated. Further study of PTZ PK following dosage escalation and in different study populations is required to confirm these observations.
With regards to the parallel linear/MM model, the population estimates for the volume of distribution and clearance in our study were similar to those observed in patients with sepsis by Roberts et al.\(^{600}\) but higher than other population analyses\(^{198,267,268}\). The enhanced or hyper-dynamic clearance conditions observed in our study were largely driven by the high average creatinine clearance observed among the patients included in our study. This is not the first time this hyper-clearance phenomenon among hospitalized patients has been described\(^{125,269-271}\). In keeping with other PK studies of critically ill patients, we observed significant variability with a coefficient of variation of around 50% for the population PK parameters\(^{272}\).

The consequences of the enhanced or hyper-clearance of piperacillin observed in our hospitalized patient study cohort were readily apparent in the Monte Carlo simulations. The stimulation suggests that for a pathogen with an MIC ≤8 mg/L, an extended infusion of 4 grams of piperacillin administered 6 or 8 hourly reaches an acceptable probability of target attainment rate of >98%. Extended infusions, administered every 6 or 8 hours, reach a satisfactory target attainment of 94% and 82%, respectively, for an MIC of 16mg/L. However intermittent administration of 4g piperacillin, either 6 or 8 hourly, only reaches satisfactory target attainments for the most sensitive of organisms. Collectively, the results of the Monte Carlo simulations suggest that changing medical practice from bolus dosing to an extended infusion would improve target attainment rates dramatically for organisms with an MIC of ≤16 mg/L. More importantly, the results of the simulations suggest that more intensive extended infusion PTZ dosing regimens (3.375- 4.5 g intravenously (3 hour infusion) every 6 hours) than those commonly used in clinical practice (3.375-4.5 g intravenously (4 hour infusion) every 8 hours) are required to maximize \(t_{>\text{MIC}}\) for higher MICs, especially in patients with augmented clearance of piperacillin. While it is
customary to make dose reductions in patients with renal impairment, there are not such recommendations for individuals with enhanced GFRs. Further PK studies are sorely needed to determine the most optimal dosage regimens in patients who present with augmented renal clearance. When designing these PK studies in patients with augmented renal clearance, the models should include a term to account of the potential non-linear clearance of piperacillin.

Based on best available data to date, the results of tazobactam simulations show that every 8 hour dosing provide reasonable concentrations against β-lactamase producing bacteria. Although very limited, in-vitro experiments suggest that the antibacterial activity of PTZ is lost when the inhibitor concentrations falls below a critical threshold of 4 mg/L. While daily AUCs were similar between intermittent and extended dosing schemes, the Monte Carlo simulations demonstrated that the time the tazobactam concentration is above 4mg/L is higher when an extended infusion is used as the method of administration (data not shown). While these results are reassuring, the PD target and its relationship to tazobactam PK are not well understood and require further elucidation.

In conclusion, we have shown a population model based on parallel linear/MM clearance best describes the observed data. The population estimates are consistent with previous studies, but show large inter-patient variability. Monte Carlo simulation suggests that PTZ at 4.5 grams administered over 3 hours every 6 hours can be used to successfully treat organisms with an MIC ≤ 16mg/L. However, there remain unanswered questions regarding the PD target of β-lactamase inhibitors and the impact that using extended infusion may have on β-lactamase/β-lactamase inhibitor interactions. Further clinical trials and in-vitro experiments are required to answer these questions. By providing the optimal sampling
schedule, from our D-optimal design analysis, we hope that future PK clinical trials are designed to capture the most accurate population estimates for both piperacillin and tazobactam with the minimal number of sampling time points.
CHAPTER 2: IMPACT OF BOLUS DOSING
VERSUS CONTINUOUS INFUSION OF
PIPERACILLIN AND TAZOBACTAM ON THE
DEVELOPMENT OF ANTIMICROBIAL
RESISTANCE IN PSEUDOMONAS AERUGINOSA

17. Abstract

Management of nosocomial pneumonia is frequently complicated by bacterial resistance. Extended infusions of beta-lactams are increasingly used to improve clinical outcomes. However, the impact of this strategy on the emergence of antimicrobial resistance is not known. A hollow fiber infection model with Pseudomonas aeruginosa (PA01) was used. Human-like pharmacokinetic (PK) profiles of piperacillin/tazobactam were simulated over five days. Three dosages of piperacillin/tazobactam were administered over 0.5 hrs or 4 hrs with re-dosing every 8hrs. Two initial bacteria densities were investigated (≈10^4 CFU/ml and ≈10^7 CFU/ml). The time courses of total bacterial population and the resistant sub-population were determined. All data were described using a mathematical model, which was then used to define the relationship between drug concentrations, bacterial kill and emergence of piperacillin resistance. There was logarithmic growth in controls in the initial 24 hrs reaching a plateau of ≈9 log_{10}CFU/mL. Bacterial killing following administration of piperacillin via bolus dosing or extended infusions was similar. For lower initial bacterial density trough total plasma piperacillin concentration:MIC ratios of 3.4 and 10.4, for bolus and extended infusion regimens, respectively, were able to suppress the emergence of piperacillin resistance. For the higher initial
bacterial density, all regimens were associated with progressive growth of a resistant sub-population. A stratified approach, according to bacterial density, is required to treat patients with nosocomial pneumonia. Antimicrobial monotherapy may be sufficient for some patients. However for patients with a high bacterial burden alternative therapeutic strategies, are required to maximize bacterial killing and prevent antimicrobial resistance.
18. Introduction

The attributable mortality of hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP) remains high despite treatment with antimicrobial chemotherapy\textsuperscript{274}. \textit{P. aeruginosa} is a common cause of nosocomial pneumonia\textsuperscript{275}. Approximately 10-50\% of patients treated for nosocomial pneumonia develop antimicrobial resistance\textsuperscript{49,276}. The development of resistance in \textit{P. aeruginosa} may account for a proportion of clinical failures following administration of standard therapeutic regimens. Emergence of antimicrobial resistance compromises the outcome of individual patients, but also has significant ramifications for treatment of critically ill populations\textsuperscript{276,277}.

For \(\beta\)-lactam antibiotics, the PD index that best links drug exposure with the antibacterial effect is the fraction of the dosing interval the free drug concentrations are above the bacterial minimum inhibitory concentration (\(fT_{>\text{MIC}}\))\textsuperscript{89,155}. Use of extended or continuous infusion maximizes the time drug concentrations are above the MIC\textsuperscript{71,105}. Compared with bolus dosing, increased bacterial killing is seen both in-vitro and in-vivo with infusions of \(\beta\)-lactam antibiotics\textsuperscript{162,278,279}. \textit{In silico} models suggest that exposures produced by infusions of \(\beta\)-lactam antibiotics generate a greater probability of target attainment compared with bolus dosing\textsuperscript{201}. Despite this, few studies have demonstrated a clinical advantage associated with \(\beta\)-lactam infusions and there is no information on the use of such regimens and the likelihood of emergence of drug resistance\textsuperscript{72,280}.

The PD index that best links drug exposure and the emergence of antimicrobial resistance is poorly defined. For meropenem, the ratio of minimum concentration to MIC (Cmin:MIC) has been linked to suppression of resistance\textsuperscript{170}. Studies using in vitro models suggest that emergence of
resistance often follows an "inverted U" shaped pattern—in this case, there is no amplification of resistant sub-populations at both low and high drug concentrations\textsuperscript{169}. Here, we use an in-vitro hollow fibre infection model (HFIM), with \textit{P. aeruginosa}, to examine the impact of administering PTZ by bolus dosing or extended infusion on the emergence of piperacillin resistance.
19. Materials and methods

19.1. Antimicrobial agent

For the HFIM, the clinical formulation of PTZ 2g/0.25g was used and supplied by Stragen UK (Surrey, UK). For in vitro susceptibility testing, development of drug-containing agar, and for HPLC, pure piperacillin and tazobactam was used, which was obtained from Sigma-Aldrich (Dorset, UK).

19.2. Microorganism

*P. aeruginosa* (PA01) was used for all experiments (kindly provided by Prof C. Winstanley (University of Liverpool, UK)). The bacterium was stored at ~80°C in cation-adjusted Mueller-Hinton II (Ca-MH) broth with 10% glycerol (Sigma-Aldrich, Dorset, UK). For each experiment, fresh isolates were grown on blood-agar plates (Oxoid Limited, Hampshire, UK) at 37°C for 24 hours. The mutational frequency to resistance was estimated, on two separate occasions, by plating aliquots of 0.1 mL of *P. aeruginosa* (PA01) onto ten Ca-MH agar plates containing 24 mg/L piperacillin and 3 mg/L of tazobactam (6×piperacillin MIC). Stability of piperacillin at 4°C was confirmed by quantification of the drug in the agar plates and reproducible enumeration of PA01 over a one week period (data not shown). The bacterial suspension was prepared as for injection into the HFIM (see below). The concentration of the bacterial suspension was determined by quantitative cultures. The ratio of the number of resistant bacteria to the total population was used to estimate the frequency of resistant isolates.
19.3. **Susceptibility studies**

The minimum inhibitory concentration (MIC) for PA01 was conducted on five occasions, in Ca-MH broth (Sigma-Aldrich, UK) using microbroth dilution methodology as described by the Clinical Laboratory Sciences Institute (CLSI).²⁸¹

19.4. **High-performance liquid chromatography**

Piperacillin concentrations were measured using a previously validated high-performance liquid chromatography (HPLC) method with a Shimadzu Prominence (Shimadzu, Milton Keynes, UK).²⁶⁰ Fifty µL of extracted sample was injected onto a Hypersil ODS C18 5um column 150x 4.6mm I.D. (Thermo Scientific, Hertfordshire, UK). A standard curve encompassing 1.56–400 mg/L was constructed in Ca-MH broth, from stock solutions of piperacillin 8,000 mg/L in water (Fisher Scientific, Loughborough, UK). The internal standard was penicillin G in water at 1000 mg/L (Sigma-Aldrich, Dorset, UK). The starting mobile phase was: 100% A 0.2M potassium phosphate monobasic buffer in 90:10 water: acetonitrile (v/v) with a gradient over 7 minutes progressing to 50% A and 50% B (acetonitrile) with a run time of 9 minutes and a flow rate of 1.5 mL/min. Piperacillin was detected using UV at 220 nm. Piperacillin and the internal standard eluted after 6.8 and 7.2 minutes, respectively. The intra- and inter-assay coefficient of variation was <5.8%. The limit of detection and quantification was 1.56 mg/L.

19.5. **Hollow-fibre infection model**

A HFIM was used to study the response of PA01 to PTZ. Two different inocula were used.²⁸² The HFIM circuit is illustrated in Figure 1. Briefly, outflow from the central compartment (containing 300 mL Ca-MH broth)
was connected, via a pump (≈7.2mL/min) (205U, Watson-Marlow, UK), to the hollow fibre cartridge (FibreCell Systems, USA), which then returned to the central compartment. Fresh Ca-MHB was pumped (≈3.3mL/min) from a reservoir into the central compartment. Drug was added to the central compartment utilizing a programmable syringe driver (Aladdin pump, World Precision Instruments, UK). Waste was removed, by pump (≈3.3mL/min), from the central compartment.

The inoculum was prepared by removing five colonies of PA01 from the blood-agar plate and suspending them in 30mL Ca-MH broth. The broth was incubated on a shaker at 37°C for four hours. The optical density of the stock inoculum was adjusted to 0.450 at 600nm (≈8x10^8 CFU/mL). For the high inoculum experiments, the stock was used without further dilution. For the low inoculum experiments, a further 1 in 4000 dilution was made in Ca-MH broth to achieve a final inoculum of ≈4 x10^5 CFU/mL. The high inoculum (≈8x10^8 CFU/mL) was chosen to be comparable with the bacterial density studied in previous in-vitro studies and, although high, is encountered in patients with VAP^170,282–284. The lower inoculum (≈4 x10^5 CFU/mL) is more typical of patients with VAP. A bacterial density of >10^4 CFU/mL is considered diagnostic of VAP while a bacterial density of 10^5 CFU/mL has been shown to have a greater diagnostic specificity for VAP^8,285. Each HFIM was then injected, via the sampling port, with 5 mL of either the high or low inoculum. The final inoculum was confirmed by quantitative culture on Ca-MH agar.

19.6. Pharmacokinetic and pharmacodynamic studies

PTZ 2g/0.25g was dissolved in 10 mL sterile 0.9% saline. The study drug was prepared for injection into the circuit in 5 mL syringes following further
dilution to achieve the desired concentrations. The effect of various dosages of PTZ (equivalent to piperacillin 3g, 9g and 17g in a human) was investigated. PTZ was administered over 30 minutes (bolus) or 4 hours (extended infusion), every eight hours for a total of 15 dosages. Each experimental run included four HFIMs, with three different piperacillin regimens and a drug-free control. A total of six experiments were performed including: the high inoculum against PTZ as a bolus (in duplicate); the high inoculum against PTZ as an extended infusion (in duplicate); the low inoculum against PTZ as a bolus; and, the low inoculum against PTZ as an extended infusion.

Piperacillin concentrations were estimated by removing 0.5 mL samples from the central compartment at 0.5, 1, 1.5, 2.5, 4.5 and 8 hours after the first and seventh dose. PK samples were stored at -80°C until processing. Drug concentrations were estimated in all HFIM experiments.

The total bacterial density and a drug-resistant sub-population in the HFIM were estimated by plating to drug-free and drug-containing Ca-MH agar plates (Sigma-Aldrich, UK). Drug-containing Ca-MH agar contained piperacillin 24 mg/L and tazobactam 3 mg/L. At 1, 25, 53, 73, 101 and 121 hours after inoculation, 0.5 mL was withdrawn from the core compartment of the HFIM. “Resistant” bacteria were defined as number of organisms enumerated on drug-containing agar. “Sensitive” bacteria were derived from the total number of bacteria, as counted on drug-free agar, minus the number of resistant bacteria.
19.7. **Mathematical modelling**

All PK and PD data were co-modelled using a population methodology with the program Pmetrics (version 0.3)\(^{255,256}\). The structural mathematical model was based on a previously published model of bacterial resistance\(^{286}\). The mathematical model comprised seven inhomogeneous ordinary differential equations.
**Equation 2.1** describes the rate of change of the amount of piperacillin (mg) in the central compartment. A one-compartment model with first-order elimination was used to describe the PK data obtained from all experiments.

**Equation 2.2** describes the rate of change of piperacillin concentrations in an effect compartment.

**Equation 2.3** describes the rate of change of burden of sensitive bacteria in the HFIM including terms that describe the theoretical maximal bacteria density (3a); the suppression of growth induced by piperacillin; (3b) and, the rate of bacterial killing induced by piperacillin (3c).

**Equation 2.4** describes the rate of change of bacterial density of resistant bacteria in the HFIM including terms that describe the theoretical maximal bacteria density (4a) and piperacillin concentration in the effect compartment associated suppression of growth (4b).

**Equation 2.5, 2.6** and 2.7 take the same form as equations 2.2, 2.3 and 2.4, but described the effect of drug on low inoculum experiments.

A schematic representation of the structural model is shown in Figure 10:

**Equation 2.1:**
\[
\frac{dX_1}{dt} = R(1) - \left(\frac{Cl}{V_c} \times X_1\right)
\]

**Equation 2.2:**
\[
\frac{dC_{eff}}{dt} = Kce \times \left(\frac{X_1}{V_c} - C_{eff}\right)
\]

**Equation 2.3a:**
\[
\frac{dCFU_s}{dt} = K_{gmax} \times CFU_s \times \left(1 - \frac{CFU_s + CFU_j}{POP\times MAX}\right)
\]
Equation 2.3b:
\[
\left(1 - \left(\frac{C_{eff}^{H_{ss}}}{C_{eff}^{H_{ss}} + (C50_{ss})^{H_{ss}}}ight)\right)^{-}
\]

Equation 2.3c:
\[
K_{max} \times CFU_s \times \left(\frac{C_{eff}^{H_{ss}}}{C_{eff}^{H_{ss}} + (C50_{ss})^{H_{ss}}}\right)
\]

Equation 2.4a:
\[
\frac{dCFU_r}{dt} = Kg_{max_r} \times CFU_r \times \left(1 - \frac{CFU_s + CFU_r}{POPMAX}\right) \times
\]

Equation 2.4b:
\[
\left(1 - \left(\frac{C_{eff}^{H_{sr}}}{C_{eff}^{H_{sr}} + (C50_{sr})^{H_{sr}}}\right)\right)
\]

In the differential equations (above) and in Figure 10, \(X_1\) is the total amount of piperacillin (mg) in the central compartment. \(R(1)\) is the infusion of piperacillin into the central compartment. \(Cl\) is the clearance of piperacillin from the central compartment (L/h), and \(Vc\) (L) is the volume of the central compartment. \(CFUs\) and \(CFUr\) are the number of sensitive and resistant organisms in the HFIM (log10CFU/mL). \(Kce\) is the rate constant governing movement of piperacillin to and from the central compartment. \(Kgmax_s\) and \(Kgmax_r\) represent the maximum rate of growth of the sensitive and resistant populations respectively (log10 CFU/h). \(POPMAX\) (CFU/mL) is the theoretical maximal bacterial burden within the HFIM. \(Kgmax_s, Kgmax,\) and \(POPMAX\) were assumed to be the same in the experiments examining both high and low inocula. \(C50ss\) and \(C50sr\) (mg/L) are the drug concentrations that produce half-maximal suppression of growth of the sensitive and resistant populations respectively. \(Hs_s\) and \(Hs_r\) are the slope functions for suppression of growth of the sensitive and resistant populations. \(Kkmax_s\) (log10CFU/mL) represent the maximum rate of kill of the sensitive
population.  C50ks (mg/L) is the drug concentration that produces half-maximal kill of the sensitive and resistant populations respectively.  Hk_s is the slope functions for killing of the sensitive population.  Killing of resistant bacteria was not observed and therefore not included in the mathematical model.  Clearance, Vc, Kgmax_s, Kgmax_r, and POPMAX were assumed to be the same in both the high and low inoculum experiments.  All the other PD parameters were specific to the studies examining the high and low inoculum.

![Figure 10. Schematic illustration of the population pharmacokinetic/pharmacodynamic model.](image)

Error polynomials were obtained by fitting the same structural mathematical model to the PK and PD data from each of the 24 individual HFIMs using the maximum likelihood estimator in the program ADAPT 5\textsuperscript{257,287}. The means, medians, and standard deviations of the population parameters were estimated. The fit of each of the structural models to the data was
further assessed by: (i) the log-likelihood value; (ii) the coefficient of determination ($r^2$) from a linear regression of the observed- predicted plots both before and after the Bayesian step; (iii) the Akaike information criterion; and (iv) comparison of the observed data with simulation based on the mean and median population parameter estimates.

19.8. Pharmacokinetic and pharmacodynamic simulations

Simulations were performed for each regimen and inoculum, using the mathematical model described above. The PK program ADAPT 5 was used\(^{257}\). Both the population and the individual median parameter estimates (i.e. after the Bayesian step) for each of the 24 HFIM were used. A number of endpoints were explored and reported in the simulations. The fraction of the final 3 dosing intervals (i.e. the final 24 hours of the experiment, on day 5) that the drug concentrations were above the MIC, 4-times the MIC and the area under the piperacillin concentration curve were estimated\(^{288}\).

A second set of simulations was performed for 17 piperacillin regimens between 0 to 20g three times daily, administered by bolus or extended infusion. The median parameter estimates for the population were used. The density of total bacteria and the resistant sub-populations after 121 hours (15 doses) and trough piperacillin concentration were estimated. The trough piperacillin concentration was adjusted for MIC (Cmin:MIC ratio).

19.9. Bridging from the hollow fibre infection model to humans

To explore the clinical implication of the experimental observations, the results of the HFIM were bridged to humans. A previously published parallel first-order/Michaelis-Menten clearance model for piperacillin plasma PK was used\(^{201}\). Four regimens were investigated using a 5,000-patient
Monte Carlo simulation. The regimens were: (a) piperacillin 4 grams administered over 30 minutes; or (b) 4 grams administered over 4 hours, with repeat dosing every 8 hours; or (c) piperacillin 4 grams administered over 30 minutes; or (d) piperacillin 4 grams administered every 3 hours with repeated dosing every 6 hours. The mean parameter values and their associated variance (obtained from the output of the original population PK model\textsuperscript{201} were embedded in subroutine PRIOR of the ADAPT 5 program\textsuperscript{257}. The parameter estimates $\pm$ standard deviations were as follows: $V_{\text{max}}$ (mg/h) 898.91 $\pm$ 402.61; $K_m$ (mg/L) 90.13 $\pm$ 74.14; $V_c$ (L) 13.67 $\pm$ 7.20; $K_{cp}$ (h$^{-1}$) 9.19 $\pm$ 10.25; $K_{pc}$ (h$^{-1}$) 20.95 $\pm$ 16.91 and $CL$ (L/h) 6.62 $\pm$ 3.81\textsuperscript{201}. A log-normal parameter distribution was used in the simulations. Protein-binding for piperacillin was assumed to be 30%\textsuperscript{146}. For each regimen, the fraction of simulated subjects who achieved the PD target of a trough total piperacillin concentration:MIC ratio of either 3.4 for bolus regimens or 10.4 for extended infusions, for a range of MICs from 0.0625 to 64 mg/L was determined.
20. **Results**

20.1. **MIC values**

The piperacillin MIC in Ca-MH broth for PA01 was 2, 4, 4, 8 and 8 mg/L. A median MIC of 4 mg/L was used in subsequent analyses.

20.2. **Mutational frequency to resistance**

The mutational frequency of PA01 to piperacillin was $1.04 \times 10^7$ at 6-times MIC (i.e. there was 1 resistant bacterium for every $1.04 \times 10^7$ bacteria).

20.3. **Results of the hollow fibre infection model**

20.3.1. **Pharmacokinetics**

Extended infusion regimens maintained the piperacillin concentration above the MIC for a greater proportion of the dosing interval. Trough piperacillin concentrations were lower with the bolus regimen when compared with the same total dosage delivered by extended infusion (Figure 11; Table 14 and Table 15).
Figure 11. Pharmacokinetic data. Panel showing the fit of the mathematical model to the observed piperacillin concentrations. [4a – 3g bolus; 4b – 9g bolus; 4c – 17g bolus; 4d – 3g extended infusion; 4e – 9g extended infusion; 4f – 17g extended infusion.]
Table 14. Table showing the mean, median and standard deviation of the parameter estimates from the mathematical model.

<table>
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<tr>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl (Litres/hr)</td>
<td>0.167</td>
<td>0.037</td>
<td>0.164</td>
</tr>
<tr>
<td>Va (Litres)</td>
<td>0.367</td>
<td>0.073</td>
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<tr>
<td>POPMAX (log$_{10}$ CFU/ml)</td>
<td>3.89 x10$^9$</td>
<td>2.91 x10$^9$</td>
<td>3.25 x10$^9$</td>
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<tr>
<td>Kgmax$<em>{s}$ (log$</em>{10}$ CFU/ml/hr)</td>
<td>0.642</td>
<td>0.035</td>
<td>0.630</td>
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<tr>
<td>Kgmax$<em>{r}$ (log$</em>{10}$ CFU/ml/hr)</td>
<td>0.450</td>
<td>0.044</td>
<td>0.445</td>
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### High inoculum

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
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<th>Median</th>
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</thead>
<tbody>
<tr>
<td>Kce (hr$^{-1}$)</td>
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<tr>
<td>C50s$_{s}$ (mg/Litres)</td>
<td>2.805</td>
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<td>Hs$_{s}$</td>
<td>8.364</td>
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<td>9.792</td>
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<tr>
<td>Kgmax$<em>{s}$ (log$</em>{10}$ CFU/ml/hr)</td>
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<td>0.017</td>
<td>0.097</td>
</tr>
<tr>
<td>Hk$_{s}$</td>
<td>2.089</td>
<td>2.854</td>
<td>1.197</td>
</tr>
<tr>
<td>C50k$_{s}$ (mg/Litres)</td>
<td>343.664</td>
<td>302.027</td>
<td>231.662</td>
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<tr>
<td>C50$_{s}$ (mg/Litres)</td>
<td>35.516</td>
<td>4.334</td>
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<tr>
<td>Hs$_{r}$</td>
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<td>2.480</td>
<td>0.978</td>
</tr>
<tr>
<td>Initial Conditions (Total)</td>
<td>3.51 x10$^7$</td>
<td>1.61 x10$^7$</td>
<td>3.20 x10$^7$</td>
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<tr>
<td>Initial Conditions (Resistant)</td>
<td>11.998</td>
<td>6.929</td>
<td>8.554</td>
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### Low inoculum

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<th>Parameter</th>
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<tr>
<td>Kce (hr$^{-1}$)</td>
<td>0.056</td>
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<td>C50s$_{s}$ (mg/Litres)</td>
<td>3.189</td>
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<td>Hs$_{s}$</td>
<td>2.510</td>
<td>1.644</td>
<td>1.670</td>
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<tr>
<td>Kgmax$<em>{s}$ (log$</em>{10}$ CFU/ml/hr)</td>
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<td>0.029</td>
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<td>Hk$_{s}$</td>
<td>3.321</td>
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<td>C50k$_{s}$ (mg/Litres)</td>
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<td>16.455</td>
<td>73.011</td>
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<tr>
<td>C50$_{s}$ (mg/Litres)</td>
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<tr>
<td>Hs$_{r}$</td>
<td>10.346</td>
<td>2.218</td>
<td>10.862</td>
</tr>
<tr>
<td>Initial Conditions (Total)</td>
<td>5.34 x10$^4$</td>
<td>2.64 x10$^4$</td>
<td>5.11 x10$^4$</td>
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<tr>
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<td>0.038</td>
<td>0.011</td>
<td>0.036</td>
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</tbody>
</table>

[Cl is the piperacillin clearance from the central compartment; Vc is the volume of the central compartment; Kce is the rate constant governing movement of piperacillin to and from the central compartment; Kgmax$_{s}$ and Kgmax$_{r}$ represent the maximum rate of growth of the sensitive and resistant populations; POPMAX is the maximal bacterial burden; C50s$_{s}$ and C50s$_{r}$ are the drug concentrations that produce half-maximal suppression of growth of the sensitive and resistant populations; Hs$_{s}$ and Hs$_{r}$ are the slope functions for suppression of growth of the sensitive and resistant populations; Kgmax$_{s}$ represent the maximum rate of kill of the sensitive population; C50k$_{s}$ is the drug concentration that produces half-maximal kill of the sensitive and resistant populations respectively; Hk$_{s}$ is the slope functions for killing of the sensitive population]
### Table 15. Table showing the PK-PD indices for the six dosage regimens.

<table>
<thead>
<tr>
<th>Piperacillin dosage</th>
<th>$T_{\text{MIC}}$ (%)</th>
<th>$T_{\text{4xMIC}}$ (%)</th>
<th>AUC (mg/L/24hr)</th>
<th>Cmin (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3g Bolus</td>
<td>81%</td>
<td>43%</td>
<td>457.8</td>
<td>2.0</td>
</tr>
<tr>
<td>3g Extended infusion</td>
<td>100%</td>
<td>59%</td>
<td>457.8</td>
<td>5.2</td>
</tr>
<tr>
<td>9g Bolus</td>
<td>100%</td>
<td>81%</td>
<td>1831.3</td>
<td>8.1</td>
</tr>
<tr>
<td>9g Extended infusion</td>
<td>100%</td>
<td>100%</td>
<td>1831.3</td>
<td>20.8</td>
</tr>
<tr>
<td>17g Bolus</td>
<td>100%</td>
<td>100%</td>
<td>3662.5</td>
<td>16.2</td>
</tr>
<tr>
<td>17g Extended infusion</td>
<td>100%</td>
<td>100%</td>
<td>3662.5</td>
<td>41.6</td>
</tr>
</tbody>
</table>

[T>MIC is the time the free piperacillin concentration in the central compartment was above the MIC; $T>4xMIC$ time the free piperacillin concentration in the central compartment was above the four times the MIC; AUC is the area under the concentration time curve and Cmin is the trough piperacillin concentration.]

### 20.3.2. Untreated controls at the high and the low inoculum

Logarithmic growth occurred in the total bacterial population with an estimated maximum bacterial density of $3.89 \times 10^9$ CFU/mL (Table 14; Figure 12). Growth of the resistant sub-population achieved a maximum density of $\approx 10^2$ CFU/mL at which point there was no further growth of the resistant sub-population. The final bacterial density of both the total population (POPMAX) and the resistant sub-population was similar for both inocula.
20.3.3. *Impact of a bolus dosage and extended infusion regimen of piperacillin to treat a high inoculum of PA01*

Suppression of total bacterial growth was observed in the initial 48-72 hours with all dosages (Table 14 and Figure 13). Amplification of a resistant sub-population occurred with all piperacillin dosages when administered as either a bolus or an extended infusion (Figure 13). Dose-dependent suppression of growth of the resistant sub-population was seen. For all three dosages, the total bacterial population was replaced by the resistant sub-population by 96 hours. For the highest dosages there was suppression of growth of the total bacterial population, but the population consisted entirely of a resistant sub-population. Expansion of the resistant sub-population occurred at a similar rate following administration of piperacillin by bolus dosing or extended infusion regimens.
Figure 13. Effect of PTZ therapy with a high inoculum.
[The observed bacteria densities for the total (□) and resistant bacterial (x) populations are shown. The solid and dotted lines represents the fit of the mathematical model to the data for 5a – 3g bolus; 5b – 9g bolus; 5c – 17g bolus; 5d – 3g extended infusion; 5e – 9g extended infusion; 5f – 17g extended infusion.]
Figure 14. Effect of PTZ therapy with a low inoculum. [Panel showing observed bacteria densities for the total (□) and resistant bacterial (x) populations. The solid and dotted lines represent the fit of the mathematical model to the data for 6a – 3g bolus; 6b – 9g bolus; 6c – 17g bolus; 6d – 3g extended infusion; 6e – 9g extended infusion; 6f – 17g extended infusion.]
20.3.4. **Impact of a bolus dosage and extended infusion regimen of piperacillin to treat a low inoculum of PA01**

At the lowest dosage, suppression of growth of the total bacterial population was observed (Table 14 and Figure 14). However, there was progressive growth of the resistant sub-population. By 120 hours, all bacterial isolates were resistant to piperacillin. Following the administration of 9g and 17g of piperacillin there was a reduction in total CFU of \( \approx 3.6 \) and \( \approx 1.4 \log_{10} \text{CFU/mL} \) for the bolus dosage, and \( \approx 3.4 \) and \( \approx 1.1 \log_{10} \text{CFU/ml} \) for the extended infusion, respectively. No resistance emerged at the highest two dosages of piperacillin.

20.4. **Mathematical modelling**

The fit of the mathematical model to the observed data was acceptable. The \( r^2 \) for the observed-versus-predicated piperacillin concentrations was 0.908 using the median model parameter values (Bayesian prior) to calculate predicted values. For the high inoculum experiments, the \( r^2 \) values of Bayesian prior predictions vs. observations for the bacterial densities were 0.329 and 0.782 for the total population and resistant sub-population, respectively. For the low inoculum experiments the \( r^2 \) values of Bayesian prior predictions for the bacterial densities were 0.884 and 0.885 for the total population and resistant sub-population, respectively. The \( r^2 \) for the linear regression for all observed-predicted values improved by using the median parameter values of the Bayesian posterior obtained by updating the prior distribution for each experiment. Faster growth occurred in the sensitive sub-population compared with the resistant sub-population (i.e. \( K_{g_{\text{max}}} = 0.642 \log_{10} \text{CFU/ml/h} \) versus \( K_{g_{\text{max}}} = 0.450 \log_{10} \text{CFU/ml/h} \)).
20.5. **Bridging study**

The simulations suggested a dose-dependent change in bacterial densities based on findings from the high and low bacterial inoculum experiments (Figure 15). The changes in bacterial densities with increasing piperacillin exposure represents an “inverted U” shaped curve similar to that described with fluoroquinolones. At high bacterial densities (\( \approx 7 \log_{10} \text{CFU/mL} \)), a trough plasma piperacillin concentration: MIC ratio of 4.6 and 11.9, for the bolus and extended infusion regimens respectively, was required to suppress growth of the total bacterial population (Table 16). However, at these trough concentrations there was expansion of the resistant sub-population.

**Figure 15.** Panel showing the change in bacterial density with trough free piperacillin:MIC ratio following 5 days of treatment and target attainment of clinical regimens. [Left hand panel refer to bolus dosing regimens and right hand panel refer to extended infusion regimens. The top two panels show the results for the lower inocula; the lower two panels show the results for the higher inocula. Solid line – total population. Dashed line – resistant sub-population. Fine dotted line – stasis line. Arrow – relevant Cmin:MIC ratio.]
Table 16. Table showing the PD targets identified in the HFIM.

<table>
<thead>
<tr>
<th></th>
<th>Bolus Cmin:MIC (mg/L)</th>
<th>Extended infusion Cmin:MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hollow Fibre</td>
<td>Predicted Plasma*</td>
</tr>
<tr>
<td><strong>Low bacterial density</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial stasis (Total bacteria)</td>
<td>1.4</td>
<td>2.0</td>
</tr>
<tr>
<td>1 log reduction in total CFU/ml</td>
<td>1.8</td>
<td>2.6</td>
</tr>
<tr>
<td>2 log reduction in total CFU/ml</td>
<td>2.4</td>
<td>3.4</td>
</tr>
<tr>
<td>3 log reduction in total CFU/ml</td>
<td>3.2</td>
<td>4.6</td>
</tr>
<tr>
<td>Suppression of resistance</td>
<td>2.4</td>
<td>3.4</td>
</tr>
<tr>
<td><strong>High bacterial density</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial stasis (Total bacteria)</td>
<td>3.2</td>
<td>4.6</td>
</tr>
</tbody>
</table>

[*protein binding is assumed to be 30%*]
At low initial bacterial densities, a trough plasma piperacillin concentration:MIC ratio of 3.4 and 10.4, for the bolus and extended infusion regimens respectively, was required to suppress bacterial resistance. At these trough concentrations there was >2 log reduction in total bacterial density. The Monte Carlo simulation showed that \( \approx 60\% \) of patients administered the bolus or extended infusion regimens were expected to achieve these trough concentrations with a regimen of 4g administered 8 hourly when the MIC was low (Figure 16). Increase in the MIC results in a reduction of the probability of target attainment with suppression of resistance occurring in 14.6% and 5.8% of patients when the MIC is 4 mg/L. Administration of 16g of piperacillin daily improved the target attainments to \( \approx 80\% \), for either regimen for highly-susceptible organisms.
Figure 16. Panel showing the results of the Monte Carlo simulation with the probability of target attainments against a range of MICs.
21. Discussion

This study investigates the impact on antibacterial activity and the emergence of piperacillin resistance following administration of PTZ by bolus versus extended infusion. Piperacillin administered by either bolus or extended infusion results in comparable antibacterial activity and emergence of antimicrobial resistance. In the setting of a high bacterial inoculum, expansion of a resistant sub-population occurs even when there is an overall decline in bacterial density. In a clinical setting a decline in bacterial density may only be achieved at the expense of the development of a resistant bacterial sub-population. These resistant bacteria may be responsible for a subsequent clinical relapse in an individual patient or serve as a source for horizontal transmission. Prevention of the emergence of a resistant sub-population in the setting of a high bacterial inoculum may require an alternative therapeutic approach, such as combination chemotherapy. In the low bacterial inoculum experiments, trough plasma total piperacillin concentration:MIC ratios of 3.4 and 10.4, from the bolus and extended infusion regimens, respectively, are required to suppress the emergence of antimicrobial resistance. Results of the Monte Carlo simulation suggest that that fewer than 11% of patients achieve these concentrations using regimens of piperacillin 12 g/day in three divided dosages for an organism with an MIC of 4mg/L. Although treatment of organisms with more susceptible MICs or increasing the total piperacillin dosage to 16 g daily leads to an increase the rates of target attainment, this is still far from optimal.

A bacterial density of >10^4 CFU/ml in bronchoalveolar lavage fluid is required for a diagnosis of VAP. However, bacterial densities as high as 10^8 CFU/ml are frequently encountered in patients with this syndrome.
Bacterial densities spanning a similar range \((10^4 - 10^8 \text{ CFU/ml})\) are also present in patients with HAP\(^{289}\). The high and low inocula used in this in-vitro study were selected to encompass the range of these clinically relevant bacterial densities. Antimicrobial management for patients with HAP and VAP is complex with many conflicting results from both clinical and pre-clinical studies\(^6\). In-vitro studies, including this study, suggest that monotherapy with a β-lactam agent to treat high densities of *Pseudomonas aeruginosa* is insufficient to suppress the emergence of antimicrobial resistance\(^{61,288}\). The addition of a second agent to a β-lactam, (e.g. an aminoglycoside or fluoroquinolone) may enable suppression of emergence of resistance, but this requires further study\(^{290}\).

The PK variability of β-lactam antibiotics in critically ill patients has led to suggestions that therapeutic drug monitoring may be a useful adjunct to therapy\(^{284}\). Trough concentrations are a clinically convenient therapeutic target because of the ease with which samples can be interpreted. The fraction of the dosing interval that drug concentrations are above a threshold (e.g. some multiple of the MIC) requires more intensive sampling. When the bacterial burden is \(\approx 10^4 \text{ CFU/mL}\) (the density required for a diagnosis of VAP), the piperacillin concentration:MIC ratio within the HFIM that suppresses the emergence of piperacillin resistance is 2.4 and 7.3 for the bolus and extended infusion regimens, respectively. This corresponds to a plasma Cmin:MIC ratio of 3.4 and 10.4 for the bolus and extended infusion regimens, respectively, assuming piperacillin protein binding is 30%. That the Cmin:MIC ratio is consistent with the ratio of 6.2 for bolus regimens of meropenem\(^{170}\). The Cmin:MIC ratio identified in the HFIM is achieved in insufficient numbers of critically ill patients suggests that
considerable dosage escalation may be required to achieve adequate drug exposure.

A limitation of the HFIM is the lack of immune-mediated bacterial killing. Nevertheless, the absence of immune function permits the direct estimation of the extent of antimicrobial activity that can be attributed to a drug. The PD targets identified in the HFIM may be different if immune mediated bacterial killing was also present. This provides a safety margin when bridging the results into humans by delineating a “worst case scenario”. Additionally, the results from the HFIM may be applied to immunocompromised patients. The PD targets identified with the HFIM require validation with additional strains of Pseudomonas with different MICs and/or mechanisms of antimicrobial resistance.

In summary, this study suggests the following: (a) bolus regimens are equivalent to intermittent infusion in terms of the antibacterial effect and the emergence of drug resistance; and (b), that bacterial burden has a significant influence on the ultimate outcome of antibacterial therapy. Patients with low bacterial burdens, (e.g. \( \approx 10^4 \) CFU/ml) of P. aeruginosa may potentially be treated with monotherapy, with little chance of driving resistance. This will minimize the potential adverse events associated with combination chemotherapy, such as nephrotoxicity from aminoglycosides. In contrast, patients with a higher bacterial density may require additional adjunctive therapies, such as combination chemotherapy, to prevent the emergence of antimicrobial resistance. The impact on bacterial density on emergence of antimicrobial resistance in other bacterial species warrants further in-vitro investigation. Future clinical management of patients with HAP and VAP may require patients to be stratified according to bacterial
species antimicrobial resistance pattern and pathogen density in order to select the optimal individual regimen.
CHAPTER 3: PLASMA AND INTRA-PULMONARY POPULATION PHARMACOKINETICS OF PIPERACILLIN AND TAZOBACTAM IN CRITICALLY ILL PATIENTS.

22. Abstract

Pulmonary infections in critically ill patients are common and associated with high morbidity and mortality. Piperacillin-tazobactam is a frequently used therapy in critically ill patients with pulmonary infection. Antibiotic concentrations in the lung reflect target site antibiotic concentrations in patients with pneumonia. The aim of this study was to assess the plasma and intra-pulmonary pharmacokinetics (PK) of piperacillin-tazobactam in critically ill patients administered standard piperacillin-tazobactam regimens. A population PK model was developed to describe plasma and intra-pulmonary piperacillin and tazobactam concentrations. The probability of piperacillin exposures reaching pharmacodynamic endpoints and the impact of pulmonary permeability on piperacillin and tazobactam pulmonary penetration was explored. The median piperacillin and tazobactam pulmonary penetration ratio was 49.3% and 121.2%, respectively. Pulmonary piperacillin and tazobactam concentrations were unpredictable and negatively correlated to pulmonary permeability. Current piperacillin-tazobactam regimens are insufficient to treat pneumonia in 14% of critically ill patients caused by organisms defined as susceptible to piperacillin-tazobactam.
23. Introduction

Pulmonary infection in critically ill patients results in an unacceptably high mortality and morbidity, which increases the length of hospital stay and associated healthcare costs\textsuperscript{15,291}. Approximately 16\% of patients admitted to intensive care units (ICU) present with a pulmonary infection\textsuperscript{1}. Additionally, the lung is the primary site of infection in over 60\% of nosocomial infections occurring within the ICU\textsuperscript{1}. Attributable mortality from ventilator-associated pneumonia is estimated to be 13\%, but may be as high as 69\% in certain subgroups\textsuperscript{41}. Pulmonary infections in critically ill patients are caused by a wide range of organisms, including difficult-to-treat organisms such as \textit{P. aeruginosa}\textsuperscript{14}. Use of appropriately targeted antimicrobial chemotherapy is associated with improved clinical outcomes\textsuperscript{91}. However, clinical outcomes in patients that are infected with a susceptible organism receiving an appropriate antimicrobial agent remain sub-optimal. This is partly due to marked PK variability occurring in critically ill patients\textsuperscript{292}. The PK of critically ill patients may be affected by physiological changes associated with illness, which typically results in a higher proportion of patients receiving sub-optimal drug exposure when a fixed regimen is used\textsuperscript{10,201,292}. Additionally, many currently licensed drug regimens are informed by studies performed in non-critically ill patients, and may not necessarily be appropriate outside that context.

PTZ is a combination of an extended-spectrum β-lactam antibiotic (piperacillin) with a β-lactamase inhibitor (tazobactam). PTZ has a broad-spectrum of action that includes Gram-positive, Gram-negative and anaerobic bacteria\textsuperscript{234}. Consequently, PTZ is a common choice for both directed and empirical treatment of critically ill patients\textsuperscript{6}. The PD index that best links piperacillin concentrations with its antimicrobial effect is the
fraction of the dosing interval that unbound piperacillin concentrations are above the minimum inhibitory concentration (MIC)\textsuperscript{157}. Near-maximal antimicrobial effect is generally observed when free piperacillin concentrations exceed the MIC for at least 50% of the dosing interval (50% fT\textsubscript{>MIC})\textsuperscript{69}. However, 100% fT\textsubscript{>MIC} may be more appropriate for critically ill patients\textsuperscript{160}. The global increase in the incidence of antimicrobial resistance has focused attention on antimicrobial drug regimens that are safe, effective and also minimise the probability of the emergence of antimicrobial resistance\textsuperscript{293}. We recently used a hollow fibre infection model of PTZ versus \textit{P. aeruginosa} to demonstrate a trough (Cmin) total piperacillin concentration-to-MIC ratio of between 3 and 10 prevents the emergence of antimicrobial resistance\textsuperscript{294}. Identification of PTZ regimens that enable the attainment of PD targets for both efficacy and suppression of emergence of antimicrobial resistance may led to improved clinical outcomes and increase the clinical longevity of this commonly used agent.

Adequate antibiotic concentrations at the site of infection are required for effective antimicrobial activity\textsuperscript{13}. For pulmonary infection, the epithelial lining fluid (ELF) represents a compartment that is both clinically relevant and accessible for measurement of drug concentrations\textsuperscript{147,148}. In general, clinical β-lactam exposure–response relationships within ELF are poorly defined\textsuperscript{147}. An understanding of drug penetration into ELF and drug exposure-response relationships within that compartment are an important consideration when bridging from pre-clinical to clinical studies\textsuperscript{295}. Healthy volunteer data suggests the area under the concentration (AUC) time curve in ELF is ≈25\% and ≈50\% of the plasma piperacillin and tazobactam AUCs, respectively\textsuperscript{235}. While there is a general paucity of information regarding ELF penetration of antimicrobial agents in critically ill patients, two studies
suggest that piperacillin and tazobactam ELF concentrations are ≈50% and 65-90% of their respective paired plasma concentrations\textsuperscript{150,151}.

The primary aim of this clinical study was to quantify the pulmonary penetration of piperacillin and tazobactam in critically ill patients. We also investigated factors that may influence the penetration of drug into the lung. A population PK model was used to describe the observed plasma and ELF concentrations of piperacillin and tazobactam in critically ill patients. Monte Carlo simulation was used to explore the impact of PK variability on plasma and ELF piperacillin exposures to achieve the desired PD target. Additionally, the influence of pulmonary permeability on pulmonary piperacillin and tazobactam concentrations was investigated.
24. Methods

24.1. Pharmacokinetic study

This was a prospective open-label single arm PK study. The study was conducted in accordance with the Declaration of Helsinki and approved in the UK by both the local Research Ethics Committee and the Medicine and Healthcare Products Regulatory Agency (EudraCT number: 2011-004470-28). Intubated patients who received PTZ for suspected or documented pulmonary infection at the University Hospital of South Manchester NHS Trust, Manchester, UK were eligible for inclusion. PTZ 4g/0.5g (Stragen, UK) was administered over less than 30 minutes every eight hours except in patients with a creatinine clearance of <20ml/min or those on renal replacement therapy who were administered the drug every 12 hours. Written informed consent was obtained from the next of kin of all patients participating in the study. Additionally retrospective informed consent was obtained from all patients that survived and regained capacity to give consent. Demographic data (including age, sex, race, height, weight), disease severity (by APACHE II and SOFA score), underlying renal function, presence of renal replacement therapy and clinical outcome were recorded.

Sampling was performed following administration of the first dose of PTZ if possible. All patients underwent sampling at steady-state. The mean half-life of piperacillin is ≈0.75 hr so patients were assumed to be at steady-state by the second dose\textsuperscript{201}. A previously published, optimally designed sampling schedule was used to inform the timings for collection of the plasma samples\textsuperscript{201}. Plasma samples were collected at ½, 1½, 2½, 3¾, 5, 6 hours after initiation of the infusion for the first dose and immediately prior
to the dose and ¼, ¾, 2, 3½ and 4½ hours following initiation of the infusion at steady state. All plasma samples were collected in lithium heparin containing tubes. As soon as collected all plasma samples were centrifuged at 1,400 x g for 12 minutes. Samples were stored at -80 °C in 0.4 mL aliquots prior to analysis.

Non-directed bronchial lavage (NBL) was used for recovery of intra-pulmonary samples\textsuperscript{296}. Two intra-pulmonary samples were collected on each patient during the steady-state dosing interval. Samples were collected at ¾ and 2 or ¾ and 3½ hours following initiation of the infusion. Patients: (a) requiring > 80% inspired oxygen; (b) requiring > 12 cmH20 positive end expiratory pressure; (c) in whom endotracheal suction leads to a severe and prolonged desaturation; (d) with severe bronchospasm; (e) with uncontrolled or persistently raised intracranial pressure or (f) with severe disseminated intravascular coagulation did not have NBL samples collected. Briefly, suitable patients were pre-oxygenated with 100% oxygen for 2 minutes prior to sampling. A suction catheter was introduced into the bronchia tree until wedged and 20 mL of sterile normal (0.9%) saline was instilled over 5-10 seconds and then immediately aspirated. Typically 10 mLs of normal saline was recovered. Patients were monitored for four hours after the NBL for signs of cardio-respiratory compromise. As soon as collected all NBL samples were filtered through a 48 µm filter and centrifuged at 2,000 x g for 10 minutes. Samples were stored at -80 °C in 0.5 mL aliquots prior to analysis.

24.2. \textbf{Piperacillin, tazobactam, urea and protein assays.}

Piperacillin and tazobactam concentrations in plasma and lavage fluid were measured using a validated liquid chromatography–tandem mass
spectrometry (LC/MS/MS) method with an Agilent 6420 Triple Quad Mass spectrometer (Agilent Technologies UK Ltd, Cheshire, UK). Twenty µL of extracted sample was injected onto a Synergi 4u Hydro RP 80A 100x2.0mm column (Phenomenex, Cheshire, UK). The standard curves for piperacillin and tazobactam encompassing the concentration ranges of 0.02-10.0 mg/L and 0.02-5.0 mg/L respectively for plasma and 0.02-10.0 mg/L for lavage fluid were constructed in plasma and blank lavage fluid, respectively. The standard curves were made from a stock solution of 1 mg/L of piperacillin and tazobactam, respectively. The internal standard was caffeine in water at 0.1 mg/L (Sigma Aldrich, Dorset, UK). The between-day coefficients of variation were <17.4% for piperacillin and <15.5% for tazobactam. The lower limit of detection for piperacillin and tazobactam in plasma and lavage fluid was 0.02 mg/L.

Urea concentrations in plasma and lavage fluid were performed using a colorimetric technique (QuantiChromTM Urea Assay Kit DIUR-500, Gentaur BVBA – Bioxs, Belgium). The standard curve for the urea assay is linear over a concentration range of 0-100 mg/dL. Plasma samples were diluted 1:5 prior to measure of urea. The ELF dilution, introduced by lavage sampling, was calculated by the urea dilution method\textsuperscript{297,298}. Here comparison of urea concentration in the plasma and lavage fluid allows estimation of the dilution caused by instillation of lavage fluid to the lung. The concentration of piperacillin and tazobactam in ELF was estimated using the following formula:

\[
[\text{Drug}]_{\text{ELF}} = \frac{[\text{Urea}]_{\text{plasma}}}{[\text{Urea}]_{\text{lavage}}} \times [\text{Drug}]_{\text{lavage}}
\]

Where \([\text{Drug}]_{\text{ELF}}\) and \([\text{Drug}]_{\text{lavage}}\) are the concentration of either piperacillin or tazobactam in ELF and lavage fluid, respectively. \([\text{Urea}]_{\text{plasma}}\) and
[Urea]_{lavage} are the concentrations of urea in the plasma and lavage, respectively.

Pulmonary permeability was assessed by the ratio of the mean total protein concentrations in plasma and ELF\(^{299}\). Total protein was quantified in plasma using the Total Protein assay on an Abbott Architect C16000 (Abbott Laboratories, IL, USA). This colorimetric assay used biuret reagent to detect the presence of peptide bonds. The limit of detection for total protein was 0.5 g/dL. The limit of quantification was 0.76 g/dL. The imprecision of the Total Protein assay is ≤ 3% total coefficient of variation. Protein in ELF was quantified using UPro assay on an Abbott Architect c8000 (Abbott Laboratories, IL, USA). This assay uses a turbidimetric procedure in which benzethonium chloride is used as the protein denaturing agent. The limit of quantification and detection for the UPro assay is 6.75 mg/dL. The imprecision of the assay is ≤ 7.8% total coefficient of variation. ELF protein concentration was corrected for dilution using the urea dilution method described above.

24.3. Population pharmacokinetic analysis

All data were analyzed using a population PK methodology with the non-parametric adaptive grid (NPAG) program Pmetrics 1.1.3\(^{255}\). For both piperacillin and tazobactam a three-compartment structural mathematical model was used.

Equation 3.1:

\[
\frac{dX_1}{dt} = R(1) - \left( \frac{Cl}{V_c} + k_{cp} + k_{cELF} \right) \times X_1 + k_{pc} \times X_2 + k_{ELF} \times X_3
\]
Equation 3.2:
\[
\frac{dX_1}{dt} = k_{cp} \times X_1 - k_{pc} \times X_2
\]

Equation 3.3:
\[
\frac{dX_3}{dt} = k_{cELF} \times X_1 - k_{ELFc} \times X_3
\]

The differential equations for the three-compartment structural mathematical model used are shown above. $X_1$, $X_2$ and $X_3$ are the amounts of piperacillin (in mg) in the central, peripheral and ELF compartments, respectively. $R(1)$ represents the infusion of piperacillin. $Cl$ (L/hr) is the clearance, and $Vc$ is the volume of the central compartment (L). $K_{cp}$, $K_{pc}$, $K_{cELF}$ and $K_{ELFc}$ are the first-order inter-compartmental rate constants between the central and peripheral and central and ELF compartments. Covariates were not included in the structural model.

Elimination and movement of drug to and from the central compartment to the peripheral or ELF compartments was a first-order process. The PK data were weighted by the inverse of the measured assay variance for both piperacillin and tazobactam. Samples with drug concentration below the limit of assay quantification were excluded from analysis. A polynomial describing the assay variance was derived from regression of the measured mean drug concentrations and the standard deviation for samples with known high and low piperacillin and tazobactam concentrations. The means, medians, and standard deviations of the population parameters were estimated. Bayesian posterior estimates for each parameter were also obtained for each patient (using the “population of one” utility in NPAG). Scatter plots of observed versus predicted piperacillin concentrations were examined for the population as a whole and for individual patients. The fit of the structural model to the data were
assessed in the following way: (a) the log-likelihood value; (b) the coefficients of determination ($r^2$), slope and y-intercept from regression of the observed-predicted plots before and after the Bayesian step; and (c) the Akaike information criterion (AIC).

24.4. **External validation of the population pharmacokinetic analysis**

All simulations were performed in ADPAT 5. Observed data from a previous PK study was used as a validation dataset. In this study, by Boselli et al., 40 patients were administered a 30 minute intravenous loading dose of PTZ 4/0.5 g followed by a daily continuous infusion of either 12/1.5 g or 16/2 g. Three plasma samples (at least 4 hours apart) and one NBL sample were collected after at least 48 hours of PTZ. Five thousand subject Monte Carlo simulations were performed of the regimens utilised by Boselli et al. The parameter estimates (i.e. estimates of clearance, volume etc) from the population PK analysis outlined in this study (rather than the Boselli study) were utilised. The median, 5th, 25th, 75th and 95th centile piperacillin and tazobactam concentrations in plasma and ELF from the simulation were plotted. The observed piperacillin and tazobactam concentrations in plasma and ELF, from Boselli et al, was overlaid on the simulated data. Visual inspection was made of the ability of the population PK model to predict the validation data.

24.5. **Simulations to estimate piperacillin-tazobactam exposure in plasma and ELF**

Monte Carlo simulation was performed using a 5,000-subject simulation. The mean parameter vector and the full covariance matrix from the
population PK analysis was embedded in subroutine PRIOR of the ADAPT 5 program. Normal and log-normal parameter distributions were explored in the simulations. The ability to recapitulate the original parameter values and their dispersions was used to select which parameter distribution was selected. For piperacillin and tazobactam the median, 5th-percentile, 25th-percentile, 75th-percentile and 95th-percentile total, unbound and ELF concentrations for the population were identified every hour. Again a regimen of PTZ 4/0.5 grams, administered over 30 minutes, every 8 hours was used for the simulations. The unbound plasma and ELF AUCs, for both drugs, were calculated for the fifth dose (32 to 40 hours after initiation of therapy).

Simulation, for each of the 17 patients, was performed using the Bayesian posterior (individual) parameter estimates (i.e. clearance, volume of the central compartment and inter-compartmental rate constants). A regimen of PTZ 4/0.5 grams, administered over 30 minutes, every 8 hours was used for the simulations except for the 3 patients administered PTZ 4/0.5 grams, administered over 30 minutes, every 12 hours due to renal impairment. For both piperacillin and tazobactam the area under the concentration time curve (AUC) was estimated in plasma, for the unbound plasma fraction, and in the total ELF. Protein binding for both piperacillin and tazobactam was assumed to be 30%. The AUCs were calculated at steady-state (5 doses/32 hours after initiation of therapy for patients with an eGFR≥20 ml/min or 4 doses/36 hours after initiation of therapy for patients with an eGFR<20 ml/min). The correlation of total ELF to unbound plasma exposure for both piperacillin and tazobactam were assessed. Similarly the correlation of pulmonary permeability to pulmonary piperacillin and tazobactam penetration ratios was assessed. All correlations were
analysed using Spearman rank test (GraphPad Prism version 5 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com).

Finally a 5,000-subject simulation was performed using piperacillin 4 grams, administered over 30 minutes, every 8 hours. The fraction of simulated subjects who achieved six pre-defined PD targets for a range of MICs from 0.5 to 128 mg/L was determined. The PD targets were an unbound plasma or ELF piperacillin concentration above the MIC for 50% of the dosing interval (50% fT>MIC), 100% of the dosing interval (100% fT>MIC) or a trough piperacillin concentration to MIC ratio of ≥ 3.4. The cumulative response of patients, with a range of MICs defined as susceptible to piperacillin and achieving each of the PD targets was estimated using a published MIC distribution for organisms causing hospital-acquired and ventilator-associated pneumonia7.
25. **Results**

25.1. **Pharmacokinetic study**

Between June 2012 and July 2013 eighteen critically ill patients were enrolled with a mean age of 56 years and mean APACHE II score of 15 (Table 17). One patient who was infected with a novel coronavirus was excluded from all analyses because of issues related to biosafety. First-dose PK was assessed in four patients. Steady state PK was assessed in seventeen patients who had received a mean of 8.8 doses (range 2-16). Four patients were on renal replacement therapy and received PTZ every 12 hours. The remaining 13 patients received PTZ every 8 hours. Five patients received PTZ over 5 minutes while the remaining 12 patients received PTZ over 30 minutes. In total, 128 plasma and 31 ELF samples were obtained for PK analyses. Three piperacillin plasma samples, 3 piperacillin ELF samples and 14 tazobactam plasma samples were below the limit of assay quantification.
Table 17. Table showing the patients underlying demographics, severity of disease and outcome.

<table>
<thead>
<tr>
<th></th>
<th>mean</th>
<th>median</th>
<th>range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56.0</td>
<td>53.5</td>
<td>31.4-80.8</td>
</tr>
<tr>
<td>Height (metres)</td>
<td>1.7</td>
<td>1.7</td>
<td>1.40-1.83</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80.0</td>
<td>75.0</td>
<td>47.0-140.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.7</td>
<td>25.4</td>
<td>20.9-44.2</td>
</tr>
<tr>
<td>CPIS</td>
<td>5.6</td>
<td>5.3</td>
<td>3.0-9.0</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>14.9</td>
<td>15.0</td>
<td>8.0-24.0</td>
</tr>
<tr>
<td>SOFA score (first dose)</td>
<td>6.1</td>
<td>6.0</td>
<td>2.0-14.0</td>
</tr>
<tr>
<td>SOFA score (steady state)</td>
<td>5.8</td>
<td>6</td>
<td>1.0-10.0</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>106.9</td>
<td>106.9</td>
<td>18.3-230.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>9</td>
<td>52.9%</td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td>47.1%</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>16</td>
<td>94.1%</td>
</tr>
<tr>
<td>Bangladeshi</td>
<td>1</td>
<td>5.9%</td>
</tr>
<tr>
<td>Renal replacement therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>13</td>
<td>76.5%</td>
</tr>
<tr>
<td>Yes</td>
<td>4</td>
<td>23.5%</td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alive</td>
<td>14</td>
<td>82.4%</td>
</tr>
<tr>
<td>Dead</td>
<td>3</td>
<td>17.6%</td>
</tr>
</tbody>
</table>

[Creatinine clearance: calculated by Cockgroft-Gault; patients on renal replacement therapy exclude.]

Three non-directed bronchial lavage (NBL) specimens (two from a single patient) were not collected because of a clinical requirement for a high fraction of inspired oxygen (FiO₂) that precluded sampling. A single patient had a drop in oxygen saturation from 95% to 88% that required a temporary increase in FiO₂. A change in oxygen saturation was not observed in any other patients. No other changes in respiratory or cardiovascular parameters were observed in the four hours following collections of the other NBL samples.
25.2. Population pharmacokinetic analysis

The fit of the mathematical model to the observed data was acceptable. A linear regression of the predicted-versus-observed plasma piperacillin and tazobactam concentrations revealed the following relationship: Observed Piperacillin Concentration = 0.884 x Predicted Piperacillin Concentration + 2.01; $r^2 = 0.901$. Similarly, the Observed Tazobactam Concentration = 0.880 x Predicted Tazobactam Concentration + 0.165; $r^2 = 0.839$. A linear regression of the predicted and observed piperacillin and tazobactam concentrations in the ELF was given by: Observed Piperacillin Concentration = 0.790 x Predicted Piperacillin Concentration – 1.65; $r^2 = 0.812$; and Observed Tazobactam Concentration = 0.827 x Predicted Tazobactam Concentration + 1.21; $r^2 = 0.878$. For plasma piperacillin and tazobactam concentrations, the mean weighted bias was -0.00999 and 0.0214, respectively; and the bias-adjusted mean weighted precision was 25.5 and 1.22, respectively. For piperacillin and tazobactam concentrations in ELF, the mean weighted bias was -0.057 and 0.169, and of bias-adjusted mean weighted precision was 0.124 and 7.23, respectively. The parameter estimates from the population analysis are summarized in Table 18.
Table 18. Piperacillin and tazobactam population pharmacokinetic parameter estimates obtained by Pmetrics.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cl (litres/hr)</th>
<th>Vc (litres)</th>
<th>kcp (hr(^{-1}))</th>
<th>kpc (hr(^{-1}))</th>
<th>kELF (hr(^{-1}))</th>
<th>kELFc (hr(^{-1}))</th>
<th>VELF (litres)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piperacillin</td>
<td>Mean</td>
<td>12.122</td>
<td>11.717</td>
<td>13.132</td>
<td>20.107</td>
<td>0.225</td>
<td>0.559</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>9.337</td>
<td>10.556</td>
<td>10.503</td>
<td>25.135</td>
<td>0.158</td>
<td>0.343</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>6.833</td>
<td>4.921</td>
<td>8.672</td>
<td>10.15</td>
<td>0.212</td>
<td>0.693</td>
</tr>
<tr>
<td>Tazobactam</td>
<td>Mean</td>
<td>9.675</td>
<td>14.795</td>
<td>9.743</td>
<td>21.317</td>
<td>0.436</td>
<td>1.074</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>7.177</td>
<td>15.661</td>
<td>5.277</td>
<td>28.681</td>
<td>0.219</td>
<td>0.235</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>5.645</td>
<td>8.762</td>
<td>8.905</td>
<td>10.847</td>
<td>0.465</td>
<td>1.847</td>
</tr>
</tbody>
</table>

[Cl (L/hr) is the clearance, and Vc is the volume of the central compartment (L). Kcp, Kpc, KcELF and KELFc are the first-order inter-compartmental rate constants (hr\(^{-1}\)) between the central and peripheral and central and ELF compartments.]
Figure 17. External validation of piperacillin and tazobactam population model.
[The top row of panels show total plasma drug concentration and the lower panels show ELF drug concentrations. Each panel shows the median drug concentration (solid black line), the inter-quartile range (shaded grey area) and the 5th and 95th centiles (dotted black lines). Overlying data points represent observed data from Boselli et al.151.]
25.3. **External validation of the population pharmacokinetic analysis**

A plot of observed piperacillin and tazobactam concentrations from a previously published study overlaid by the predicted 5th, 25th, 50th, 75th and 95th centile drug concentrations from this study (simulated using the population PK model) revealed a high degree of concordance (Figure 17).151

25.4. **Plasma piperacillin-tazobactam concentrations and pulmonary penetration**

Simulated concentration-time profiles, showing the median, 5th, 25th, 75th and 95th centile drug concentrations in both plasma and ELF, following administration of five simulated doses of piperacillin 4 grams and tazobactam 0.5 grams, each as a 30 minute infusion every eight hours, are shown in Figure 18. The median AUC_{ELF}/AUC_{unbound plasma} penetration ratio was 49.3% (range: 2.0% - 515.9%) for piperacillin and 121.2% (range: 11.0% - 391.3%) for tazobactam.

Simulated plasma and ELF exposures for each individual patient (using the Bayesian posterior parameter estimates) allowed an assessment of the drug penetration from plasma to ELF and the inter-relationship between the two co-administered drugs. There was no statistically significant correlation between ELF piperacillin exposure (AUC_{ELF}) and unbound plasma piperacillin exposure (AUC_{unbound plasma}) (r=0.369, P=0.159; Figure 19). Similarly, there was no statistically significant correlation between ELF tazobactam exposure (AUC_{ELF}) and unbound plasma tazobactam exposure (AUC_{unbound plasma}) (r=0.306, P=0.248; Figure 19). Unbound tazobactam
exposure in the plasma of critically ill patients was statistically significantly positively correlated with unbound piperacillin plasma exposures (AUC\textsubscript{unbound, plasma}) \( (r=0.864; \ P<0.0001; \ \text{Figure 21}) \). There was also a statistically significant positive correlation between tazobactam and piperacillin ELF exposures (AUC\textsubscript{ELF}) \( (r=0.604; \ P=0.013; \ \text{Figure 21}) \).

\textbf{Figure 18. Concentration-time profiles for piperacillin and tazobactam.}

[The top panel compares total drug concentration (dotted line), unbound drug concentration (solid, black line) and ELF drug concentration (solid grey line). The middle panels show unbound plasma concentration while the lower two panels show ELF concentrations with median drug concentration (solid black line), the inter-quartile range (shaded grey area) and the 5\textsuperscript{th} and 95\textsuperscript{th} centiles (dotted black lines).]
Figure 19. Relationship between unbound plasma and ELF drug concentrations for piperacillin and tazobactam.
[Figure shows each of the observed trial patients (black dots) and for 5,000 simulated patients (small grey dots).]

Mean pulmonary permeability, as estimated by the ratio of urea-corrected total protein in ELF to plasma total protein concentration, was 0.1226 (median = 0.0795; S.D. = 0.1155). A statistically significant negative correlation was observed between the piperacillin penetration ratio (AUC_{ELF}/AUC_{unbound plasma}) and pulmonary permeability (r=-0.593; P=0.016, Figure 20). In contrast, no statistically significant correlation was seen between the tazobactam penetration ratio (AUC_{ELF}/AUC_{unbound plasma}) and pulmonary permeability (r=-0.064; P=0.815, Figure 20).
25.5. Probability of target attainment analysis

Monte Carlo simulation was used to estimate the probability of achieving predefined PD targets. The results of the probability of target attainment analysis for piperacillin are shown in Figure 22. The administration of piperacillin 4g three times daily, as a 30 minute infusion to treat an organism with an MIC of 1 mg/L resulted in 96%, 77% and 64% of patients achieving a PD target of 50% \( f_{T>MIC} \), 100% \( f_{T>MIC} \) and \( C_{min/MIC}>3.4 \), respectively. The treatment of an organism with an MIC of 16 mg/L (i.e. the current CLSI and EUCAST breakpoint for \( P. \) aeruginosa\(^{300,301} \)) resulted in 54%, 20% and 6% patients achieving a PD target of 50% \( f_{T>MIC} \), 100% \( f_{T>MIC} \) and \( C_{min/MIC}>3.4 \), respectively.
Figure 21. Comparison of the piperacillin and tazobactam exposures in the plasma, ELF and the plasma:ELF ratio.

[The dotted lines illustrate the 8:1 ratio of piperacillin to tazobactam in the administered piperacillin 4.0 gram/tazobactam 0.5 gram preparation.]
Figure 22. Results of the Monte Carlo simulation with the probability of target attainments, for unbound and ELF piperacillin, against a range of MICs.

[The pharmacodynamic targets are the fraction of patients whose drug concentration was above the MIC for 50% (left panel) or 100% (middle panel) of the dosing interval and the fraction of patients whose trough piperacillin concentration to MIC ratio was ≥ 3.4. Histogram shows MIC distribution for organisms causing hospital-acquired and ventilator-associated pneumonia.]

The predicted target attainments in plasma and ELF, for each MIC, were similar. For example, the target attainment rate using an endpoint of unbound piperacillin concentrations that were 50% $fT_{>\text{MIC}}$ was 96% and 54% for MICs of 1 and 16 mg/L, respectively. In comparison, the use of the same PD target in ELF (i.e. 50% $T_{>\text{MIC}}$) resulted in target attainment rates of 94% and 48% for MICs of 1 and 16 mg/L, respectively. For the most susceptible organisms (i.e. MICs in the range 0.25-1 mg/L) both the unbound plasma and ELF concentrations were above the MIC for 50% of the dosing interval in over >90% of simulated patients.

From the frequency distribution of PTZ susceptibilities of isolates causing hospital-acquired and ventilator-associated pneumonia the overall response rate of critically ill patients with VAP can be estimated (Figure 22)\(^7\). If piperacillin was administered empirically (i.e. the MIC is not known) 80% of critically ill patients with VAP would achieve plasma 50% $fT_{>\text{MIC}}$ and 77% of patients would achieve ELF 50% $T_{>\text{MIC}}$. In contrast, if piperacillin was administered in critically ill patients with VAP caused by a susceptible organism (i.e. MIC ≤ 16 mg/L) 86% of patients would achieve plasma 50% $fT_{>\text{MIC}}$ and 82% of patients would achieve ELF 50% $T_{>\text{MIC}}$. For suppression of emergence of antimicrobial resistance following empirical administration of piperacillin, 38% of critically ill patients with VAP would achieve plasma $C_{\text{min}}/\text{MIC}>3.4$ and 41% of patients would achieve ELF $C_{\text{min}}/\text{MIC}>3.4$. If piperacillin was administered to critically ill patients with VAP caused by a susceptible organism, 42% of patients would achieve plasma $C_{\text{min}}/\text{MIC}>3.4$ and 45% of patients would achieve ELF $C_{\text{min}}/\text{MIC}>3.4$.\[^{157}\]
Discussion

Inspection of the concentration-time profiles for both piperacillin and tazobactam illustrates marked PK variability in critically ill patients (Figure 18). The PK variability is notably more evident in the lung compared with plasma for both compounds. The estimates of clearance and volume of the central compartment from the population PK model are consistent with previously published values\textsuperscript{198,201,302}. The validity and generalisability of our results are further suggested by the concordance of simulated concentrations from the population PK model with data from a previously published group of critically ill patients (see Figure 17)\textsuperscript{151}.

Beta-lactam antibiotics penetrate the lung by passive diffusion\textsuperscript{147,303}. Diffusion into tissues is dependent on the concentration gradient across biological membranes, the surface area of the membrane and a diffusion coefficient\textsuperscript{143}. The diffusion coefficient is principally influenced by physicochemical characteristics of the drug (e.g. the degree of lipophilicity) and the extent of protein binding\textsuperscript{109,144}. There was a positive correlation between plasma and ELF exposures for both piperacillin and tazobactam. However, these relationships did not reach statistical significance which is unexpected and perhaps due to the modest number of patients in the study. In this study we used the ratio of total protein in ELF to plasma as a surrogate measure of lung permeability\textsuperscript{299}. We expected to see an increase in diffusion of drug with increasing pulmonary protein penetration. However, we observed a statistically significant negative correlation between the piperacillin pulmonary penetration ratio (AUC\textsubscript{ELF}/AUC\textsubscript{unbound plasma}) and pulmonary permeability (Table 20). As pulmonary permeability increased, there was a reduction in the relative proportion of piperacillin penetrating the lung. For tazobactam there was no statistically significant
correlation between the pulmonary penetration ratio \((\text{AUC}_{\text{ELF}}/\text{AUC}_{\text{unbound plasma}})\) and pulmonary permeability. There are a number of potential explanations for the relationship between piperacillin lung penetration and permeability. Methodologically, this is a small study with extreme PK variability in both the observed plasma and ELF drug concentration. Multiplication of the measured pulmonary sample concentration by a dilution factor, derived from comparison of urea concentrations in plasma and pulmonary samples\(^{297,298}\), may contribute to the greater variability observed in the pulmonary drug concentrations when compared with plasma concentrations. A possible biological explanation includes dilution of intra-pulmonary piperacillin due to larger ELF volumes that are associated with increasing pulmonary permeability. Alternatively, \(\beta\)-lactam antibiotics are substrates for organic anion transporters in other organs such as the kidney\(^ {238}\). Disruption of an active transport system may occur in the injured lungs of critically ill patients, which exhibit increased permeability to protein. Another explanation may be that an increase in pulmonary protein permeability preferentially affects diffusion of piperacillin and tazobactam in and out of the lung. Therefore in lungs with low protein permeability, piperacillin diffuses into the lung faster than it diffuses out. The reverse occurs in lungs with higher protein permeability. Validation of negative correlation of pulmonary piperacillin penetration and pulmonary permeability is required in a similar clinical cohort.

Measurement of antimicrobial agents in ELF may not truly reflect drug concentration within other pulmonary sub-compartments. Micro-dialysis techniques may provide a better estimate of pulmonary drug concentrations than bronchoalveolar lavage for quantifying drug concentrations in ELF\(^ {304}\). However, due to practical difficulties with pulmonary micro-dialysis, ELF
sampling remains the most commonly utilised technique in both pre-clinical and clinical studies\textsuperscript{13,305}. In this study we used NBL. NBL is a safe and effective way of sampling the lung and quantifying antimicrobial drug concentrations in the ELF of critically ill patients\textsuperscript{296}. NBL is less invasive than bronchoscopy and bronchoalveolar lavage (BAL), allowing multiple NBL samples to be collected throughout the dosing interval. The collection of two NBL samples from each patient, rather than one BAL provides a more robust estimate of the concentration-time profile in the ELF of individual patients. Only one minor adverse event was reported following NBL.

For β-lactam antibiotics, the PD index that best links drug exposure with the antibacterial effect is the fraction of the dosing interval that the free drug concentrations are above the MIC\textsuperscript{157}. For piperacillin the unbound piperacillin concentration must be above the MIC for at least 50% of the dosing interval is generally associated with near maximal efficacy (50% fT\textsubscript{>MIC})\textsuperscript{89}. We recently demonstrated a trough unbound piperacillin concentration to MIC ratio of >3.4 is required to suppress the emergence of antimicrobial resistance (Cmin/MIC>3.4)\textsuperscript{294}. From the target attainment analysis (Figure 22) the empirical administration of piperacillin 4g three times daily, as a 30 minute infusion (i.e. the MIC is not known) results in an 80% probability of attainment of a PD target of plasma 50% fT\textsubscript{>MIC} or a 77% probability of attainment of a PD target of 50% ELF T\textsubscript{>MIC}. The probability of achieving the same PD targets increases to 86% and 82%, for plasma and ELF respectively, when the MIC is known and organisms with MICs beyond the breakpoint (i.e.>16 mg/L) are excluded. Therefore, 14-18% of patients with a “susceptible” organism will have sub-optimal drug exposure. Furthermore, approximately 60% of patients will not achieve the plasma or
ELF PD targets associated with suppression of antimicrobial resistance (Cmin/MIC>3.4). This analysis identifies two important issues. Firstly, piperacillin 4g three times daily, as a 30 minute infusion, is inadequate for effective treatment and suppression of emergence of antimicrobial resistance in an unacceptably high proportion of critically ill patients, and especially those with pneumonia resulting from infection with a less susceptible organism. Secondly, the probability of achieving each of the PD targets (i.e. fT>MIC, Cmin/MIC etc) in plasma and ELF are similar. Plasma piperacillin concentrations do not precisely predict ELF piperacillin concentrations. Consequently, some individuals with “sufficient” plasma piperacillin exposure will have inadequate ELF piperacillin exposures and vice-versa. ELF rather than plasma exposure has been shown to predict outcome for other antimicrobial agents. The causative organisms were not isolated in our 17 patients which makes exploration of the relationship between piperacillin plasma and ELF PK, PD (fT>MIC, Cmin/MIC) and clinical outcome impossible. Further appropriately powered clinical studies are required to examine whether piperacillin exposure in plasma or ELF better predicts clinical outcomes.

The addition of tazobactam to piperacillin extends the activity of the β-lactam to β-lactamase producing strains of organisms such as Enterobacteriaceae, Staphylococcus aureus, H. influenzae and M. catarrhalis. The current regimen of PTZ, at a fixed 8:1 ratio, is supported by in-vitro studies. However, the PD index that best links β-lactamase inhibitor exposure with effect is poorly defined. Both: (i) the fraction of the dosing interval the β-lactamase inhibitor concentration is above a threshold (T>threshold) and (ii) the area under the β-lactamase inhibitor concentration time curve have been suggested as the relevant PD
The required concentration of β-lactamase inhibitor is dependent on the amount and type of β-lactamase. Tazobactam penetrates the lung of most critically ill patients, but there is marked variability. Therefore, a subset of patients may have insufficient pulmonary tazobactam concentrations to adequately inhibit some β-lactamases that cause hydrolysis of piperacillin and clinical failure despite adequate piperacillin exposure. Increasing the tazobactam dosage (while maintaining the piperacillin dosage) may overcome β-lactamase production as has been demonstrated in an in-vivo meningitis model. As plasma tazobactam exposure does not reflect tazobactam exposure in ELF, the identification of patients with poor pulmonary tazobactam penetration is difficult and appears to require direct sampling from the lung.

In conclusion, the primary aim of this study was to develop and validate a mathematical model to describe piperacillin and tazobactam concentration in plasma and the lung of critically ill patients. Additionally we show an unexpected relationship of increased pulmonary permeability being associated with a reduction in pulmonary piperacillin penetration. We also demonstrated that predicting pulmonary piperacillin and tazobactam exposures on the basis of plasma drug exposures may be unreliable. Appropriately powered clinical trials are required to further defined the relationship between plasma and pulmonary drug exposures and establish the impact of pulmonary, rather than plasma, drug exposure on clinical outcome. Additionally pre-clinical and clinical studies are required to investigate mechanisms of lung penetration in patients with pneumonia. Biomarkers related to pulmonary permeability or drug penetration could be incorporated as covariates into mathematical models to improve predictions of pulmonary drug exposures. New regimens of PTZ may be required
which optimise drug concentrations in the lung, at the site of infection. It is likely that a single regimen is not suitable for all individuals. If ELF exposure is shown to predict clinical outcome, with greater accuracy than plasma exposure, and covariates for pulmonary drug penetration cannot be identified then direct measurement of drug concentrations in the pulmonary compartment, and adjustment of individual regimens may be required.
CHAPTER 4: SOFTWARE FOR DOSAGE

INDIVIDUALISATION OF PIPERACILLIN-TAZOBACTAM FOR CRITICALLY ILL PATIENTS

27. Abstract

Piperacillin-tazobactam is frequently used for empirical and targeted therapy of infections in critically ill patients. Considerable pharmacokinetic (PK) variability is observed in critically ill patients. By estimating an individual’s PK, dosage optimization Bayesian estimation techniques can be used to calculate the appropriate piperacillin regimen to achieve desired drug exposure targets. The aim of this study was to establish a population PK model for piperacillin in critically ill patients then analyse the performance of the model in the dose optimization software “BestDose”. Linear, with estimated creatinine clearance and weight as covariates, Michaelis-Menten (MM) and parallel linear/MM structural models were fitted to the data from 146 critically ill patients with nosocomial infection. Piperacillin concentrations measured in the first dosing interval, from each of 8 additional individuals, combined with the population model were embedded into the dose optimization software. The impact of the number of observations was assessed. Precision was assessed by (a) the predicted piperacillin dosage and by (b) linear regression of the observed-versus-predicted piperacillin concentrations from the second 24 hours of treatment. We found that a linear clearance model with creatinine clearance and weight as covariates for drug clearance and volume of distribution, respectively, best described the observed data. When informed with at least two observed piperacillin concentrations the dose
optimization software predicted a mean piperacillin dosage of 4.02 grams in the 8 patients administered piperacillin doses of 4.00 grams. Linear regression of the 8 individuals observed-versus-predicted piperacillin concentrations after 24 hours of piperacillin dosing demonstrated an $r^2 > 0.89$. In conclusion, for most critically ill patients' individualized piperacillin regimens delivering a target serum piperacillin concentration is achievable. Further validation of the dosage optimization software in clinical trial is required.
Infection in critically ill patients is associated with excessive morbidity, mortality, length of hospital stay and healthcare costs\textsuperscript{15,291}. Early and appropriate antimicrobial therapy is associated with improved clinical outcomes in a variety of clinical contexts\textsuperscript{68,91}. Many licensed antimicrobial regimens are derived from studies in non-critically ill patients and when applied in the intensive care unit (ICU), may result in sub-optimal drug exposure for a significant proportion of critically ill patients\textsuperscript{92,201}. Marked PK variability is characteristic of critically ill patients, and may result from alterations in cardiac output, tissue perfusion, end-organ dysfunction, increased capillary permeability, hypoalbuminemia and use of extracorporeal circuits. This PK variability results in a wide range of drug exposures\textsuperscript{292}. Low drug exposures increase the probability of clinical failure and emergence of antimicrobial resistance, while high drug exposures are associated with an increased likelihood of toxicity\textsuperscript{89}.

PTZ is widely used to treat infections in critically ill patients\textsuperscript{262}. For β-lactam agents, the fraction of the dosing interval that free drug concentrations are above the MIC (fT_{MIC}) is the PD index that best links drug exposure with the antibacterial effect\textsuperscript{157}. For PTZ, the fT_{MIC} of 50% of the dosing interval (50% fT_{MIC}) is associated with favourable clinical outcomes\textsuperscript{89}. The use of extended (or continuous) infusions of β-lactam antibiotics increases the fT_{MIC}, and may potentially improve overall efficacy\textsuperscript{72,201}. Nevertheless, as many as 20% of patients receiving such regimens may still have sub-optimal drug exposure\textsuperscript{71}. The exposure-response relationships determining emergence of antimicrobial resistance and occurrence of adverse events are less well defined. Trough (pre-dose or Cmin) total β-lactam concentrations to MIC ratio of between 3 and 10 have been shown to
prevent the emergence of antimicrobial resistance in dynamic in vitro infection models\textsuperscript{170,234}. PTZ is usually well tolerated and adverse events are typically detected in <2% of patients, but toxicity has been reported in as many as \textasciitilde50% of patients in specific cohorts\textsuperscript{234}. Dosage adjustment of antimicrobial regimens allows delivery of optimal drug exposures, allowing for variability in PK, aiming to maximise clinical efficacy, reduce the chance of adverse events and suppress the emergence of antimicrobial resistance.

Therapeutic drug monitoring (TDM) is a standard of care for some antimicrobial agents such as gentamicin, vancomycin and voriconazole\textsuperscript{175,212,213}. Increasing evidence suggests that TDM of β-lactam antibiotics (including PTZ) may improve clinical outcomes in critically ill patients\textsuperscript{190}. Dose adaptation using Bayesian approaches offers a potential way of individualising regimens for critically ill patients. This approach estimates a patient's (Bayesian posterior) PK using a combination of measured drug concentrations and information about the drug, gained from previous experiences with that drug (quantified using population PK analysis). Dose optimizing software can then identify the optimal dosage to achieve a pre-defined target drug concentration.

The aim of this study was to develop a population PK model to describe a large dataset of critically ill patients. The population PK model was incorporated into the Bayesian dose optimisation software, “BestDose”. We then assessed the accuracy of the dose optimisation software using in silico validation experiments using multi-dose PK data from a small cohort of patients not included in the original population PK model.
29. Methods

An overview of the development of the population PK model and the building, testing and demonstration of the dosage optimisation software is shown in Figure 23.

29.1. Pharmacokinetic studies in critically ill patients

PK data from 146 patients from three, previously published, studies were obtained\textsuperscript{151,198,201}. A total of 803 piperacillin concentrations were available with each patient contributing 2-10 observations. Patients were administered between 2 and 4 grams of piperacillin by either 30 minutes infusion every eight hours, a 4 hourly infusion every 8 hours or by continuous infusion.

Lodise et al provided data from 76 patients undergoing abdominal or thoracic surgery who received 2 grams of piperacillin over 30 minutes\textsuperscript{198}. Plasma concentrations were measured in the first dosing interval. A further 18 patients undergoing colorectal surgery who had received 4 grams of piperacillin over 30 minutes every 6 hours were included. Plasma samples were obtained in the first and second dosing interval.

Plasma concentrations from a further 12 hospitalised patients, who had received 3 grams of piperacillin over 4 hours every 8 hours were included\textsuperscript{201}. These patients were predominantly sampled on day 3 of therapy.
Figure 23. An overview of the development of the population pharmacokinetic model and the building, testing and demonstration of the dosage optimisation software.
Finally, 40 ventilated patients with ventilator–associated pneumonia, administered piperacillin 12 grams or 16 grams by continuous infusion were included. Plasma samples were collected during the second 24 hours of therapy. Weight and estimated creatinine clearance by Cockcroft-Gault was known for each patient. Piperacillin concentration was measured, in all three studies, using well-validated high performance liquid chromatography assays.

29.2. Development of a population pharmacokinetic model of piperacillin

Data were analyzed using a population PK methodology using the non-parametric adaptive grid program Pmetrics 1.1.1. Since no estimates of uniform assay error was available from the original studies, we utilized an assay error polynomial (i.e., $SD = C_0 + C_1Y + C_2Y^2 + C_3Y^3$) with inputs of 1.04, 0.14, 0, and 0 (where C is the error polynomial input and Y is the drug concentration in mg/L). The polynomial was estimated by linear regression of the means and associated standard deviation for each of the three piperacillin concentrations for the 40 patients administered piperacillin by continuous infusion by Boselli et al. In the fitting process, each concentration was weighted by its Fisher information, which is the inverse of the variance. Additionally, we chose the option in Pmetrics to multiply the variance by gamma ($\gamma$), which is an adaptive scalar that captures additional process noise such as errors in timing of samples or doses.

We evaluated several structural PK models. The details of these structural models are shown in below. The models differed in the way in which piperacillin was cleared from the central compartment, and by the covariates that were included. The models were parameterized in the
following ways: (a) elimination by a first-order process (Equation 4.1a); (b) elimination by a Michaelis-Menten process (Equation 4.1b); (c) elimination by parallel first-order and Michaelis-Menten processes (Equation 4.1c); and (d) elimination by a first-order process with creatinine clearance as a covariate and body mass as a covariate for the volume of the central compartment (Equation 4.1d). Piperacillin elimination, by a Michaelis-Menten process, is biologically plausible and has previously been identified to best describe the observed data in a population PK analysis\textsuperscript{201}.

Equation 4.1a: (Linear):

\[
\frac{dX_1}{dt} = R(1) - \left(\frac{Cl}{Vc} + k_{cp}\right) \times X_1 + k_{pc} \times X_2
\]

Equation 4.1b: (Michaelis-Menten):

\[
\frac{dX_1}{dt} = R(1) - \left(\frac{V_{max}}{(k_m \times Vc) + X_1} + k_{cp}\right) \times X_1 + k_{pc} \times X_2
\]

Equation 4.1c: (Michaelis-Menten/Linear):

\[
\frac{dX_1}{dt} = R(1) - \left(\frac{V_{max}}{(k_m \times Vc) + X_1} + \frac{Cl}{Vc} + k_{cp}\right) \times X_1 + k_{pc} \times X_2
\]

Equation 4.1d: (Linear with covariates):

\[
\frac{dX_1}{dt} = R(1) - \left(\frac{Cl + (Crcl \times Cl_{l})}{V_{cl} + (Wt \times V_{cl})} + k_{cp}\right) \times X_1 + k_{pc} \times X_2
\]

Equation 4.2:

\[
\frac{dX_2}{dt} = k_{cp} \times X_1 - k_{pc} \times X_2
\]

The ordinary differential equations are listed above where $X_1$ and $X_2$ are the amounts of piperacillin (mg) in the central and peripheral compartments, respectively. $R(1)$ represents the infusion of piperacillin. $Cl$
(L/hr) is the clearance, and Vc is the volume of the central compartment (L). Vmax is the maximum rate of clearance by the Michaelis-Menten clearance mechanism (mg/hr), and Km is the concentration of piperacillin where clearance by the Michaelis-Menten clearance mechanism is half maximal (mg/L). Kcp and Kpc are the first-order inter-compartmental rate constants. Cls, fraction of piperacillin clearance due to creatinine clearance (L/hr). Cli, clearance due to non-renal means (L/hr). Vi, volume of the central compartment not related to body mass (L). Vs, volume of the central compartment proportional to body mass (L).

For an individual patient without data, i.e. prior to any measured piperacillin concentrations, his/her parameter value joint distribution is the same as for the population. However, if observed data are available, the population distribution (i.e. the Bayesian prior) may be updated to a new distribution (i.e. the Bayesian posterior) that better predicts the individual's observations. The support points do not move, but their relative probabilities change, based on ability to predict the patient's observed concentrations for his/her dosing history.

For each model, the final-cycle population parameter value distributions are summarized in terms of measures of central tendency (e.g. means, medians) and dispersion (e.g. standard deviation). Scatter plots of the observed-versus-predicted for the population (i.e. Bayesian prior) and individual patients (i.e. Bayesian posterior) were examined. The fit of each of the four structural models to the data was assessed using a combination of the following: (a) the log-likelihood value; (b) the Akaike information criterion (AIC); and (c) the coefficients of determination ($r^2$) from a linear regression of the observed-predicted plots before and after the Bayesian step. Differences between the various models were also assessed.
statistically by calculating the difference in log-likelihood values and comparing this value to a $\chi^2$ distribution with the degrees of freedom equal to the difference in the number of parameters between each model.

29.3. **Building the piperacillin dose optimisation software**

We used the dose optimisation software, “BestDose”, to estimate each individual patient’s PK and the optimal individual dosages for each patient. BestDose is based on software originally developed nearly 20 years ago, by the Laboratory of Applied Pharmacokinetics, University of Southern California, Los Angeles\textsuperscript{313}. This dose optimisation software has previously been used to individualise therapy with vancomycin, voriconazole and antiretroviral therapy\textsuperscript{314,315}.

The software requires four specific components: (a) a structural mathematical model that best describes the PK (we used the fourth structural PK model above - see results); (b) the “density” file (one of the outputs of the Pmetrics analysis), which serves as the Bayesian prior; (c) a patient “past” file that contains the observed drug concentrations and details of the administered regimen, (d) a patient “future” file which contains the target drug concentrations deemed to be appropriate for that patient and initial estimates of the required drug dosages and frequency of administration.

The dose optimisation software uses the equations in the model file and the population Bayesian prior in the density file, together with the individual patient’s observed drug concentrations in the past data file to calculate a Bayesian posterior parameter value distribution for that patient. The dose optimisation software then calculates the drug dose that minimizes the expected weighted squared error (over the Bayesian posterior distribution)
between the predicted and user-specified target drug concentrations in the future data file.

29.4. **Testing the performance of the piperacillin dosing predictions**

A separate external dataset from Roberts et al\textsuperscript{200} was then used to test the performance of the piperacillin dose optimisation software. Piperacillin concentrations obtained from eight patients receiving piperacillin 4 grams as a 20 minute infusion every six (n=7) or eight hours (n=1), were used to validate the performance of the dose optimisation software. Patients had a mean of 14 observations (range: 12 to 21) during the first dosing interval and a further 9 observations (range: 6 to 12) during a dosing interval 24 hours later. The creatinine clearance was calculated for each patient using the Cockcroft-Gault equation.

Piperacillin concentrations collected during the first dosing interval were entered into the dose optimisation software. To determine the optimal number of observations required a comparison was made of one, two, three or six observations. Piperacillin concentrations collected approximately 6 hours (1 observation) or 0.5 and 6 hours (2 observation) or 0.5, 3 and 6 hours (3 observation) or 0.5, 1, 2, 3, 4 and 6 hours (6 observations) after the start of the first dosage were utilised to describe each individual patient’s PK. Using 1, 2 or 3 observations was investigated as these are reasonable for routine clinical care while 6 observations is sufficient to optimally estimate each of the PK parameters in a 6 parameter model. Each patient was administered piperacillin 4 grams over either 20 or 40 minutes. The dose optimisation software “past” data file contained the observed first dosing interval piperacillin concentrations for each of the
eight individual validation patients. The “future” data file contained the required timings of the future dosages, an initial ‘guess’ at the likely future dose that would be required to achieve desired concentration targets (in this case 4 grams), the required piperacillin target concentration and the timing of the target. For this in silico experiment, the target piperacillin concentrations, at 24.5, 25, 26, 27, 28 and 30 hours (6 observations) after the start of the first dosage, were used as the observed piperacillin values after the first piperacillin dose on the second day of piperacillin dosing. The output from the dose optimisation software was an estimate of: (a) the individual’s (post-Bayesian) PK parameters and (b) the dosage required to achieve the observed piperacillin concentrations.

The ability of the dose optimisation software was tested in two ways. Firstly, the capability of the software to estimate each individual’s PK was assessed by comparison of the observed-versus-predicted piperacillin concentration after 24 hours of therapy. Simulations, utilising parameter estimates from each of the eight validation individuals, was performed in ADAPT 5\textsuperscript{257}. The piperacillin concentrations, at the corresponding time points to the observed data during the second 24-hours of treatment were estimated. The ability of the dose optimisation software to predict the observed data was evaluated by: (a) visual inspection of the simulated piperacillin concentration-time profiles for the eight individual patients including the observed piperacillin concentrations; (b) linear regression of the observed-versus-predicted piperacillin concentrations for each of the eight validation individuals and (c) linear regression and estimation of the mean weighted prediction error (bias) and the bias-adjusted squared prediction error (precision) of the observed-versus-predicted piperacillin concentrations for all eight patients combined.
Secondly, the dose optimisation software was tested by comparing the estimated delivered piperacillin dosage predicted from the observed piperacillin concentrations after 24 hours of therapy. Assessment was made by comparison of actual and the predicted piperacillin dosage after 24 hours of treatment.

Linear regression was performed using GraphPad Prism version 5 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. Estimation of the mean weighted prediction error and the bias-adjusted squared prediction error was performed in R 3.0.1\textsuperscript{256}.

29.5. Simulations to demonstrate the utility of the dose optimisation software

The dose optimisation software was finally used to predict the required dosage to achieve a pre-determined piperacillin concentration. In this analysis the same six time points, from the first 24 hours of piperacillin therapy administered to the eight validation patients, were used. For each of the eight individuals, three doses of drug were administered in the first 24 hours at 8-hour intervals. This was to simulate the time required to measure the drug concentration after the first dose. Dosage adjustment was then performed for the fourth to seventh doses because day 2 of treatment represents an early opportunity for TDM intervention. The piperacillin target concentration was a pre-dose (trough) concentration estimated from an \textit{in vitro} hollow fibre infection model, containing \textit{P. aeruginosa}. The identified concentration is associated with suppression of emergence of piperacillin resistance\textsuperscript{294}. The target trough total plasma piperacillin concentrations used were 13.6 mg/L and 41.6 mg/L, for 30 minute and four hour infusion regimens, respectively. These
concentrations would be applicable to a strain of *P. aeruginosa* with a minimum inhibitory concentration of 4 mg/L as used in the in-vitro model.
30. **Results**

30.1. **Population pharmacokinetics of piperacillin in critically ill patients**

The demographics and clinical characteristics of the 146 patients used in this study are summarized in Table 19. A comparison of the fit of each of the four structural mathematical models to the data is shown in Table 20. All four models performed well. Evaluation of the log-likelihood values against a \( \chi^2 \) distribution and comparison of Akaike information criterion indicated the Michaelis-Menten model was superior to the other mathematical models. Examination of the linear regression of the observed-versus-predicted plots revealed each model showed an \( r^2 > 0.9 \) but the linear clearance model with covariates had a y-intercept value closest to zero. As all the models perform similarly, the linear clearance model with covariates, as the most clinically relevant model was used for predicting individual critically ill patients’ PK and their optimal piperacillin dosage despite being statistically inferior to the Michaelis-Menten model. The mean, median and standard deviation of the parameter estimates for the linear clearance with covariates population PK model are shown in Table 21.
**Table 19. Summary of each of the three studies included in the population pharmacokinetic model.**

<table>
<thead>
<tr>
<th></th>
<th>Felton et al</th>
<th>Boselli et al</th>
<th>Lodise et al</th>
<th>Combined</th>
<th>Roberts et al</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Estimated creatinine clearance (ml/min)</strong></td>
<td>Mean</td>
<td>115.0</td>
<td>69.8 (52.0)</td>
<td>89.0</td>
<td>85.9</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>111.5</td>
<td>52.0</td>
<td>85.5</td>
<td>81.0</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>38.0-169.1</td>
<td>14.0-245.7</td>
<td>27.0-221.0</td>
<td>14.0-245.7</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>Mean</td>
<td>77.0</td>
<td>73.0</td>
<td>70.5</td>
<td>71.7</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>71.5</td>
<td>68.5</td>
<td>69.0</td>
<td>69.0</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>38.1-122.5</td>
<td>49.0-113.0</td>
<td>50.0-98.5</td>
<td>38.1-122.5</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>Male</td>
<td>8</td>
<td>25</td>
<td>54</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>4</td>
<td>15</td>
<td>40</td>
<td>59</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>Mean</td>
<td>46.8 (49.5)</td>
<td>62.3 (64.0)</td>
<td>54.2 (55.0)</td>
<td>55.8 (56.0)</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>20.0-69.0</td>
<td>34.0-88.0</td>
<td>18.0-78.0</td>
<td>18.0-88.0</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>N/A</td>
<td>4 or 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Number of patients</strong></td>
<td>12</td>
<td>40</td>
<td>94</td>
<td>146</td>
<td>8</td>
</tr>
<tr>
<td><strong>Number of doses</strong></td>
<td>Mean</td>
<td>9.58</td>
<td>48 hour</td>
<td>1 or 2</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>9</td>
<td>continuous infusion</td>
<td>4 or 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>4-24</td>
<td>21-26</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Observations per patient</strong></td>
<td>Mean</td>
<td>5.9</td>
<td>3.0</td>
<td>6.5</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>6.0</td>
<td>3.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>4.0-8.0</td>
<td>3.0-3.0</td>
<td>2.0-10.0</td>
<td>2.0-10.0</td>
</tr>
</tbody>
</table>
Table 20. Evaluation of the predictive performance of piperacillin and tazobactam population models.

<table>
<thead>
<tr>
<th>Model</th>
<th>Number of variables</th>
<th>Log likelihood</th>
<th>Probability compared to Linear model</th>
<th>Number of cycles to convergence</th>
<th>AIC</th>
<th>Linear regression of observed-predicted for each patient</th>
<th>R²</th>
<th>Intercept</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear</td>
<td>4</td>
<td>-2973.5</td>
<td>n/a</td>
<td>1310</td>
<td>2988</td>
<td>0.931</td>
<td>2.43</td>
<td>0.903</td>
<td></td>
</tr>
<tr>
<td>Michaelis-Menten</td>
<td>5</td>
<td>-2892.5</td>
<td>2.26x10⁻¹⁹</td>
<td>1490</td>
<td>2914</td>
<td>0.933</td>
<td>3.35</td>
<td>0.921</td>
<td></td>
</tr>
<tr>
<td>Parallel Linear/MM</td>
<td>6</td>
<td>-2894.5</td>
<td>7.00 x10⁻¹⁸</td>
<td>2182</td>
<td>2922</td>
<td>0.933</td>
<td>3.42</td>
<td>0.908</td>
<td></td>
</tr>
<tr>
<td>Linear with covariates*</td>
<td>6</td>
<td>-2899.0</td>
<td>6.65 x10⁻¹⁷</td>
<td>1061</td>
<td>2930</td>
<td>0.925</td>
<td>2.27</td>
<td>0.918</td>
<td></td>
</tr>
</tbody>
</table>

[* indicates model selected for the dose optimisation software validation].
Table 21. Piperacillin population pharmacokinetic parameter estimated obtained from Pmetrics.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cli</td>
<td>3.83</td>
<td>2.79</td>
<td>3.35</td>
</tr>
<tr>
<td>Cls</td>
<td>0.11</td>
<td>0.1</td>
<td>0.09</td>
</tr>
<tr>
<td>Vi</td>
<td>4.54</td>
<td>1.83</td>
<td>4.45</td>
</tr>
<tr>
<td>Vs</td>
<td>0.12</td>
<td>0.06</td>
<td>0.14</td>
</tr>
<tr>
<td>Kcp</td>
<td>6.74</td>
<td>0.85</td>
<td>11.4</td>
</tr>
<tr>
<td>Kpc</td>
<td>9.14</td>
<td>1.65</td>
<td>13.15</td>
</tr>
</tbody>
</table>

[Cls, fraction of piperacillin clearance due to creatinine clearance (litres per hour). Cli, clearance due to non-renal means (litres per hour). Vi, volume of the central compartment not related to body mass (litres). Vs, volume of the central compartment proportional to body mass (litres). Kcp, transfer rate constant from the central compartment to the peripheral compartment (per hour). Kpc, transfer rate constant from the peripheral compartment to the central compartment (per hour).]

30.2 Predicting individual critically ill patients pharmacokinetics

Linear regression of each of the individual patients revealed \( r^2 \) values ranging from 0.861 to 0.987 using a single observation, 0.893 to 0.987 using two observations, 0.883 to 0.979 for three observations and 0.833 to 0.967 for six observations (Table 22). Linear regression of the combined observed-predicted values, from all eight individuals, revealed \( r^2 \) values of 0.727 using a single observation, 0.805 using two observations, 0.738 using three observations and 0.681 using six observations (Figure 24). The mean weighted prediction error (bias) and bias-adjusted squared prediction error (precision) were 3.66 mg/L and 184.26 mg^2/L^2 using a single observation, 4.73 mg/L and 130.32 mg^2/L^2 using two observations, 2.32 mg/L and 91.45 mg^2/L^2 mg/L using three observations and 1.01 and 117.32 mg^2/L^2 using six observations, respectively. Visual inspection of the concentration-time profiles for each of the eight patients showed predicted
concentrations were higher than the observed concentration following a single observation. In seven of the eight validation patients utilising more than one observation resulted in a satisfactory agreement between the observed piperacillin concentration at 24 hours and the piperacillin concentration predicted by the population PK model (Figure 25). For patient 3 the model did not predict the observed data well, but inspection of the raw data showed considerable differences between the observed piperacillin concentrations resulting from the first and fifth dosage.

![Figure 24. Observed-versus-predicted piperacillin concentration for the in silico validation cohort after 24 hours of therapy.](image)
Table 22. Table showing the effect of the number of observations on the predicted dose.

<table>
<thead>
<tr>
<th>Pt</th>
<th>r²</th>
<th>Predicted piperacillin dose (grams)</th>
<th>% of delivered dose</th>
<th>r²</th>
<th>Predicted piperacillin dose (grams)</th>
<th>% of delivered dose</th>
<th>r²</th>
<th>Predicted piperacillin dose (grams)</th>
<th>% of delivered dose</th>
<th>r²</th>
<th>Predicted piperacillin dose (grams)</th>
<th>% of delivered dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.86</td>
<td>2.68</td>
<td>-0.33</td>
<td>0.92</td>
<td>5.22</td>
<td>0.31</td>
<td>0.97</td>
<td>4.87</td>
<td>0.22</td>
<td>0.94</td>
<td>4.97</td>
<td>0.24</td>
</tr>
<tr>
<td>2</td>
<td>0.93</td>
<td>1.76</td>
<td>-0.56</td>
<td>0.95</td>
<td>1.15</td>
<td>-0.71</td>
<td>0.90</td>
<td>1.71</td>
<td>-0.57</td>
<td>0.90</td>
<td>1.67</td>
<td>-0.58</td>
</tr>
<tr>
<td>3</td>
<td>0.98</td>
<td>9.49</td>
<td>1.37</td>
<td>0.98</td>
<td>8.72</td>
<td>1.18</td>
<td>0.88</td>
<td>11.64</td>
<td>1.91</td>
<td>0.96</td>
<td>17.65</td>
<td>3.41</td>
</tr>
<tr>
<td>4</td>
<td>0.93</td>
<td>3.10</td>
<td>-0.23</td>
<td>0.92</td>
<td>3.05</td>
<td>-0.24</td>
<td>0.89</td>
<td>3.53</td>
<td>-0.12</td>
<td>0.83</td>
<td>3.42</td>
<td>-0.15</td>
</tr>
<tr>
<td>5</td>
<td>0.99</td>
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[Pt is patient; Linear regression from the predicted piperacillin concentrations made after administration of the first piperacillin dose, of drug concentrations after 24 hours of treatment and the predicted piperacillin dose (grams), and expressed as a percentage of the 4 grams actually delivered, required to achieve the observed concentration.]
Figure 25. Panel showing piperacillin concentration-time profiles for eight validation patients generated from six observed piperacillin concentrations during the first dosing interval.

[●: Observed data, entered into the software package; ○: Observed data, unknown to the software package; solid line (-): predicted piperacillin concentration-time profile.]
30.3. Predicting of the piperacillin dosage delivered to individual critically patients

From a single post-dose observation, collected following 24 hours of therapy the mean and median predicted piperacillin dose for the cohort of eight validation patients was 3.58 and 2.86 grams compared with a delivered dose of 4.00 grams (Table 22 and Figure 26). For two post-dose observations the mean and median predicted piperacillin dose was 4.02 and 3.47 grams compared to a mean and median predicted piperacillin dose 4.55 and 3.65 grams following three observations and 5.52 and 4.20 grams if six observations were used. When utilising at least two observed piperacillin concentrations, the dose optimisation software predicted that patient 3 required at least twice the administered dose of piperacillin to achieve the observed piperacillin concentrations. For this patient, inspection of the observed piperacillin concentrations (Figure 25) shows the piperacillin concentrations after 24 hours of therapy are markedly higher than the piperacillin concentrations achieved after the first dose, but with little sign of significant drug accumulation.
Figure 26. Comparison of the impact of number of observed piperacillin concentrations.
[One, two, three or six known first-dose piperacillin concentrations were entered into the dose optimisation software on predicted piperacillin dosage for patients actually administered piperacillin 4 grams.]

30.4. Examples of the clinical utility of the piperacillin dose optimisation software

The dose optimisation software was used, in silico, to predict a specified trough piperacillin concentration following a 30 minute administration of piperacillin every eight hours. In order to achieve the target piperacillin concentration piperacillin 4.7 grams and 8.8 grams, eight hourly by 30 minute administration, were required for patients 1 and 8, respectively (Figure 27).
Figure 27. Panel showing two examples of the dose optimisation software.
[●: Observed data, entered into the software package; solid line (-): predicted piperacillin concentration; each arrow head represent an administered dose with ▼ for dosages administered prior to individualisation (all bolus doses) and ▲ for dosages administered following individualisation (administration over 30 minutes for bolus doses); therapeutic drug target of a trough piperacillin concentration of 13.6 mg/L.]
Discussion

Infection remains a commonly encountered problem in critically ill patients and is associated with high morbidity, high mortality and increased healthcare costs. Variation in the PK of β-lactam antibiotics occurs in critically ill patients\cite{316}. The use of a fixed regimen in critically ill patients will result in a wide range of drug exposures\cite{264}. Therapeutic drug monitoring (TDM) enables adjustment of the dose based on observed drug concentrations to achieve optimal drug exposure for an individual patient. TDM is a standard of care for some agents such as gentamicin, vancomycin and voriconazole and is associated with improved clinical outcomes\cite{175,213}. Limited evidence suggests that TDM improves achievement of PD targets for β-lactam antibiotics\cite{161,190}.

Here, we develop and validate the necessary tools to enable dosage individualisation of piperacillin in critically ill patients. Administration of β-lactams by extended or continuous infusion or through application of TDM has been suggested to exploit relatively detailed understanding of β-lactam PD. Continuous infusions have been shown to only achieve desired PD target in \( \approx 80\% \) of critically ill patients\cite{71}. This could, potentially, be improved using TDM and dose optimisation. Typically TDM is performed using dosing nomograms. However, the use of dosing nomograms in critically ill patients poorly predicts the required drug regimen\cite{317,318}. This is due the considerable PK variability in study populations. Additionally dosing nomograms require assessment of a subject with the drug at PK steady state whereas Bayesian dose optimisation may be performed after the first dose. Finally, Bayesian dose optimisation offers a truly personalised dosage for each patient rather than forcing the individual into one of several potential dosing bands. A pragmatic interpretation of the dosage identified by the dosing software may be required so a practical and easy to
administer dose could be prescribed to the patient. This dosage would result in a drug concentration that safely exceeds the identified plasma therapeutic target.

Both inter- and intra-subject PK variability may be important. Inter-subject variability is fundamental to the argument for using TDM - i.e. inherent variability results in too many patients receiving sub-optimal drug exposures. Intra-subject variability (due to the continually changing clinical state and PK observed in critically ill patients) has an impact on the ability to accurately predict a regimen that enables attainment of a desired therapeutic target in an optimally precise manner. This is illustrated by patient 3 in Figure 25. Visual inspection of the concentration-time profiles (Figure 25) illustrates the difference between the observed and predicted piperacillin concentrations after 24 hours of therapy. This was due to a marked and rapid change in both piperacillin clearance and volume of distribution. Use of a mathematical non-linear clearance model may have estimated the accumulation of piperacillin seen in patient 3. Additionally optimal design of plasma sampling time points may maximise the information gained from each sample and allow better estimation of parameters such as clearance and volume of distribution. It is likely that repeated assessment of a patient’s observed drug concentration will be required, especially in patients with discernible changes in their clinical condition.

The change in PK parameters results from pathophysiological changes in critically ill patients. In order for a mathematical model to predict a patient’s PK changes a greater understanding of pathophysiological alterations is required. Quantification of these pathophysiological changes and subsequent incorporation as covariates into a mathematical model may allow better prediction of evolving PK and requires further study.
Alternatively, a more pragmatic approach would be frequent observation and dosage adjustment performed with a minimum of delay between observation and adjustment. In this *in silico* validation the time between the two sets of observations was 24 hours. This is a reasonable estimate for the current amount of time it would take to process, measure and then model the observed data before being able to change the regimen. Reducing the turnaround time would mean less time for pathophysiological alteration to change the dosing requirements. In unstable, critically ill patients the process of measuring drug concentrations and establishing the optimal antimicrobial regimen may be a near continuous process. Reformatting drug assays to platforms such as enzyme immunoassay and away from chromatography may reduce time delays in producing drug concentration data. Additionally, moving the equipment required for TDM closer to the patient will minimise the turn-around-time\(^{319,320}\). In the future, we anticipate that drug quantification will be performed in a similar way to glucose measurement using handheld devices at the bedside with built-in dosing software.

The drug exposure target used for dosage adjustment in the *in silico* validation experiment was identified in an in-vitro dynamic PK-PD experiment\(^{294}\). Here a strain of *P. aeruginosa*, with a minimum inhibitory concentration (MIC) of 4 mg/L was exposed to a range of PTZ dosages. The identified target piperacillin concentration, expressed as a product of the MIC, was the lowest pre-dose concentration required to suppress the emergence of piperacillin resistance. Two different TDM target concentrations were identified, both for piperacillin administered every eight hours, one by 30-minute bolus injection and a separate target for four-hour extended infusions. To achieve this trough concentration, piperacillin dosages markedly higher than currently licensed may be required. This is
illustrated by patient 8 who was predicted to require piperacillin 7.4 grams or 11.8 grams, depending on the regimen, to achieve the target concentration. Use of these high piperacillin dosages would require attention to patient safety, although the resulting concentrations appear to be well tolerated\textsuperscript{190}.

The results of this in silico validation experiment illustrate the complexities of optimising treatment for critically ill patients. The dose optimisation software allows precise targeting of drug concentrations as previously demonstrated with voriconazole\textsuperscript{223}. The changing pathophysiology of critically ill patients and variability in PK makes delivery of optimised regimens challenging. Despite these challenges the dose optimisation software, when informed with at least two observed concentrations, was able to provide a satisfactory response in seven of the eight patients. Rapid, near-patient drug quantification would reduce the impact of PK variability and may be required to further personalise and optimise antimicrobial therapies in the ICU. The ability of TDM and Bayesian dose adaptation of $\beta$-lactam antibiotics to improve outcomes in critically ill patients requires evaluation in prospective randomised studies.
32. Final Discussion

Infection is commonly encountered in critically ill patients leading to an increased morbidity, high mortality and excess healthcare costs\textsuperscript{15}. Data from studies presented in this thesis show that the current strategy of using fixed population-based antibiotic regimens results in a wide range of drug exposures in critically ill individuals. This is concordant with previously published data in ICU patients\textsuperscript{321}. Low drug exposures are associated with clinical failure and emergence of resistance while high drug exposures increase the risk of drug-induced toxicity.

Combining data from clinical PK and pre-clinical (either animal and/or in-vitro) PK-PD studies to identify optimal dosing strategies is rapidly becoming established as a fundamental tool in maximising the efficacy of both new and existing therapeutic agents\textsuperscript{322,323}. This concept of bridging clinical PK and pre-clinical PK-PD studies, utilised in the thesis, has the advantage of reducing the costs associated with running large randomised controlled trials and is particularly useful for developing regimens against rare pathogens (such as multi-resistant Gram negative bacteria or biohazard organisms) where there are insufficient patient numbers for formal clinical trials. However, validation of the key components is still required. The PK-PD indices associated with efficacy, emergence of antimicrobial resistance and toxicity require further exploration in clinical cohorts. The process of bridging will involve multiple iterative steps with results of the clinical validation steps likely to further inform the future pre-clinical studies.
Throughout the thesis the non-parametric population modelling software Pmetrics was utilised\(^{255}\). For clinical data, particularly from critically ill patients, the assumption that parameter estimates are non-parametrically distributed may be appropriate. For analysis of in-vitro PK-PD models, such as the hollow fibre infection model, parameter estimates may be normally distributed making the non-parametric analysis unnecessary. There are no published comparisons of Pmetrics with parametric software programmes (e.g. NONMEN or MONOLIX) for the analysis of experimental PK-PD infection model data. In order to complete a non-parametric analysis Pmetrics requires a fixed error model. This is in contrast to alternative parametric modelling software options where the error is estimated in the analysis. The Pmetric error model is derived from the assay variance. The assay variance can be measured for PK studies but is more difficult to estimate for PD data. Additionally the error model is multiplied by a scalar ($\gamma$) which is estimated by Pmetrics and is made up of the error not described by the assay variance. Inclusion of this scalar therefore assumes that all the error in the data is proportional to the assay variance, which it may not be. At the time of performing the analysis in this thesis a non-parametric Monte Carlo simulator was not available in Pmetrics. The simulations within this thesis were performed using ADAPT 5 which assumes the parameter estimates are normally or log-normally distributed. The impact of using parameter values estimated using non-parametric software in a simulator which assumes a parametric distribution is unknown. Future Monte Carlo simulation will be performed using the newly developed non-parametric simulator in Pmetrics.

In order to progress the development of the analytical skills exhibited in this thesis a number of improvements could be made. Three clinical PK analysis were included in this thesis, each finding a different structural
model to best fit the data. Each structural model was developed to answer a specific question (e.g. to describe the mechanism of piperacillin clearance) and fitted to the available data but a more consistent approach to model development and fitting would be optimal. Exploration and inclusion of covariates into the structural models may have improved the models ability to fit the data and help reduce the inter-individual variability. In the design of future PK studies measurement of potential covariates will be included.

Currently all patients are administered the same fixed regimen of β-lactam antibiotics with dose reduction suggested in patients with severe renal impairment. Over the next 10-20 years treatment of critically ill patients could markedly progress with application of personalised antimicrobial regimens. Delivery of personalised antimicrobial regimens could provide a cost-effective improvement in patient outcomes by reducing length of hospital stay and overall mortality. Here, a hypothetical case is used to illustrate how a pulmonary infection could be treated in a critically ill patient using the Bayesian dose optimisation software described in this thesis. Finally the steps required to evolve from current practice to this vision of personalised critical care are discussed.

32.1. **Personalised critical care - a vision of the future**

A 68 year old gentleman is admitted to the ICU following an emergency laparotomy for a ruptured abdominal aortic aneurysm. During surgery he has his aorta clamped for a prolonged period and suffers significant blood loss requiring transfusion of blood products. The patient is intubated and mechanically ventilated on the ICU. Over the first 24 hours of his ICU admission the patient develops multiple organ failure with acute kidney and lung injury. The patient develops marked peripheral oedema as a result of
capillary leak, typical of sepsis, and large volume fluid resuscitation. On
day 3 of his ICU admission the patient develops fever and copious purulent
secretions are suctioned from his endotracheal tube. A chest radiograph
shows a new area of left lower lobe consolidation. A clinical diagnosis of
probable ventilator associated pneumonia is made and bronchoscopy is
performed. A bronchoalveolar lavage (BAL) sample is collected. The BAL
sample undergoes bacterial culture and sensitivity testing. Direct detection
of bacterial DNA in BAL is performed to identify both the bacterial species
and presence of antimicrobial resistance mechanism. A small vascular
cannula containing a microdialysis catheter is inserted into a vein in the
patient’s forearm.

Empirical piperacillin-tazobactam therapy is commenced. The patient’s
body weight, cardiac output, creatinine clearance and a measure of extra-
vascular fluid are entered in Bayesian dose optimisation software installed
on a bedside tablet computer. Using these covariates the dosing software
proposes an optimal initial dose of piperacillin-tazobactam 6g/0.75g
administered over three hours. The initial dose is followed by additional
doses of piperacillin-tazobactam 3g/0.375g, administered over three hours,
every six hours. The software predicts this dose will generate an exposure
associated with maximal antimicrobial effect, suppress emergence of
antimicrobial resistance and minimise the risk of drug-induced toxicity.
Microdialysis and BAL samples are collected immediately after the
completion of the infusion and an hour before the next dose is due to be
administered. Pulmonary permeability is estimated from the BAL fluid by
the ratio of pulmonary to plasma protein concentration, corrected for
dilution. The microdialysis and BAL samples are injected into a bedside
analyser on the ICU. The analyser is able to estimate unbound piperacillin,
tazobactam and urea concentrations. Three hours later and immediately
prior to the next dosage administration a further pair of microdialysis and BAL samples are collected and assayed. A strain of *E. coli*, without any known resistance mechanism, is identified by PCR from the BAL fluid.

The microdialysis and pulmonary piperacillin and tazobactam concentrations, pulmonary permeability, patient’s body weight, cardiac output, updated creatinine clearance and a measure of extra-vascular fluid are entered into Bayesian dose optimisation software. Pulmonary permeability is markedly reduced and the dosing software suggests increasing the dose to piperacillin-tazobactam to 5g/0.625g to achieve adequate pulmonary exposures. Over the next 24 hours the patient becomes anuric and requires continuous veno-venous haemofiltration. Daily measurement of pre- and post-infusion piperacillin and tazobactam concentrations in microdialysis and BAL fluids allows optimal pulmonary drug exposures to be maintained despite changes in cardiac output, extra-vascular fluid volume and renal function. After 48 hours of antimicrobial therapy the MIC of the organism is known, allowing for further refinement and delivery of an optimal piperacillin-tazobactam regimen.

32.2. **Next steps to allow delivery of personalised antimicrobial regimens to critically ill patients**

32.2.1. *Identification of optimal piperacillin exposure for critically ill patients*

For β-lactam antibiotics such as piperacillin, near maximal antimicrobial efficacy is observed when the unbound drug concentration is above the MIC for at least 50% of the dosing interval (50% fT\textsubscript{MIC} \textsuperscript{89}). Experimental data from this thesis, which is similar to published data, shows emergence of a resistant bacterial sub-population still occurs when β-lactam antibiotics
are administered at an exposure associated with near-maximal antimicrobial effect\textsuperscript{170}. Both antimicrobial efficacy and suppression of emergence of antimicrobial resistance are key considerations in identifying the optimal piperacillin exposure which will improve outcomes for critically ill patients and protect future populations from multi-drug resistant organisms.

Within this thesis, bacterial killing and suppression of emergence of antimicrobial resistance were studied in the hollow fibre infection model at bacterial densities ranging from $\approx 10^5$ to $10^8$ CFU/ml. These bacterial densities are clinically relevant and frequently observed in critically ill patients\textsuperscript{7,8,283}. At $\approx 10^5$ CFU/ml a trough, total piperacillin concentration:MIC ratios of 3.4 and 10.4, for bolus and extended infusion regimens, respectively, were associated with both suppression of the emergence of piperacillin resistance and a two log reduction in bacterial density. These trough targets are similar to those identified for meropenem and could be used as a therapeutic target for dose optimisation software as illustrated in the case study\textsuperscript{170}. At high bacterial densities ($\approx 10^8$ CFU/m) large piperacillin exposures, delivered by short or extended infusion, were unable to prevent the emergence of resistant bacteria. This suggests that critically ill patients with higher bacterial densities may need different antimicrobial regimens compared with those with lower bacterial densities. For example, combination of piperacillin-tazobactam with a second agent (e.g. an aminoglycoside or a fluoroquinolone) may be required to suppress emergence of resistant bacteria at high bacterial densities. In this thesis \textit{Pseudomonas aeruginosa} was studied in the HFIM. The applicability of these results to other bacterial species, particularly the Enterobacteriaceae, needs to be established. The drug exposures identified in this thesis need to be evaluated in different bacterial species and densities in both \textit{in vitro} HFIM studies and clinical pharmacodynamic studies.
There are currently no published clinical studies linking drug exposure with suppression of emergence of antimicrobial resistance. These clinical trials are required to cross validate the results of in vitro studies. Clinical pharmacodynamic studies for resistance are complex and require (a) properly phenotyped patients (e.g. where the clinical diagnosis, bacterial species and organism density is established); (b) collection of multiple microbiology samples with bacterial isolation of sufficient sensitivity to identify emergence of resistant organisms; (c) larger patient numbers than PK studies (i.e. 50-100 patients) and (d) measurement and estimation of drug exposure from the appropriate compartment (e.g. plasma and/or tissue samples). These clinical studies could assess whether plasma or a relevant tissue (i.e. lung) exposure correlates with the PK-PD index.

32.2.2. Identification of optimal tazobactam exposure for critically ill patients

Tazobactam is a β-lactamase inhibitor which extends the spectrum of activity of piperacillin to include β-lactamase producing strains of organisms such as Enterobacteriaceae, *S. aureus, H. influenzae* and *M. catarrhalis*. The PK-PD index that best links β-lactamase inhibitor exposure with efficacy is poorly defined. The area under the β-lactamase inhibitor concentration time curve and the fraction of the dosing interval the β-lactamase inhibitor concentration is above a threshold, have both been suggested as relevant PK-PD indices. Proposed threshold concentrations are either 4 mg/L or related to the β-lactam MIC of the organism. Further in vitro and clinical studies are urgently required to establish the PK-PD index which links drug exposure with β-lactamase inhibition. Currently a number of novel β-lactam/β-lactamase inhibitor combinations
(e.g. ceftazidime-avibactam, meropenem-RPX7009) are in phase III trials of critically ill patients with VAP and/or urinary tract infections. These clinical studies may provide a clinical estimate of the exposure β-lactamase inhibition relationship. So far, pre-clinical studies of these compounds have highlighted their potency as β-lactamase inhibitors rather than define the PK-PD index required for β-lactamase inhibition.

32.2.3. *Delivery of optimal plasma piperacillin and tazobactam exposures to critically ill patients*

Marked piperacillin and tazobactam inter-subject PK variability is demonstrated in critically ill patients by the concentration-time profiles from each of the three clinical cohorts in this thesis (Figure 6, Figure 18 and Figure 24). This variability in concentration-time profiles results from differences in individual patients' volumes of distribution and clearance. The parameter estimates from each of the three population analyses are in keeping with each other and previously published values.\(^{198,200,267,268}\). Parameters in each of the population analysis have a coefficient of variation of around 50% which is commonly observed in PK studies of critically ill patients.\(^{272}\). The data set used for validation of the dosage optimisation study demonstrates intra-subject, as well as inter-subject, PK variability. That is to say, marked changes in clearance and volume of distribution occur between administrations of sequential dosages (Figure 24). Therefore, administration of the same dose to a patient, at a different time in their illness, results in a different drug exposure. Administration of piperacillin-tazobactam either by extended infusion or aided by Bayesian dose optimisation software are options which could overcome the challenge of PK variability.
32.2.3.1. Delivering piperacillin by extended infusion

Piperacillin delivered by extended infusion, rather than bolus dosage, results in improved target attainment against all but the most susceptible organisms where both methods of administration are satisfactory (Figure 7). The potential advantages of extended infusion are most obvious when treating less susceptible but not frankly resistant organisms (i.e. MIC 8-16 mg/L). The results of the Monte Carlo simulation are in keeping with previously published simulations and clinical trial data\textsuperscript{71,321,324}. The HFIM and subsequent Monte Carlo simulations (Figure 16) demonstrates that emergence of antimicrobial resistance may occur in a significant number of patients irrespective of whether piperacillin-tazobactam is administered by bolus or extended infusion.

Currently a large, randomised, controlled, double-blind, double-dummy phase IIb trial of bolus dosing versus extended infusions for β-lactam antibiotics is underway in Australia, New Zealand and Hong Kong\textsuperscript{325}. This may provide the first evidence that administration of β-lactam antibiotics by extended infusions is associated with reduced mortality in critically ill patients.

32.2.3.2. Piperacillin dose optimisation

Simulation demonstrates that fixed dosage regimens of piperacillin-tazobactam even when delivered by extended infusion, will not deliver an optimal regimen to all critically ill patients (Figure 7). Dosage optimisation, sometime called therapeutic drug monitoring, ensures a personalised dosage to all patients. This is performed by quantification of drug concentrations and adjusting drug dosages to deliver optimal drug exposures. Dosage optimisation, sometimes using Bayesian techniques, is routinely used for nephrotoxic drugs, such as gentamicin and vancomycin,
and may be useful for β-lactam antibiotics like piperacillin\cite{161,175,190,213}. Currently adjustment of dosages is primarily utilised in clinical practice to reduce the risk of drug-related toxicity rather than optimise antimicrobial effect. However, as demonstrated in chapter 4 of this thesis, Bayesian dose optimisation can deliver regimens capable of suppressing emergence of antimicrobial resistance. \textit{In silico} validation of Bayesian dose optimisation software appears satisfactory for both β-lactam antibiotics and voriconazole\cite{223}. Evaluation of the impact of Bayesian β-lactam antibiotic dose optimisation on clinical outcomes in critically ill patients requires assessment within prospective randomised clinical trials. The control group in the clinical trial could be the current therapeutic regimen utilising bolus dosing or administration using extended infusion, if shown to be superior. Dose optimisation would be aimed at achieving sufficient piperacillin exposures to induce bacterial killing and suppress emergence of antimicrobial resistance. Outcome measures of clinical trials include clinical cure, microbiological cure, emergence of antimicrobial resistance, length of ICU stay and mortality. Clinical trials evaluating Bayesian dose optimisation would need to include economic analysis to justify the increase healthcare cost of bioanalysis, software development and licences.

The PK of an antimicrobial agent may change in an individual during a treatment episode. Repeat sampling and Bayesian optimisation of the dose may be required each time a significant change in a patient’s pathophysiology was observed. Pharmacokinetic variability arises due to: (a) an increase in the volume of distribution due to changes in extravascular water, (b) either an increase or reduction in the drug clearance due to acute kidney injury or augmented renal clearance and (c) alterations in protein binding in the presence of hypoalbuminemia\cite{99}. Population pharmacokinetic models incorporating renal function, markers of tissue
permeability, vascular permeability and disease severity as covariates may improve the predictive power of Bayesian dose optimisation software. Incorporating covariates may allow a more accurate prediction of the required first dose allowing early and rapid attainment of PK-PD targets. Furthermore a population model incorporating covariates may be able to predict the impact of changing pathophysiology on pharmacokinetics. This could reduce the number of samples that are required to inform the Bayesian dose optimisation software during the course of an infection. This can be highlighted by using the hypothetical case described in section 32.1 of this discussion, where the patient develops worsening renal impairment. Deterioration in renal function would currently require re-estimation of piperacillin and tazobactam clearance necessitating frequent and repeated collection and measurement of samples. Bayesian dose optimisation software, utilising a population pharmacokinetic model with creatinine clearance as a covariate, should be able to predict the effect of renal impairment on drug clearance and suggest modification of the dose without collection of a new set of samples. This would reduce the number of samples collected in a patient. Sampling of both tissue and venous compartments of critically ill patients is associated with a low complication rate but the number of samples is best kept to a minimum. Decreasing the number of assays would reduce the cost associated with dose optimisation. A further PK study with collection of plasma, microdialysis and BAL samples as well as potential covariates would need to be performed. The PK study would require samples to be collected at multiple time points associated with a number of dosing intervals during an infective episode. Subsequently a new population PK model would need to be developed incorporating covariates found to improve the fit of the mathematical model to the observed PK data. The improved predictive power of mathematical models including multiple covariates would ideally be assessed in silico.
prior to implementation in prospective clinical trials. Controlled clinical trials could be undertaken which use Bayesian dose optimisation software based on simple mathematical models without covariates as the control intervention.

Piperacillin renal clearance may be non-linear due to the presence of an active tubular secretion mechanism\textsuperscript{238}. Dose optimisation above current licenced regimens may result in saturation of piperacillin clearance mechanisms\textsuperscript{191,264}. Therefore, piperacillin dosage escalation studies or particular attention to piperacillin-related toxicity during dose optimisation studies would be required when using high piperacillin doses.

32.2.3.3. Tazobactam dose optimisation

Pharmacokinetic variability affects tazobactam as well as piperacillin. Dose optimisation for tazobactam may also be required for the same reasons outlined above for piperacillin. In order to optimise the dose of the β-lactamase inhibitor, tazobactam may need to be administered independently from piperacillin. In current clinical practice piperacillin and tazobactam are administered concurrently. Identification of a PK-PD target is fundamental for dose optimisation of tazobactam. Currently, there is no clinical trial evidence of separate administration of a β-lactam and a β-lactamase inhibitor. \textit{In vitro} studies would be required to estimate the optimal time interval between administrations of the two agents. Clinical trials of efficacy and toxicity, including economic analysis, would be required if optimised doses of both piperacillin and tazobactam were administered. Patient selection into clinical trials would require careful identification of patients with β-lactamase producing organisms. Outcome measures should include clinical cure, microbiological cure, emergence of antimicrobial resistance, length of ICU stay and mortality.
32.2.4. **Delivery of optimal pulmonary piperacillin and tazobactam exposures to critically ill patients**

Piperacillin and tazobactam PK variability is more extreme in the lung of critically ill patients than the plasma (Figure 18). Dose optimisation could be applied to samples collected from the lung as illustrated in the case summary. There are no previous studies of dose optimisation using tissue concentration of antimicrobial agents. Clinical pharmacodynamic studies are required to establish whether plasma or tissue exposures determine clinical efficacy and suppression of emergence of resistance. *In silico* studies, similar to the validation study in plasma in this thesis, would be the appropriate first investigation of the utility of dose optimisation using tissue concentration of antimicrobial agents. Following *in silico* validation studies both *in vivo* and prospective clinical trials of dose optimisation using tissue concentration of antimicrobial agents would be required. If plasma exposure of an antimicrobial agent is shown in clinical trials to be the best determinant of outcome then dose optimisation of tissue exposure would not be required.

A positive correlation (although not reaching statistical significance) between plasma and ELF exposures for both piperacillin and tazobactam was observed. This suggests that plasma PK variability partially contributes to PK variability in the lung. Other factors, including pulmonary permeability, are involved in producing such marked changes in pulmonary piperacillin and tazobactam concentrations. Unexpectedly a statistically significant negative correlation between the piperacillin pulmonary penetration and pulmonary permeability was observed. Inclusion of a validated measure of pulmonary permeability as a covariate may improve the predictive ability of the Bayesian dose optimisation software.
32.2.5. Improved understanding of the mechanisms of pulmonary penetration of antimicrobial agents

Beta-lactam antibiotics have previously been shown to enter the lung by passive diffusion\(^ {147,303}\). The inverse relationship between pulmonary piperacillin penetration and pulmonary permeability suggests that diffusion may not be the only mechanism for pulmonary piperacillin penetration and that, like the kidney, active transport may be involved. Pulmonary epithelium has been shown to express a wide range of drug transporters including P-glycoprotein, multidrug resistance proteins, members of the family of organic cation transporters and organic anion transporters as well as peptide transporters\(^ {327,328}\). There are sparse data on antibiotic interactions with pulmonary drug transporters. Limited in vitro evidence suggests ciprofloxacin may be actively transported through pulmonary epithelial cells\(^ {329,330}\). Pulmonary penetration by piperacillin and tazobactam requires further investigation in clinical trials, animal models and in in vitro alveolar cell culture model\(^ {331,332}\). Clinical trials could be performed in both healthy volunteer and patient groups. Intra-pulmonary PK, in humans and animal models, with ascending dosages will establish the relationship between pulmonary and plasma exposures. A linear relationship would be expected with diffusion. A non-linear relationship, with concentrations in the lung higher than plasma, may suggest the presence of active transport mechanisms. In vitro cell culture models allow isolation and investigation of the separate roles of endothelial and epithelial cells (pneumocytes) in pulmonary penetration. Inhibitors of specific active transport mechanisms can be added to cell culture and animal models so a specific mechanism can be interrogated. If pulmonary penetration of antimicrobial agents is dependent on active transport this may be an important site of drug-drug interactions or be a potential therapeutic target.
32.2.6. *Near-patient quantification of piperacillin and tazobactam*

In the case summary above, bedside quantification of the antimicrobial agents in microdialysis fluid and BAL is performed. The use of microdialysis catheters would remove the need for separation of plasma from blood cells. Microdialysis samples allow estimation of the unbound concentration of a drug, which is biologically active. Clinical and assay data would need to be entered into a user friendly interface on a ward based computer. Technical development of near patient drug assays and software is not a feature of this thesis. However, for the full potential of dose optimisation to be realised near patient testing and improvements to dose optimisation software is required. The current delays in sample collection, transport, processing and measurement adversely impact on the real-life utility of dose optimisation. Development of rapid, simple near patient assays are required to implement dose optimisation into clinical practice.
33. Conclusion

The hypothesis of this thesis had two key components. Firstly, that mathematical modelling techniques could be used to identify drug exposure targets that are associated with effective antimicrobial regimens. This was shown to be feasible using a population analysis of data from an *in vitro* HFIM with identification of piperacillin exposure that result in both near-maximal efficacy and minimise the development of drug resistance. Secondly, that mathematical modelling techniques could be used to optimise outcomes for individual critically ill patients. Computerised dosing software was constructed using a population pharmacokinetic model of plasma piperacillin concentrations in critically ill patients. This software was shown to be capable of recommending an individualised piperacillin-tazobactam dose for critically ill patients using the exposure identified in the HFIM as a therapeutic target.

The process outlined in this thesis serves as a paradigm for designing optimal regimens for current and novel therapeutic agents. PK must be defined in diverse patient populations with differing degrees of illness and organ dysfunction. From these studies the impact of critical illness on PK can be assessed and using D-optimal design, sampling in future studies can be collected at maximally informative time points. Simulation using PK-PD models allows identification of optimal regimens to inform dosing regimens in phase III studies. PD studies should be performed in *in vitro* systems (like the HFIM), animal models and in appropriately powered clinical trials allowing estimation of drug exposures and cross validated between the different studies. Several PD endpoints should be considered including efficacy (bacterial killing), suppression of emergence of antimicrobial resistance and toxicity. Mathematical modelling should be used to link an antimicrobial agent’s PK (relating dose to exposure) and PD
(defining the exposure-response relationship for effect, resistance suppression and toxicity). Mathematical models can then be used to define the appropriate dose and method of administration for a patient population or, if required, develop alternative strategies for drug delivery such as Bayesian dose optimisation.
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APPENDICES

Clinical trial documents.

The following documents are included in the appendices as examples of the documentation developed for the PROPEL clinical trial.

1. PROPEL protocol v4 22 07 13.pdf
2. PROPEL CRF v3.2 11 3 13.pdf
3. PROPEL Pat Info Sheet (first dose and steady state) v2.1.docx
4. PROPEL consent (first dose and steady state) v2.0.docx

The following clinical trial documentation was also developed but is not included to reduce duplication.

1. PROPEL Pat Info Sheet (steady state) v1.1.docx
2. PROPEL consent (steady state) v1.0.docx
3. PROPEL (retrospective) Pat Info Sheet (first dose and steady state) v2.1.docx
4. PROPEL consent to cont (first dose and steady state) v2.0.docx
5. PROPEL (retrospective) Pat Info Sheet (steady state) v1.1.docx
6. PROPEL consent to cont (steady state) v1.0.docx
7. PROPEL PerLR Info Sheet (first dose and steady state) v2.1.docx
8. PROPEL PerLR consent (first dose and steady state) v2.0.docx
9. PROPEL PerLR Info Sheet (steady state) v1.1.docx
10. PROPEL PerLR consent (steady state) v1.0.docx
11. PROPEL ProfLR consent (first dose and steady state) v2.0.docx
12. PROPEL ProfLR consent (steady state) v1.0.docx
Appendix 1: PROPEL protocol version 4 22/07/2013
PROTOCOL

PROPEL

Plasma and intrapulmonary population pharmacokinetics of piperacillin/tazobactam in critically ill patients

EudraCT number: 2011-004470-28
CTA: 21463/0218/001
REC number: 11\NW/0680
Reference number: PROPEL-01
Sponsors protocol number: 2011RM010
Version Number: 4.0
Release Date: 22nd July 2013
## Protocol

**PROPEL:** Plasma and intra-pulmonary population pharmacokinetics of piperacillin/tazobactam in critically ill patients

EudraCT number: 2011-004470-28  
CTA: 21463/0218/001  
REC number: 11/NW/0680  
Reference number: PROPEL-01  
Version Number: 4.0  
Release Date: 22.07.2013

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### DOCUMENT HISTORY

<table>
<thead>
<tr>
<th>Document</th>
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<th>Summary of Changes</th>
</tr>
</thead>
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<td>v1 24.08.2011</td>
<td>N/A</td>
</tr>
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</table>
| Protocol Amendment 1      | V2 16.07.2012    | 1. Study of first dose and/or steady-state pharmacokinetics  
                          | 2. Addition of Cardiothoracic Intensive Care Unit (CICU)  
                          | 3. Adverse event reporting and exempt events  
                          | 4. CRF as source data  
                          | 5. Changes to consent at end of study  
                          | 6. Removal of three medical qualified individual who are not longer involved in the study  
                          | 7. Additions of comment regarding timing of steady-state |
| Protocol Amendment 2      | V3 05.12.2012    | 1. Patient numbers changed to a range of 25 to 40.  
                          | 2. Raising of the upper age limit from 75 to 85  
                          | 3. Removal of “suspected pulmonary infection” from enrolment criteria  
                          | 4. Removal of requirement for 48 hours intubation and mechanical ventilation prior to commencing IMP  
                          | 5. Translation research facility now Clinical Research Facility |
| Protocol Amendment 3      | V3 22.07.2013    | 1. Patient numbers changed to a range of 20 to 40.  

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Approved Version (Signed)........................................Print Name..................................Date.............
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Department of Microbiology, Clinical Sciences Building, University Hospital of South Manchester, Southmoor Road, Manchester. M23 9LT
Abbreviations

ACU  Adult intensive care unit
APACHE II  Acute Physiology and Chronic Health Evaluation II
ARDS  Acute respiratory distress syndrome
AUC  Area under the curve
CICU  Cardiothoracic Intensive Care Unit
CNS  Central nervous system
CPIS  Clinical Pneumonia Infection Score
CRF  Case Report Form
CVS  Cardiovascular system
CVVHDF  Continuous veno-venous hemodiafiltration
CVVHF  Continuous veno-venous hemofiltration
DNA  Deoxyribonucleic acid
DSUR  Development Safety Update Report
ELF  Epithelial lining fluid
ETT  Endotracheal tube
hr  Hour
ICU  Intensive Care Unit
IMP  Investigational medicinal product
L  litre
LIS  Lung Injury Score
mg  Milligrams
MHRA  Medicines and Healthcare products Regulatory Agency
min  minute
NBL  Non-direct bronchial lavage
NHS  National Health Service
NIHR  National Institute for Health Research
PCR  Polymerase chain reaction
PEEP  Positive end expiratory pressure
PerLR  Personal Legal Representative
PIS  Patient Information Sheet
PK  Pharmacokinetics
ProfLR  Professional Legal Representative
RRT  Renal replacement therapy
SAEs  Serious Adverse Events
SOFA  Sequential organ failure assessment
SUSARs  Suspected Unexpected Serious Adverse Reactions
UHSM  University Hospital of South Manchester
VAP  Ventilator Associated Pneumonia
VAP-PIRO  Ventilator associated pneumonia- predisposition, insult, response, organ dysfunction
**Introduction**

Ventilator-associated pneumonia (VAP) is a severe respiratory infection occurring in patients mechanically ventilated for greater than 48 hours. VAP is defined as the presence of new or progressive radiographic infiltrates on chest radiograph associated with either a temperature >38.5°C or <36.5°C, white cell count >10/L or <1.5/L or purulent tracheal secretions.\(^1\) VAP affects between 8 – 28% of ICU patients and increases the risk of a patient dying by 27%.\(^2\) Additionally, VAP is associated with an increase in the length of mechanical ventilation, length of hospital stay and healthcare costs.\(^3\) The use of broad-spectrum antibiotics is associated with increasing rates of antimicrobial resistance, which in turn is associated with an increase in mortality from VAP.\(^4\) The choice of antimicrobial therapy is crucial with an inappropriate choice associated with adverse events.\(^5\) Even in patients given the correct antimicrobial agent low drug exposures increase the risk of therapeutic failure and the emergence of antibiotic resistance.\(^6\) Low drug exposures are as a result of the extreme pharmacokinetic variability seen in critically ill patients.\(^7\) Equally high drug exposures increase the risk of drug toxicity.

Concentration of a drug at its site of action is an important determinant of efficacy. Measurement of drug concentration in pulmonary epithelial lining fluid provides an estimate of effective concentration at the site of infection. Non-directed bronchial lavage (NBL) is a less invasive method of collecting samples from the lower respiratory tract compared with bronchoalveolar lavage. NBL has previously been used to collect fluid for measurement of antibacterial drug concentration.\(^8\) Serial determination of drug concentration both in plasma and at the target site in multiple patients allows construction of a population pharmacokinetic model. This mathematical model can be used to provide a robust estimate of the degree of variability seen in the patient cohort and to individualise future regimens to achieve drug exposure targets that: (1) maximise the chance of therapeutic success; (2) minimise the probability of emergence of drug resistance; and (3) minimise the probability of toxicity.

The cause of the pharmacokinetic variability in critically ill patients is not well understood. Tissue penetration, volume of distribution and clearance of drugs are altered in critically ill patients.\(^9\) Intrapulmonary levels of vancomycin, in patients with acute lung injury, have previously been shown to be affected by changes in pulmonary permeability.\(^10\) Pulmonary permeability can be measured by the ratio of plasma to alveolar protein concentration with pulmonary protein concentration increasing with worsening lung injury.\(^11\) The variability of pharmacokinetics in critically ill patients may in part be related to the systemic inflammatory response seen in these patients. A feature of the systemic inflammatory response is alteration in the level of circulating cytokines. Both pulmonary albumin concentration and circulating plasma inflammatory markers will be measured in this study. This data will be incorporated in the mathematical population pharmacokinetic model to assess the impact of these covariates as a cause of pharmacokinetic variability.

Piperacillin/tazobactam is used extensively throughout the NHS and is a first-line agent for the empirical treatment of VAP. Monte Carlo simulations suggest that 1 in 5 patients with hospital acquired pneumonia may fail therapy due to low exposure.\(^12\) Hypersensitivity reactions are the most common adverse event attributed to piperacillin/tazobactam. Data from patients with cystic fibrosis suggests this may be due to non-reversible binding of piperacillin in albumin. The kinetics of this piperacillin-albumin adduct are not described. This study will aim to determine the kinetics of adduct formation and correlate these kinetics with drug exposure.
2. Pharmacokinetics and Product Metabolism in Humans

3. Distribution

Peak plasma piperacillin and tazobactam concentrations are achieved immediately following intravenous infusion. Both piperacillin and tazobactam are approximately 25% bound to plasma proteins with the protein binding of either piperacillin or tazobactam unaffected by the presence of the other agent. Following administration, piperacillin and tazobactam are widely distributed in tissue and body fluids.

4. Biotransformation

Piperacillin is metabolised to a less active N-desethyl metabolite while tazobactam is metabolised to a single inactive metabolite (M1).

5. Elimination

Both piperacillin and tazobactam are eliminated by the kidney via glomerular filtration and tubular secretion. Following administration, approximately 50% piperacillin and approximately 60% tazobactam are excreted in the urine over 24 hours. Administration of piperacillin reduces the excretion of tazobactam by ≈10% while the presence of tazobactam has no effect on elimination of piperacillin. Piperacillin, tazobactam, and desethyl piperacillin are also secreted into the bile. The plasma half-lives of piperacillin and tazobactam range from 0.7 to 1.2 hours. Therefore steady state plasma concentrations are achieved approximately 2.8 to 6 hours after the first dose, and before the second dose, is administered (assuming an 8 or 12 hour dosing interval).

6. Impaired Renal Function

The half-lives of both piperacillin and tazobactam are increased by a decrease in creatinine clearance. For a creatinine clearance of below 20 ml/min there is a two-fold and four-fold decrease in the clearance of piperacillin and tazobactam respectively. Both piperacillin and tazobactam are removed by methods of renal replacement therapy with variation in clearance rates observed between different methods of renal replacement therapy and different filter membrane materials.

7. Impaired Liver Function

The half-life of both piperacillin and of tazobactam are increased (by approximately 25% and 18% respectively) in patients with hepatic cirrhosis when compared to healthy subjects. Dosage adjustment in patients with hepatic disease is not required.

8. Summary of pharmacokinetics in healthy volunteers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>Oral bioavailability</td>
<td>0%</td>
</tr>
<tr>
<td>Plasma protein binding</td>
<td>20-30%</td>
</tr>
<tr>
<td>Volume of distribution (L)</td>
<td>12.3</td>
</tr>
<tr>
<td>Clearance (L/hr)</td>
<td>14.52</td>
</tr>
<tr>
<td>Peak plasma concentration (mg/L)</td>
<td>277</td>
</tr>
<tr>
<td>AUC (mg.hr/L)</td>
<td>278</td>
</tr>
<tr>
<td>Elimination half-life (hr)</td>
<td>0.88</td>
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</table>

Trade: Piperacillin/Tazobactam (Stragen, UK)
Table 1. Summary of pharmacokinetic parameters of piperacillin/Tazobactam 4.5g given over 30 minutes in healthy volunteers. [AUC = area under the plasma concentration-time curve] 19

9. Summary of trials of piperacillin/tazobactam in critical illness and VAP

10. Pharmacokinetics in critically ill patients with VAP

Comparison of the volume of distribution and clearance of piperacillin in critically ill patients with healthy volunteers reveals both values to be significant higher in critically ill patients 19, 20.

<table>
<thead>
<tr>
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<th>Healthy volunteers 19</th>
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<tbody>
<tr>
<td>Volume of distribution (L)</td>
<td>12.3</td>
<td>10.4</td>
</tr>
<tr>
<td>Clearance (L/hr)</td>
<td>17.2</td>
<td>11.3</td>
</tr>
</tbody>
</table>

Table 2. Comparison of volume of distribution and clearance of piperacillin in patients with VAP and healthy volunteers.

Microdialysis studies in patients with severe sepsis and pneumonia demonstrate penetration of the lung by both piperacillin and tazobactam 21. The ratio of $AUC_{\text{Lung}} / AUC_{\text{Plasma}}$ was 0.63 ± 0.29 and 1.93 ± 1.56 for piperacillin and tazobactam respectively. Two studies by Boselli et al suggest that piperacillin and tazobactam penetrate epithelial lining fluid (ELF) but at concentrations that may be insufficient for some organism nosocomial pathogens 22, 23.

<table>
<thead>
<tr>
<th></th>
<th>Piperacillin/Tazobactam 4.5g over 30 minutes at steady state 22</th>
<th>Piperacillin/Tazobactam 18/2g continuous infusion 23</th>
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<tbody>
<tr>
<td></td>
<td>ELF (@5 hours)</td>
<td>ELF:Plasma Ratio</td>
</tr>
<tr>
<td>Piperacillin (mg/l)</td>
<td>13.6</td>
<td>0.56</td>
</tr>
<tr>
<td>Tazobactam (mg/l)</td>
<td>2.1</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Table 3. Penetration of piperacillin and tazobactam into the ELF of patients with VAP

11. Pharmacokinetics in patients undergoing renal replacement therapy

Piperacillin and tazobactam are both cleared by continuous veno-venous haemofiltration (CVVHF) but with a longer half life than in healthy subjects 24, 25. Tazobactam accumulates to a greater extent than piperacillin. Clearance of piperacillin and tazobactam is greater with continuous veno-venous haemodiafiltration (CVVHDF) than with CVVHF 26. Clearance of piperacillin and tazobactam is affected by the filter membrane material 27. Patients with some residual renal function on CVVHF have significantly greater clearance of piperacillin/tazobactam compared to anuric patients 28.

12. Randomised control trials

Three randomised controlled trials have studied the efficacy of piperacillin/tazobactam in the management of VAP. Two studies compared piperacillin/tazobactam with ceftazidime while amikacin was used in both study groups 29, 30. Both studies showed the study agents were comparable for both clinical outcome and tolerability. Jaccard et al compared piperacillin/tazobactam with imipenem-cilastatin in a heterogenous group of patients that included patients diagnosed with VAP 31. Overall clinical and safety outcome was the same but emergence of resistance to imipenem was observed in patients infected with Pseudomonas.

Trial Design

13. Trial summary

This study will investigate the plasma and lung concentration of piperacillin and tazobactam in critically ill patients. Patients will be included either when piperacillin/tazobactam is initiated or has previously been commenced by the critical care medical team. Patients will be given the standard dose of piperacillin/tazobactam as per hospital guidelines. Patients will be followed up until discharge from UHSM or thirty five days from the commencing the study drug.
14. **Primary objective**
The primary object of this study is to investigate the plasma and intra-pulmonary population pharmacokinetics of piperacillin and tazobactam in critically ill patients.

15. **Secondary objectives**
Secondary objectives of this trial are:
- To relate measures of clinical and microbiological (e.g. pathogen culture and sensitivity, antigen and DNA) outcome to measures of drug exposure.
- To investigate the impact of pulmonary permeability on lung penetration of piperacillin and tazobactam.
- To relate levels of inflammatory markers (e.g. cytokine profiles, procalcitonin) to measures of drug exposure.
- To investigate the formation of covalently bound piperacillin-albumin adducts.
- To construct an anonymised collection of wild-type bacteria for future in vitro laboratory investigations.
- To investigate the impact of antibiotic exposure on endotracheal colonisation and flora.

16. **Primary outcome measure**
The primary outcome measure is blood and lung concentrations of piperacillin and tazobactam.

17. **Secondary outcome measures**
Secondary outcome measures for this trial are:
- All cause mortality 28 days after commencing antibiotics
- Microbiological clearance of infection
- Number of ventilator-free days

18. **Null hypothesis**
Piperacillin/tazobactam (at the recommended doses) given intravenously does not achieves adequate blood and intrapulmonary levels of piperacillin/tazobactam for treatment of all patients with VAP.

19. **Trial design**
This is an open-label pharmacokinetic study without a control group. The pharmacokinetics of piperacillin/tazobactam may be assessed with the first dose and/or at steady-state.

20. **Justification of numbers needed**
A total of 20 to 40 patients will be recruited for this study. There are no statistical techniques that enable an estimate for the minimum sample size that is required to adequately describe the population pharmacokinetics and elucidate pharmacodynamic relationships. However as the population size increases progressively better estimates of the PK parameters are obtained with previous experience suggesting the population PK parameter values stabilise after approximately 20 healthy participants. For this study significant PK variability is expected therefore requiring a larger sample size.

21. **Dosing strategy**
Dose of piperacillin/tazobactam will be given as described in the summary of product characteristics. Patients will be given piperacillin/tazobactam (4g/0.5g), by slow intravenous injection over at least 3-5 minutes, every 8 hours. Patients with a creatinine clearance <20ml/min will be given piperacillin/tazobactam (4g/0.5g) every 12 hours. Patients undergoing continuous veno-venous haemofiltration will be given piperacillin/tazobactam (4g/0.5g) every 12 hours. For patients entering the study prior to piperacillin/tazobactam being administered (i.e. at episode 1) piperacillin/tazobactam will be administered as the sole antibacterial agent. For patients entering the study to investigate steady-state pharmacokinetics, (i.e. who are already on piperacillin/tazobactam; at episode 2) a second antibacterial agent may be co-administrated.

**Investigational medicinal product management**
Most of the Investigational medicinal product (IMP) will be stored in the pharmacy department. However, a supply of IMP will require storage on the Acute Intensive Care Unit (AICU) and Cardiothoracic Intensive Care Unit (CICU) as patients may start therapy out of pharmacy opening hours. All IMP will be temperature monitored and stored under appropriate conditions. The IMP on AICU and CICU will be segregated from normal stock and labelled in accordance with Annex 13. The receipt, distribution and returned IMP will be recorded by the pharmacy. Any unused IMP maybe re-issued. The IMP on the AICU and CICU accountability and prescribing will be the responsibility of the investigator.

For patients entering the study prior to piperacillin/tazobactam being administered (i.e. at episode 1) the IMP will be administered to the patients for five days. At the end of 5 days patients requiring further piperacillin/tazobactam will be administered ward stock drug. For patients entering the study to investigate steady-state pharmacokinetics, (i.e. who are already on piperacillin/tazobactam) the IMP will be just the single studied dosage.

22. Scoring systems

A number of scoring systems will be used to assess patients in this study. These include Acute Physiology and Chronic Health Evaluation II (APACHE II)32, Clinical Pneumonia Infection Score (CPIS)33, Ventilator associated pneumonia- predisposition, insult, response, organ dysfunction (VAP-PIRO)34 Lung Injury Score (LIS)35 and Sequential organ failure assessment (SOFA)36 (see appendix 1- 5).

23. Study episodes

Patients may enter the study at episode 1, where first-dose, steady-state pharmacokinetics and pharmacodynamics will be assessed or at episode 2, where steady-state pharmacokinetics alone will be assessed.

4. Episode 1

Before antibiotic administration a venous blood sample and non-direct bronchial lavage sample will be collected. The aim of this episode is to collect information regarding the aetiology of the infection and to assess the plasma pharmacokinetics during the first dose of piperacillin/tazobactam 4.5g. Serial blood sampling for plasma piperacillin and tazobactam levels will subsequently be collected at five or six further time points during the dosing interval (approximately ½, 1, 2, 4 and 6 hours after the first dose). The exact timing for the collection of the samples may be changed so as to ensure future samples are collected at the most informative time points. Prior to administration of piperacillin/tazobactam all women of childbearing age will have a pregnancy test, if this has not already been performed by the clinical care team. Anonymised data describing the patients’ demographic, underlying clinical condition, previous antibiotic use and selected scoring systems (including APACHE II (from admission), CPIS, VAP-PIRO, SOFA) will be collected. Serum creatinine will be measured at each time point.

25. Episode 2

The second episode will assess the pharmacokinetics of piperacillin/tazobactam during the maintenance phase of treatment. This episode will on the third or fourth day of piperacillin/tazobactam administration for patients entering the study at episode 1. For patients entering the study at episode 2, this episode can occur from the second dose onwards. Pre-dose (trough) piperacillin and tazobactam level will be collected followed by repeat blood samples at approximately ½, 1, 2, 4 and 6 hours post dose (this may be adjusted for the same reason as episode 1). Non-directed lavage samples will be collected at 1-2 and 6-7 hours post dose (the exact sampling times may change for the same reason as the plasma timings). The exact timing for the lavage samples may be changed so as to ensure they are collected at the most informative time points. These samples will be used to measure the concentration of study drugs, alveolar cells, urea, protein and markers of bacterial and fungal infections. A paired plasma urea sample must be taken with each NBL to calculate the dilution of epithelial lining fluid. Patients who have been extubated at this time will not have airway samples collected for drug levels but sputum will be collected for microbiological culture. Renal function will be measured by changes in serum creatinine between the first and last samples taken in this dosing interval (i.e. Pre-dose and at 6 hours). The exact extent of drug clearance by renal replacement will be estimated by measurement of piperacillin/tazobactam in both the ultrafiltrate and venous limb of the renal replacement therapy. Anonymised data describing the patients’ demographic, underlying clinical condition, previous antibiotic use and selected scoring systems (including CPIS, VAP-PIRO, SOFA, LIS) will be collected. Patients entering the study at episode 2 will have
demographic data and scoring systems information for episode 1 collected retrospectively. Women of child-bearing age entering the study at episode 2 will have a pregnancy test performed prior to administration of the IMP if this has not already been performed by the clinical care team.

26. **Episode 3**

After five days of therapy a blood and NBL sample will be collected. If the patient has been extubated a sputum sample for microbiological culture will be collected. Anonymised data describing the patients’ demographic, underlying clinical condition, previous antibiotic use and selected scoring systems (including CPIS, VAP-PIRO, SOFA) will be collected. Patients entering the study at episode 2 will not have blood or NBL sampling collected during episode 3.

27. **Episode 4**

When the patient is extubated the endotracheal tube (ETT) will be collected, labelled and frozen. The sample will be analysed by microbiological culture and identification of pathogen DNA. Endotracheal tubes will not be collected from patients who die whilst Intubated.

28. **Episode 5**

Fourteen to seventeen days after starting piperacillin/tazobactam a single blood sample will be collected to determine the concentration of the piperacillin-albumin adduct. The timing of this sample may change during the study when the piperacillin-albumin adduct from an initial group of patients has been assessed. There are no published data on the kinetics of this adduct to guide the timing of sample acquisition. Patients entering the study at episode 2 will not have blood or NBL sampling collected during episode 5.

29. **Changes to antibiotics**

30. **During the study drug administration period**

During the course of the study microbiological cultures results may become available that necessitate a change to the antibiotic prescription (study drug). If this occurs piperacillin/tazobactam should be changed by the clinical critical care or microbiology teams. Outcome data and the ETT should continue to be collected but no further blood samples for pharmacokinetics should be collected.

31. **After the study drug administration period**

Following five days of piperacillin/tazobactam (after episode 3) the drug may be stopped, continued or a second antibiotic agent added. The patient will continue in the trial and have samples and data collected as per protocol.

32. **End of Trial**

The trial will end when 40 patients have been recruited and followed up until discharge from hospital or 30 days after the end of piperacillin/tazobactam administration – whichever is sooner.

The trial will be stopped prematurely if:

- Mandated by the Ethics Committee
- Mandated by the Medicines and Healthcare products Regulatory Agency (MHRA)
- Funding for the trial ceases

33. **Stopping/discontinuation rules**

Any patient presenting with significant side effects from piperacillin/tazobactam will not have any further piperacillin/tazobactam administered. Pharmacokinetic and outcome data will still be collected.
### Study overview

<table>
<thead>
<tr>
<th>Episode</th>
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<th>2</th>
<th>3</th>
<th>4</th>
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<tr>
<td>Culture</td>
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<tr>
<td>PCR</td>
<td></td>
<td>x</td>
<td>x</td>
<td></td>
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</tr>
</tbody>
</table>

*If the patient has been extubated airway sample for microbiological culture will be sputum rather than NBL.

**Only for women of child-bearing age
Selection and withdrawal of subjects

35.  **Enrolment criteria**
Subjects will be included in the study if they meet the following inclusion criteria:

1. Subject is an adult aged 18 to 85
2. Subject requires or has been commenced on piperacillin/tazobactam as directed by clinical Intensive Care Unit medical staff

36.  **Exclusion criteria**
Subjects will be excluded from the study if they meet any of the exclusion criteria:

1. Subject is known to be intolerant of β-lactam antibiotics
2. Subject has an infection with a piperacillin/tazobactam resistant organism (i.e. *P. aeruginosa* with MIC ≥16 mg/L, other organism with MIC ≥ 8mg/L) or organisms with inherent resistance (e.g. *Stenotrophomonas*)
3. Subject is immunocompromised (neutropenia, HIV/AIDS)
4. Subject is unlikely to survive longer the 48 hours
5. Subject is pregnant or breast-feeding or plans to become pregnant during the course of the study
6. Subject is enrolled in another Clinical Trial of an Investigational Medical Product.

Safety

All procedures will be performed by qualified and appropriately trained personnel within the Intensive Care Unit.

Setting

This study will be performed within the Adult Intensive Care Unit and Cardiothoracic Intensive Care Unit at the University Hospital of South Manchester (Wythenshawe Hospital).

Interventions

37.  **Venepuncture**
The preferred method for collecting blood is via the patient existing arterial line. This allows collection of multiple samples without multiple venepuctures. If blood cannot be withdrawn from the cannula then conventional venepuncture will be performed. The total volume of blood per patient per study episode is shown below.

<table>
<thead>
<tr>
<th>Episodes</th>
<th>Total per patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<tr>
<td>2</td>
<td>32</td>
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<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

| Patients not on RRT | 68 |
| Addition blood volume for patients requiring RRT | 14 |
| Total volume per episode | 82 |

**Table 4.** Volume of blood taken from each patient per episode. (RRT= renal replacement therapy)

38.  **Non-directed Bronchial Lavage (NBL)**
During each NBL, 20ml of 0.9% saline is instilled through a catheter placed in the bronchial tree through the endotracheal tube, following pre-oxygenation with 100% oxygen for 5 minutes. The fluid is then immediately aspirated and the catheter removed. Typically 5-10ml of fluid is returned. Specific contra-indications to NBL are the patient requiring greater than 80% inspired oxygen, greater than 12 cmH20 PEEP, having severe bronchospasm or an uncontrolled intracranial pressure. During the five day dosing window routine samples will not be collected.
NBL is performed three times a week as routine, standard care of mechanically ventilated patients on the AICU at UHSM. The complication rate of NBL is 1.2-1.6%. The most common complication is oxygen desaturation with a return to baseline oxygen levels seen at a mean of 45 minutes (range: 5 minutes to 3 hours).

39. **Pregnancy test**
Prior to administration of the first dose of the IMP, all women of childbearing age will have a urinary pregnancy test.

40. **Renal replacement therapy**
Initiation of renal replacement therapy (RRT) is at the discretion of the clinical team. Patients will receive continuous veno-venous haemofiltration (CVVH) deliver by a Gambro Prisma machine.

**Sample collection and storage**
Samples will not be analysed until informed consent has been obtained with the exception of the urine and microbiological culture samples which will be processed in real time. Before analysing any samples the consent log will be checked to ensure consent has been obtained.

41. **Blood**
Samples will be collected in Lithium Heparin tubes and placed immediately onto ice. Plasma will be separated, following centrifugation, into coded labelled tubes. The sample for inflammatory markers will be transferred immediately to the laboratory in the Transplant centre at UHSM. Samples will be coded with a unique study number and stored in a secure room (usually at −80°C) in a locked freezer with limited access. Records will be kept of the samples obtained and tracking logs will be completed for the transfer of samples from the North West Lung Centre to the University of Manchester and the University of Liverpool.

42. **Non-directed bronchial lavage**
The NBL samples collected during episode 1 and 3 will be processed as routine clinical samples by the clinical microbiology laboratory at UHSM. The NBL samples collected during episode 2 will be split into alloquotes. One sample will be frozen for a cell count while the remaining sample will be centrifuged immediately to allow separation of the cell pellet from supernatant. 0.5ml of supernatant will be separated off for a urea assay. The cell pellet will be re-suspended in a known volume of saline and frozen. The lavage fluid will be separated into multiple aliquots, coded with a unique study number and stored in a secure room (usually at −80°C) in a locked freezer with limited access. Records will be kept of the samples obtained and tracking logs will be completed for the transfer of samples from the North West Lung Centre to the University of Manchester and the University of Liverpool.

43. **Endotracheal tube**
The endotracheal tube will be labelled and stored in a secure room in a locked freezer (at −80°C) with limited access. The tube will be kept for future analysis by microbiological culture and for pathogen (bacterial and fungal) DNA.

44. **Urine sample**
The urine sample for the pregnancy test will be processed on the Intensive Care unit. Following the pregnancy test the sample will be discarded.

**Adverse events, adverse reactions and safety reporting**
The most commonly reported adverse reactions are diarrhoea, nausea, vomiting and rash, each having a frequency of between 1 and 10%.

45. **Known adverse drug reactions**
System Organ Class Adverse drug reactions

**Investigations**
Uncommon
- Leucopenia
- Neutropenia
- Thrombocytopenia

Rare
- Anaemia
- Bleeding manifestations (including purpura, epistaxis, bleeding time prolonged)
- Eosinophilia
- Haemolytic anaemia

Very rare
- Agranulocytosis
- Coombs direct test positive
- Pancytopenia
- Prolonged partial thromboplastin time
- Prothrombin time prolonged
- Thrombocytosis

Nervous system disorders
Uncommon
- Headache
- Insomnia

Rare
- Muscular weakness
- Hallucination
- Convulsion
- Dry mouth

Gastrointestinal disorders
Common
- Diarrhoea
- Nausea
- Vomiting

Uncommon
- Constipation
- Dyspepsia
- Jaundice
- Stomatitis

Rare
- Abdominal pain
- Pseudomembranous colitis
- Hepatitis

Renal and urinary disorders
Uncommon
- Blood creatinine increased

Rare
- Interstitial nephritis
- Renal failure

Very rare
- Blood urea nitrogen increased

Skin and subcutaneous tissue disorders
Common
- Rash

Uncommon
- Pruritus
- Urticaria
- Erythema

Rare
- Bullous dermatitis
- Erythema multiforme
Increased sweating
Eczema
Exanthema

**Very rare**
Stevens-Johnson Syndrome
Toxic epidermal necrolysis

**Musculoskeletal and connective tissue disorders**

Rare
Arthralgia
Myalgia

**Metabolism and nutrition system disorders**

**Very rare**
Hypoalbuminaemia
Hypoglycaemia
Hypoproteinaemia
Hypokalaemia

**Infections and infestation**

Uncommon
Candidal superinfection

**Cardiovascular disorders**

Uncommon
Hypotension
Phlebitis
Thrombophlebitis

Rare
Flushing

**General disorders and administrative site conditions**

Uncommon
Fever
Injection site reaction

Rare
Rigors
Tiredness
Oedema

**Immune system disorders**

Uncommon
Hypersensitivity reaction

Rare
Anaphylactic/anaphylactoid reaction (including shock)

**Hepato-biliary disorders**

Uncommon
Alanine aminotransferase increased
Aspartate aminotransferase increased

Rare
Bilirubin increased
Blood alkaline phosphatase increased
Gamma-glutamyltransferase increased
Hepatitis

**Table 5.** Undesirable effects of piperacillin/tazobactam (very common ≥1/10, common ≥1/100 and <1/10, uncommon ≥1/1000 and <1/100, rare, ≥1/10 000 and <1/1000 and very rare, <1/10 000). Adapted from www.medicines.org.uk/EMC/medicine/21655/SPC/Piperacillin+Tazobactam+4+g+0.5+g+Powder+for+Solution+for+Injection+or+Infusion/ [accessed 3rd May 2011]

**46. Safety reporting**

AEs will be recorded and reported in accordance with the Medicines for Human Use (the Regulations) (Clinical Trials) Regulations 2004, incorporating Directive 2001/20/EC. The definitions of AE, adverse reaction, serious adverse reaction, unexpected serious adverse reaction and serious adverse events (SAEs) will be as given in the regulations. The outcome of an AE will be defined according to ICH Topic
47. **Adverse Event**

An AE is defined as any untoward medical occurrence in a subject to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.

48. **Adverse Event Reporting**

AEs will be elicited at the time indicated in the schedule. Any adverse or unexpected events, signs and symptoms, will be fully recorded on the AE form including details of intensity, onset, duration, outcome and relationship to the drug. AEs may also be reported spontaneously at any time. The type and duration of follow up of subjects after AEs will also be documented.

49. **Pre-dose Event**

A pre-dose event is any medical occurrence occurring after consent and prior to dosing. Any pre-dose event reported or observed will be recorded as a pre-dose event.

50. **Laboratory Results**

Laboratory results coded ‘Clinically Significant’ will be recorded as an AE. The definition of clinical significance will be at the discretion of the Investigator.

51. **Assessment of Adverse Event Severity**

The severity of each AE will be assessed at onset by a nurse and/or physician. When recording the outcome of the AE the maximum severity of the AE experienced will also be recorded.

The following guidelines will be used to assess severity:

- **Mild:** Awareness of sign or symptom but easily tolerated.
- **Moderate:** Discomfort enough to cause interference with usual activity.
- **Severe:** Incapacitating with inability to work or do usual activity.

52. **Assessment of Adverse Event Relationship to Study Drug**

The relationship of an AE to investigational product(s) will be classified as:

**Certain:** a clinical event, including laboratory test abnormality, occurring in a plausible time relationship to drug administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be definitive pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary.

**Probable/likely:** a clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfil this definition.

**Possible:** a clinical event, including laboratory test abnormality, with a reasonable time relation to administration of the drug, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.

**Unlikely:** a clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations.

**Conditional/Unclassified:** a clinical event, including laboratory test abnormality, reported as an adverse reaction, about which more data are essential for a proper assessment or the additional data are under examination.
Not assessable/Unclassifiable: a report suggesting an adverse reaction which cannot be judged because information is insufficient or contradictory and cannot be supplemented or verified.

Not related: a clinical event, including laboratory test abnormality, with sufficient evidence to accept that there is no causal relationship to drug administration (e.g., no temporal relationship to drug administration, because the drug was administered after onset of event; investigation shows that the drug was not administered; proof of other cause; etc.).

53. Assessment of Adverse Event Outcome

Outcome of AEs will be defined as:

- Resolved
- Resolving
- Not resolved
- Resolved with sequelae
- Fatal
- Unknown

54. Action Taken for Adverse Event

Any action taken will be recorded on the CRF according to the following:

- None
- Concomitant medication required
- Trial drug discontinued
- Withdrawal from study
- Other

55. Collection of Extra Laboratory Samples / Investigations

In the event of a clinically important AE a suitable sample will be collected for drug assay or for additional laboratory tests. The Investigator must ensure that the sample is properly labelled and stored. The Investigator and others responsible for care of the subjects should institute any supplementary investigations of significant AEs based on the clinical judgement of the likely causative factor. This may include seeking a further opinion from a specialist in the field of the AE. The sponsor may suggest special tests based on expert advice.

56. Follow-up of Adverse Events Present at Last Scheduled Study Visit

AEs present at the last scheduled study visit that require follow-up or a repeat laboratory test will be followed-up initially for 5 working days to allow for at least one cycle of repeat laboratory tests if required. AEs requiring further follow-up or ongoing at two weeks post last subject, last visit, will be reviewed with the Sponsor on an individual basis to determine whether the database will be locked and subsequently updated once the events of ongoing AEs are resolved or whether data base lock will be held.

57. Adverse Reaction

58. Adverse Reaction

An adverse reaction is any untoward and unintended response in a subject to an investigational medicinal product which is related to any dose administered to that subject.

59. Unexpected Adverse Reaction

An unexpected adverse reaction is an adverse reaction the nature and severity of which is not consistent with the information in the SPC relating to the trial (summarised in Table 5). The Sponsor will be responsible for ensuring that the Investigator is provided with an up to date SPC/Investigator’s Brochure for the investigational product.
60. **Serious Adverse Event, Serious Adverse Reaction or Unexpected Serious Adverse Reaction**

A SAE, serious adverse reaction or unexpected serious adverse reaction means any AE, adverse reaction or unexpected adverse reaction respectively that:

- results in death
- is life threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability or incapacity, or
- consists of a congenital anomaly or birth defect

61. **SUSAR**

Where the adverse reaction is unexpected and serious it may be termed Suspected Unexpected Serious Adverse Reaction (SUSAR). The Investigator will ensure all relevant information is provided to the Sponsor to allow the Sponsor to meet their obligations to report to the MHRA (and where applicable, the competent authorities of any other European Economic Area (EEA) State in which the trial is being conducted) any SUSAR.

62. **Reporting requirements**

The reporting requirements are summarised in Figure 1.

To report SAEs to the Sponsor, the following 24h contact details should be used:

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<thead>
<tr>
<th>Telephone – (0161) 291 5773</th>
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<tr>
<td>OR</td>
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<td>Fax completed forms to - 0161 291 5771</td>
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<td>OR</td>
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</table>

**Adverse events/Adverse reactions**

All AEs, not requiring expedited reporting or reporting to the sponsor, will be recorded on an electronic database. The investigator will assess the severity of the event and relationship to the study drug. All AEs will subsequently be included in the Development Safety Update Report (DSUR) which will be submitted annual by the R&D department to the REC and MHRA.

63. **Severe adverse events/Severe adverse reactions**

All SAEs and SARs (listed in Table 5) will be recorded in a serious adverse event form, electronic database and the annual DSUR. Events expected in the critically ill population (listed in Table 6) will be exempt from SAE reporting to the sponsor other than recording in the annual DSUR. All other SAEs (i.e an event not listed in Table 6) will be reported to the Sponsor by telephone and a written alert will follow by fax or other method of fast transmission as soon as possible and within 24 hours of the Investigator’s awareness.

64. **SUSAR**

For a SUSAR that is fatal or life-threatening this should be reported as soon as possible and not later than 7 days after the Sponsor was first advised, for any other SUSAR this should be within 15 days. The Investigator will ensure that the ethics committee is notified as soon as possible and no later than 7 days after any life threatening or fatal SUSAR or within 15 days for any other SUSAR.

In other situations as defined in ICH Topic E2A, ICH Guideline (CPMP/ICH/377/95) expedited reporting of AEs may also be appropriate. In the following situations the process detailed above for SAEs will also take place:

- AEs that require intervention to prevent them becoming serious
- Unexpected non-serious AEs (to be defined following discussion with Sponsor)
- AEs of special interest (to be defined following discussion with Sponsor).

<table>
<thead>
<tr>
<th>Exempt event</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular events</td>
<td>Atrial fibrillation/flutter</td>
</tr>
<tr>
<td></td>
<td>Ventricular fibrillation/tachycardia</td>
</tr>
<tr>
<td></td>
<td>Percutaneous coronary intervention</td>
</tr>
<tr>
<td></td>
<td>Coronary Artery Bypass Graft</td>
</tr>
<tr>
<td></td>
<td>Non-fatal MI</td>
</tr>
<tr>
<td></td>
<td>Acute coronary syndrome</td>
</tr>
<tr>
<td></td>
<td>New or worsening heart failure</td>
</tr>
<tr>
<td></td>
<td>Shock / hypotension</td>
</tr>
<tr>
<td></td>
<td>Stroke / Transient ischaemic attack</td>
</tr>
<tr>
<td></td>
<td>Malignant hypertension</td>
</tr>
<tr>
<td></td>
<td>Vascular surgery</td>
</tr>
<tr>
<td></td>
<td>Amputation</td>
</tr>
<tr>
<td></td>
<td>Deep venous thrombosis</td>
</tr>
<tr>
<td>Surgical / Post-operative care</td>
<td>Wound debridement / re-exploration</td>
</tr>
<tr>
<td></td>
<td>Wound dehiscence</td>
</tr>
<tr>
<td>Infections</td>
<td>New or worsening pneumonia</td>
</tr>
<tr>
<td></td>
<td>New or worsening bacteraemia</td>
</tr>
<tr>
<td></td>
<td>New or worsening sepsis</td>
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<tr>
<td></td>
<td>Urinary tract infection</td>
</tr>
<tr>
<td></td>
<td>Pyelonephritis</td>
</tr>
<tr>
<td></td>
<td>Cellulitis</td>
</tr>
<tr>
<td></td>
<td>Sinusitis</td>
</tr>
<tr>
<td>Neurological event</td>
<td>Stroke / Transient ischaemic attack</td>
</tr>
<tr>
<td></td>
<td>Confusion / agitation</td>
</tr>
<tr>
<td></td>
<td>Reduced conscious level</td>
</tr>
<tr>
<td></td>
<td>Intra-cerebral oedema</td>
</tr>
<tr>
<td></td>
<td>Intra-cerebral haemorrhage</td>
</tr>
<tr>
<td></td>
<td>Sub-arachnoid haemorrhage</td>
</tr>
<tr>
<td></td>
<td>Polyneuropathy</td>
</tr>
<tr>
<td>Renal</td>
<td>Acute kidney injury</td>
</tr>
<tr>
<td></td>
<td>Urinary retention</td>
</tr>
<tr>
<td></td>
<td>Hyper / Hypokalaemia</td>
</tr>
<tr>
<td></td>
<td>Hyper / Hyponatraemia</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>Acute lung injury / ARDS</td>
</tr>
<tr>
<td></td>
<td>Pulmonary embolus</td>
</tr>
<tr>
<td></td>
<td>Pleural effusion / Empyema</td>
</tr>
<tr>
<td></td>
<td>Video-assisted thoracic surgery</td>
</tr>
<tr>
<td></td>
<td>Thoracotomy</td>
</tr>
<tr>
<td></td>
<td>Bronchospasm</td>
</tr>
<tr>
<td></td>
<td>Tracheostomy insertion</td>
</tr>
<tr>
<td></td>
<td>Re-intubation</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Abdominal pain</td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>Reduced absorption</td>
<td>Pancreatitis</td>
</tr>
<tr>
<td>Gastroparesis</td>
<td>Nausea / vomiting</td>
</tr>
<tr>
<td>Cholecystitis</td>
<td>Gastro-oesophageal reflux</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>Gastro-intestinal tract ulceration</td>
</tr>
<tr>
<td></td>
<td>Ischaemic colitis</td>
</tr>
<tr>
<td></td>
<td>Laparotomy</td>
</tr>
<tr>
<td></td>
<td>Acute liver impairment</td>
</tr>
<tr>
<td></td>
<td>Pseudomembranous colitis</td>
</tr>
<tr>
<td>Other</td>
<td>Pressure sore</td>
</tr>
<tr>
<td></td>
<td>Anaemia</td>
</tr>
<tr>
<td></td>
<td>Blood transfusion</td>
</tr>
<tr>
<td></td>
<td>Hypoaalbinaemia</td>
</tr>
</tbody>
</table>

Table 6. Table of clinical adverse events expected in a critically ill population.
Figure 1. Summary of adverse event reporting structure.
Data Management

65. **Training Issues**
To ensure accurate and reliable data each member of the research team will be provided instructional material and a training session regarding the trial.

66. **Data collection**
All data for an individual patient will be collected by the Principal Investigator or delegated nominees and recorded in a CRF. Patient identification in the CRF will be through their unique Patient Trial Number allocated at the inclusion into the study. Data will be collected from the time the patient is considered for entry into the trial until discharge from hospital or 30 days after the end study drug administration. As patients are continuously monitored whilst on the intensive care unit and data is recorded in the patients notes approximately hourly discrepancies may occur between what is observed by the investigators on the patients’ monitors at the relevant time and what is recorded. For this reason the CRF will be the source data for this study.

67. **Data Handling and Record Keeping**
During the course of the study data will be transferred onto an electronic CRF (eCRF). The eCRF will include the Patient Trial Number as the method of patient identification. The eCRF will be held on a secure database system at the University of Manchester. The samples will be link anonymised. Data will be stored on a password protected database with access limited to study personnel. USC*PACK and NONMEM pharmacokinetic programs will be used to mathematically model changes in both the blood and intrapulmonary blood levels. Due care will be taken to ensure data safety and integrity, and compliance with the Data Protection Act 1998.

68. **Data monitoring**
The research will be monitored using University Hospital of South Manchester NHS Foundation Trust R&D department processes.

69. **Archiving**
Trial documentation and data will be archived for at least five years after completion of the trial.

**Patient information and consent form**
The Principal Investigator is responsible for ensuring that informed consent is given by each patient or their legal representative. The task of taking consent may be delegated to other investigators or suitably trained professionals as laid out in the Medicines for Human Use (Clinical Trials) Regulations 2004. It is expected that the majority of patients will be unable to give informed consent, due to alteration in their conscious level caused by critical illness and sedation. In this circumstance informed consent will be sought from a Personal Legal Representative (PerLR) or Professional Legal Representative (ProfLR). Treatment of infection (including VAP) in critically ill patients is a medical emergency and delay in the administration of antibiotics is associated with a significant increase in mortality. Patients will be treated with piperacillin/tazobactam as they would if not enrolled in the study and samples may be collected without prospective consent (as laid out in The Medicines for Human Use (Clinical Trials) Amendment (No.2) Regulations 2006).

No collection of demographic data or analysis of samples will be performed until consent is obtained. Patient, PerLR or ProfLR consent must be obtained within 48 hours of entering the study (i.e. patients will have the initial samples but will not have further samples collected until consent has been obtained). (See Figure 2a and 2b).
70. **Patient Consent**

If possible, informed consent will be obtained from the patient. The patient will be informed about the study by a member of the research team and given a copy of the Patient Information Sheet (PIS). Patients will be given sufficient time to consider entry into the study. If the patient enters decides to enter the study they will be asked to sign two copies of the Patient Consent Form which will be counter-signed by the member of the research team taking consent.

71. **Personal Legal Representative Consent**

If the patient is unable to give informed consent the research team will identify a Personal Legal Representative (PerLR) who is:

- not connected to the research project, and
- engaged in caring for the participant, or is interested in his/her welfare but not in a professional capacity or for remuneration, and
- is willing to be consulted

The identified PerLR will be given information about the project so they can inform the research team of the proposed participant’s wishes, feelings and values regarding being involved in the project. If the PerLR assessment of the person’s wishes, feelings and values indicate that the patient would want to participate in the research then the PerLR should make a declaration that the research can proceed with the involvement of that person. The PerLR will be given up to 48 hours after the first dose of the study drug has been given to consider the patients participation in the study. If the PerLR is unable to make a decision they may defer the decision to a ProLR. The PerLR will be asked to sign three copies of the PerLR Consent Form which will then be counter-signed by the responsible clinician.

72. **Professional Legal Representative Consent**

In the event that the patient is unable to give informed consent and no PerLR is available, a senior doctor, who is not connected with the conduct of the trial, may act as a Professional Legal Representative (ProfLR). The ProLR may also give consent for a patient to be enrolled in the study if the PerLR is undecided. The doctor will be informed about the trial by a member of the research team and given a copy of the PIS. The doctor will be asked to sign two copies of the Professional Legal Representative Consent Form.

73. **Retrospective Patient Information**

Patients for whom consent is given by a PerLR or ProfLR will be informed of their trial participation by a member of the research team once they have regained the capacity to understand the trial details. The research team will discuss the study with the patient and the patient will be given a copy of the PIS. The patient will be given 24 hours to read the information sheet and consider their participation in the study. The patient will be asked for consent to continue their participation in the study and asked to sign the Consent to Continue Form. Patients who do not give consent will have their data entered into the trial analysis up to the point when they declined to consent to continue in the study. Patients who have not regained capacity at the end of the study (i.e. 30 days after the end of piperacillin/tazobactam administration) will not be asked to consent to continue in the study.

During the taking of retrospective consent all women of child-bearing age will be advised to consider contraception for at least one month after the last dose of antimicrobial agent.

74. **Withdrawal of Consent**

Patients may withdraw or be withdrawn by PerLR or ProfLR from the trial at any time. Samples collected and data recorded up to the point of withdrawal will be included in the trial analysis.

75. **Consent log**

A consent log will be kept to record when consent was obtained from each patient or their representative.
Identification of patients

The trial will be advertised widely on the AICU and CICU at UHSM. Patients will be identified by medical, pharmacy, nursing or other health-care professionals. On identification of a suitable patient the clinical team will liaise with the research team. An anonymous record of all patients screened will be held by the research team with reasons for non-inclusion.

Research teams approach to patients or their representatives

76. Patients

The initial approach will always be made by a member of the clinical team who will ask permission for the researcher to visit the patient. If permission is not granted anonymous details of the patients will be entered into the screening log.

77. PerLR

The initial approach will always be made by a member of the clinical team who will ask permission for the researcher to visit the patient representative. If permission is not granted anonymous details of the patients will be entered into the screening log. If a patient has already had samples collected a joint approach between the clinical and research team may be more appropriate with the clinical member of staff taking the lead. It must be made clear to the ProLR that the patient has had samples collected before the family has been approached. Informed consent is required to collect any demographic data or process any samples. Informed consent must be obtained with 48 hours of the first dose of pip/taz.

78. ProLR

The ProLR may be approached directly by the research team.
Figure 2a. Flow diagram illustrating the consent process for patients entering the study at episode 1.
Figure 2b. Flow diagram illustrating the consent process for patients entering the study at episode 2.
**Ethics and Regulatory Approval**

The trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and outlined by the Medicines and For Human Use (Clinical Trials) Regulations 2004. Approval from a Research Ethics Committee and Clinical Trial Authorisation from the Medicines and Healthcare products Regulatory Agency (MHRA) are required before the start of the trial. The trial has been registered with the UK National Institute for Health Research (NIHR) Clinical Research Portfolio.

**Financing and Insurance**

All patients enrolled in this study have clinical indications for commencing piperacillin/tazobactam therefore the drug costs will be incurred by the NHS. All other cost for this study, including laboratory costs and investigator time are supported by the Medical Research Council and University Hospital of South Manchester Endowment Award.

NHS indemnity covers NHS staff and medical academic staff with honorary contracts. UHSM provides indemnity for any harm caused to patients by the design of the research protocol.

**Dissemination of results**

The results of this study will be presented at major international conferences and published in international journals. Study information and results will be posted on the study website. A patient results newsletter will be sent to all patients who request one at the time of consent.
Trial summary

**PROPEL**

**Enrolment criteria**
1. Adult aged 18 to 75
2. Requires piperacillin-tazobactam as directed by clinical Intensive Care Unit medical staff for suspected pulmonary infection
3. Intubated and mechanically ventilated for greater than 48 hours

**Exclusion criteria**
1. Intolerant of β-lactams antibiotics
2. Infection with a piperacillin-tazobactam resistant organism
3. Subject is immunocompromised
4. Unlikely to survive longer the 48 hours
5. Pregnant or breast-feeding

**Patient may enter study at Episode 1 or Episode 2**

**Episode 1 (Day 1)**
- Blood sample for pharmacokinetic profile (6-7 samples)
- Non-directed bronchial lavage fluid (1 sample)
- Urine for pregnancy test (if applicable)
- Severity scores (Collected in retrospect for patients entering study in episode 2)

**Episode 2 (approximately Day 2-5; steady state)**
- Bloods sample for pharmacokinetic profile (6-7 samples)
- Non-directed bronchial lavage fluid (2 samples)
- Demographic data
- Severity scores
- Urine for pregnancy test (only applicable if patient entering study in episode 2)

**Episode 3 (Day 5)**
- Bloods sample for pharmacokinetic profile (1 sample)*
- Non-directed bronchial lavage fluid (1 sample)*
- Severity scores

**Episode 4**
- Endotracheal tube

**Episode 5 (Day 14-17)**
- Bloods sample for pharmacokinetic profile (1 sample)*

**Trial endpoint**
- Discharge from hospital or 30 days after the end of piperacillin-tazobactam administration

* Not collected for patients

**References**
References

## Appendices

### 79. Appendix 1 – Clinical Pulmonary Infection Score (CPIS)

<table>
<thead>
<tr>
<th>Score</th>
<th>Temp (°C)</th>
<th>Blood leukocytes (x mm$^{-3}$)</th>
<th>Airway secretions</th>
<th>PiO$_2$/FiO$_2$ (kPa)</th>
<th>Chest radiograph</th>
<th>Microbiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&gt; 36 to &lt; 38.4</td>
<td>&gt; 4000 to &lt; 11000</td>
<td>No secretions</td>
<td>&gt; 33 or ARDS</td>
<td>No infiltrate</td>
<td>No growth</td>
</tr>
<tr>
<td>1</td>
<td>&gt; 38.5 to &lt; 38.9</td>
<td>&lt; 4000 or &gt; 11000</td>
<td>Non-purulent</td>
<td></td>
<td>Patchy, diffuse infiltrate</td>
<td>Moderate or heavy growth</td>
</tr>
<tr>
<td>2</td>
<td>&lt; 36.0 or &gt; 39.0</td>
<td>Purulent</td>
<td></td>
<td>&lt; 33 without ARDS</td>
<td>Localised infiltrate</td>
<td>pathogen bacteria also seen on the Gram stain</td>
</tr>
</tbody>
</table>

(Abbreviations: ARDS: Acute respiratory distress syndrome)

<table>
<thead>
<tr>
<th>Score</th>
<th>Total score analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 5</td>
<td>Low probability of VAP</td>
</tr>
<tr>
<td>≥6</td>
<td>High probability of VAP</td>
</tr>
</tbody>
</table>
## Appendix 2 – Sequential organ failure assessment (SOFA)

<table>
<thead>
<tr>
<th>Organ System</th>
<th>Score</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Respiration</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{PaO}_2/\text{FiO}_2$, kPa</td>
<td>&gt;53.3</td>
<td>40-53.3</td>
<td>0-39.9</td>
<td>0-25.2 R</td>
<td>0-13.3 R</td>
<td></td>
</tr>
<tr>
<td><strong>Blood</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets, x10E9/L *</td>
<td>&gt;150</td>
<td>101-150</td>
<td>51-100</td>
<td>21-50</td>
<td>0-20</td>
<td></td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilirubin, µmol/l mg/dL</td>
<td>0-19</td>
<td>20-32</td>
<td>33-101</td>
<td>102-204</td>
<td>&gt;204</td>
<td>&gt;12.0</td>
</tr>
<tr>
<td></td>
<td>&lt;1.2</td>
<td>1.2-1.9</td>
<td>2.0-5.9</td>
<td>6.0-11.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CNS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glasgow Coma Score</td>
<td>15</td>
<td>13-14</td>
<td>10-12</td>
<td>6-9</td>
<td>&lt;6</td>
<td></td>
</tr>
<tr>
<td><strong>CVS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>&gt;70</td>
<td>0-70</td>
<td>Dopamine ≤5.0 or dobutamine (any dose) a</td>
<td>Dopamine 5-14.9 or epi ≤0.1 or norepi ≤0.1 a</td>
<td>Dopamine ≥15 or epi &gt;0.1 or norepi &gt;0.1 a</td>
<td></td>
</tr>
<tr>
<td><strong>Renal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>s-creatinine, µmol/l mg/dL</td>
<td>&lt;110</td>
<td>110-170</td>
<td>171-299</td>
<td>300-440</td>
<td>&gt;440 or dialysis &gt;5.0</td>
<td></td>
</tr>
<tr>
<td>Or urine output</td>
<td></td>
<td>&lt;1.2</td>
<td>1.2-1.9</td>
<td>2.0-3.4</td>
<td>3.5-4.9</td>
<td>Or &lt;500 mL/24h</td>
</tr>
<tr>
<td>Or &lt;200 mL/24h</td>
<td></td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

* R) With respiratory support

* * corresponds to x10³/mm³

* a Adrenergic agents administered for at least 1 hr (doses given are in µg/kg/min).

The $\text{PaO}_2/\text{FiO}_2$ ratio is calculated without reference to the use or mode of mechanical ventilation, and without reference to the use or level of PEEP.

The Glasgow Coma Score is preferably calculated by the patients nurse, and is scored conservatively (for the patient receiving sedation or muscle relaxants, normal function is assumed unless there is evidence of intrinsically altered mentation).

Mean arterial pressure (MAP) = diastolic + ($1/3$*(systolic-diastolic))
81. **Appendix 3 – Ventilator associated pneumonia- predisposition, insult, response, organ dysfunction (VAP-PIRO)**

### VAP-PIRO Score

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>P</td>
<td>Comorbidities</td>
<td>□ 1 point</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Bacteremia</td>
<td>□ 1 point</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>Systolic BP&lt;90mmHg*</td>
<td>□ 1 point</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>ARDS</td>
<td>□ 1 point</td>
<td></td>
</tr>
</tbody>
</table>

*Comorbidities: COPD, Immunocompromise, CHF, Chronic Renal Failure, Chronic Hepatopathy

* - Or need of vasopressor drugs to maintain blood pressure

---

**Total Score**

**Interpretation**

<table>
<thead>
<tr>
<th>Score</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1 point</td>
<td>Low risk (1 in 8) ICU mortality</td>
</tr>
<tr>
<td>2 points</td>
<td>High risk (1 in 2) ICU mortality</td>
</tr>
<tr>
<td>3-4 points</td>
<td>Very high risk (4 in 5) ICU mortality</td>
</tr>
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</table>
### THE APACHE II SEVERITY OF DISEASE CLASSIFICATION SYSTEM

<table>
<thead>
<tr>
<th>PHYSIOLOGIC VARIABLE</th>
<th>HIGH ABNORMAL RANGE</th>
<th>LOW ABNORMAL RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEMPERATURE (rectal °C)</td>
<td>37.5-38.5 °C</td>
<td>36.1-36.5 °C</td>
</tr>
<tr>
<td>MEAN ARTERIAL PRESSURE (mm Hg)</td>
<td>85-95</td>
<td>65-75</td>
</tr>
<tr>
<td>HEART RATE (heartbeats/min)</td>
<td>100-120</td>
<td>90-110</td>
</tr>
<tr>
<td>RESPIRATORY RATE (breaths/min)</td>
<td>15-25</td>
<td>14-24</td>
</tr>
<tr>
<td>BLOOD GLUCOSE (mmol/L)</td>
<td>4.7-8.3</td>
<td>4.4-7.2</td>
</tr>
<tr>
<td>S. Na+ (mmol/L)</td>
<td>135-145</td>
<td>130-140</td>
</tr>
<tr>
<td>SERUM SODIUM (mmol/L)</td>
<td>130-140</td>
<td>120-130</td>
</tr>
</tbody>
</table>

**CHRONIC HEALTH POINTS**

- **AGE POINTS**: Assign points to age as follows:
  - 0-64: 0
  - 65-74: 1
  - 75-84: 2
  - 85 or older: 3

- **ABSORBED Points**
  - 0
  - 4.5+2
  - 3+3
  - 2.5+5
  - 2+6

**DEFINITIONS**

- **Cardiovascular**: New York Heart Association Class IV.
- **Respiratory**: Chronic obstructive, obstructive or restrictive disease resulting in severe exertion restriction, i.e., unable to climb stairs or perform household duties, or documented chronic hypoxia, hypercapnia, secondary polycythemia, severe pulmonary hypertension (pulmonary arterial hypertension).
- **Renal**: Requiring chronic dialysis.
- **Immunocompromised**: The patient has received therapy that suppresses resistance to infection, i.e., chronic immunosuppression, chemotherapy, radiation, long-term glucocorticoids, or is a patient that is sufficiently advanced to suppress resistance to infection, e.g., leukemia, lymphoma, AIDS.

**APACHE II SCORE**

- Sum of **APS points** + **Age points** + **Chronic Health points**

---

### Protocol

**PROPEL**: Plasma and intra-pulmonary population pharmacokinetics of piperacillin/tazobactam in critically ill patients

EudraCT number: 2011-004470-28

CTA: 21463/0218/001

REC number: 11/NW/0680

Reference number: PROPEL-01

Version Number: 4.0

Release Date: 22.07.2013

Page 287 of 338
### Appendix 5 – The Lung Injury Score (Murray score)

<table>
<thead>
<tr>
<th>Chest Xray score</th>
<th>score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No alveolar consolidation</td>
<td>0</td>
</tr>
<tr>
<td>Alveolar consolidation confined to 1 quadrant</td>
<td>1</td>
</tr>
<tr>
<td>Alveolar consolidation confined to 2 quadrant</td>
<td>2</td>
</tr>
<tr>
<td>Alveolar consolidation confined to 3 quadrant</td>
<td>3</td>
</tr>
<tr>
<td>Alveolar consolidation in all 4 quadrant</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hypoxemia score</th>
<th>score</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO$_2$/FiO$_2$ &gt; 300</td>
<td>0</td>
</tr>
<tr>
<td>PaO$_2$/FiO$_2$ 225-299</td>
<td>1</td>
</tr>
<tr>
<td>PaO$_2$/FiO$_2$ 175-224</td>
<td>2</td>
</tr>
<tr>
<td>PaO$_2$/FiO$_2$ 100-174</td>
<td>3</td>
</tr>
<tr>
<td>PaO$_2$/FiO$_2$ &lt; 100</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PEEP score (when ventilated)</th>
<th>score</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEEP $\leq$ 5 cm H$_2$O</td>
<td>0</td>
</tr>
<tr>
<td>PEEP 6-8 cm H$_2$O</td>
<td>1</td>
</tr>
<tr>
<td>PEEP 9-11 cm H$_2$O</td>
<td>2</td>
</tr>
<tr>
<td>PEEP 12-14 cm H$_2$O</td>
<td>3</td>
</tr>
<tr>
<td>PEEP $\geq$ 15 cm H$_2$O</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Respiratory system compliance score (when available)</th>
<th>score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compliance $\geq$ 80 ml/cmH$_2$O</td>
<td>0</td>
</tr>
<tr>
<td>Compliance 60-79 ml/cmH$_2$O</td>
<td>1</td>
</tr>
<tr>
<td>Compliance 40-59 ml/cmH$_2$O</td>
<td>2</td>
</tr>
<tr>
<td>Compliance 20-39 ml/cmH$_2$O</td>
<td>3</td>
</tr>
<tr>
<td>Compliance $\leq$ 19 ml/cmH$_2$O</td>
<td>4</td>
</tr>
</tbody>
</table>

The final value is obtained by dividing the aggregate sum by the number of components that were used.

<table>
<thead>
<tr>
<th>Score</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No lung injury</td>
<td>0</td>
</tr>
<tr>
<td>Mild-to-moderate lung injury</td>
<td>0.1-2.5</td>
</tr>
<tr>
<td>Severe lung injury (ARDS)</td>
<td>$&gt; 2.5$</td>
</tr>
</tbody>
</table>

* Abbreviations: PaO$_2$/FiO$_2$ = arterial oxygen tension to inspired oxygen concentration ratio
  PEEP = positive end-expiratory pressure.
35. Appendix 2: PROPEL Case Report Form v3.2 11.0 3.20 13
Plasma and intrapulmonary population pharmacokinetics of piperacillin/tazobactam in critically ill patients

Case Report Form

Subject Number: 
Patient Recruitment Date: 
Date patient consent completed: 
Date ProfLR completed: 
Date PerLR completed: 

Study Arm (please tick)
- First Dose and Steady State □
- Steady State only □

Principal Investigator: Dr Tim Felton
MRC Clinical Research Fellow
The University of Manchester
Univeristy Hospital of South Manchester
M23 9LT
Recruitment

Patient to commence pip/taz

- 1st dose PK
- NBL
- Demographic data
- Scoring data

Episode 1

Day 1
Prior to starting pip/taz

Patient already commenced pip/taz

- Demographic data
- Scoring data

Collected in retrospect

Episode 2

Day 3
Steady-state (SS)

- SS dose PK
- NBL x2
- Demographic data
- Scoring data

Episode 3

Day 5

- NBL
- Demographic data
- Scoring data

Episode 4

Extubation

- Collect ETT

Episode 5

Day 14 to 17

- Blood sample

Recruitment Episode 5
Day 14 to 17
Episode 4
Extubation
Episode 3
Day 5
Episode 2
Day 3
Steady-state (SS)
Episode 1
Day 1
Prior to starting pip/taz
Patient already commenced pip/taz
- Demographic data
- Scoring data
Collected in retrospect
Consent Flowchart – patients entering prior to first dose of pip/taz

Patient to commence Piperacillin/Tazobactam and meets enrolment and does not fulfil any exclusion criteria?

Yes → Trial discussed and consent obtained from patient

Patient unable to give consent → No → Patients not meeting enrolment or exclusion criteria will be treated in the usual way

Yes → Trial discussed and consent obtained from Personal legal representative (PerLR) or Professional legal representative (ProfLR) [only use ProfLR if PerLR not available]

No → PerLR or ProfLR not available → No → Patient, PerLR or ProfLR refuses consent – patient will be treated in the usual way

Yes → Patient enrolled into trial
Start collecting additional demographic and underlying diagnosis data

No → Patient has “episode 1” samples collected without consent [see notes p.24]
DO NOT collect additional demographic and underlying diagnosis data
Samples not to be analysed until informed consent is obtained

Patients whose initial samples are collected without consent must have consent obtained with 48 hours of entering the study.

Patient enrolled in trial (episode 2) only when consent is obtained
Start collecting additional demographic and underlying diagnosis data
Samples may be analysed
Consent Flowchart – patients already commence on pip/taz

Patient already commenced Piperacillin/Tazobactam and meets enrolment and does not fulfil any exclusion criteria?

Yes

Trial discussed and consent obtained from patient

No

Patients not meeting enrolment or exclusion criteria will be treated in the usual way

Patient unable to give

Yes

Trial discussed and consent obtained from Personal legal representative (PerLR) or Professional legal representative (ProfLR) [only use ProfLR if PerLR not available]

No

Patient, PerLR or ProfLR refuses consent – patient will be treated in the usual way

Yes

Patient enrolled into trial for “episode 2” sampling only

Start collecting additional demographic and underlying diagnosis data related to admission

No

Patient enrolled into trial only when consent from PerLR or ProfLR is obtained

Patients whose initial samples are collected without consent must have consent obtained within 48 hours of entering the study

Trial discussed and consent

Patient unable to give consent

No

Trial discussed and consent obtained from Personal legal representative (PerLR) or

Yes

Patient enrolled into trial for “episode 2” sampling only

Start collecting additional demographic and underlying diagnosis data related to admission

PerLR or ProfLR not available

Patient has “episode 2” samples collected without consent [see notes p.24]

DO NOT collect additional demographic and underlying diagnosis data

Samples not to be analysed until informed consent is obtained
Please complete the CRF as thoroughly as possible and/or attach any anonymised reports (e.g. drug kardex, heamofiltration records etc)

The structure of the CRF is shown in the following diagram;

<table>
<thead>
<tr>
<th>Even Pages</th>
<th>Odd Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contain notes on</td>
<td>To be completed</td>
</tr>
</tbody>
</table>

All forms should be completed in black ink in a clear manner. Any changes or corrections should be made by drawing a line through the data, entering the corrected information and initialling and dating the change.

Following standard notation should be used in the event that values or answers cannot be provided:

- NA: Not applicable
- NK: Not known
- ND: Not done
- NR: Not retrievable/Not available
[1] All women of childbearing age must have a negative pregnancy test to enter the study
### Inclusion/Exclusion Criteria

Please tick ‘yes’ or ‘no’ to all questions

<table>
<thead>
<tr>
<th>A</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Is the patient aged 18 to 85?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Subject requires or has been commenced on piperacillin/tazobactam as directed by clinical Intensive Care Unit medical staff</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Exclusion Criteria

Please tick ‘yes’ or ‘no’ to all questions

<table>
<thead>
<tr>
<th>B</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>4. Patient is known to be intolerant of β-lactams antibiotics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Patient has an infection with a piperacillin/tazobactam resistant organism (i.e. P. aeruginosa with MIC ≥16 mg/L, other organism with MIC ≥ 8mg/L) or organisms with inherent resistance (e.g. Stenotrophomonas)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Patient is immunocompromised (neutropenia (&lt;500 neutrophils/µL, HIV/AIDS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Patient is unlikely to survive longer the 48 hours</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Patient is pregnant or breast-feeding or plans to become pregnant during the course of the study [1]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Patient is enrolled in another Clinical Trial of an Investigational Medical Product.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Pregnancy Test**

- Positive
- Negative
- Not Applicable

**Patient Eligible for study**

- Yes
  - Patient included in the study
  - Please complete CRF
- No
  - Patient not included in the study
  - Please complete screening log
Recruitment Information – Notes

[1] **DO NOT COLLECT DEMOGRAPHIC DATA UNTIL A CONSENT FORM HAS BEEN SIGNED**

[2] Please enter both the patients date of birth and age at the time of recruitment.

[3] Ethnic origin as self reported, by the patient or documented in casenotes.

**Please use codes as listed:**
1. White
2. White Irish
3. Other White
4. Mixed: White and Black Caribbean
5. Mixed: White and Black African
6. Mixed: White and Asian
7. Other mixed background
8. Indian
9. Pakistani
10. Bangladeshi
11. Other Asian background
12. Caribbean
13. African
14. Other Black background
15. Chinese
16. Other ethnic group (please specify)
### Recruitment Information

#### Patient Demographics [1]

<table>
<thead>
<tr>
<th>Sex</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of Birth [2]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Admission Information

Please state name of patient hospital consultant

| Date & time of hospital Admission | DD/MM/YYYY HH:MM |
| Date & time of ICU Admission     | DD/MM/YYYY HH:MM |
| Specify which ICU                | Acute | Cardiothoracic |
| Date & time of ICU Discharge     | DD/MM/YYYY HH:MM |
| Date & time of hospital Discharge| DD/MM/YYYY HH:MM |
| Date & time of Death             | DD/MM/YYYY HH:MM |
| Date & time of Intubation        | DD/MM/YYYY HH:MM |

1. Admission type

<table>
<thead>
<tr>
<th>Surgical</th>
<th>Medical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-op</td>
<td>Post-op</td>
</tr>
</tbody>
</table>
[1] Record primary ICU admission diagnosis
### Admission Clinical Information [ 1 ]

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Specify</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cardiovascular Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Neurological Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Respiratory Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Gastrointestinal Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Trauma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Sepsis</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>7.</td>
<td>Renal/Urological</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Other Disease</td>
<td></td>
<td></td>
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</tbody>
</table>
**Anti-infective Agents - Notes**

[1] List of indications for piperacillin/tazobactam
   1. Community acquired pneumonia
   2. Hospital acquired pneumonia
   3. Ventilator associated pneumonia
   4. Abdominal
   5. Bloodstream
   6. Renal/Urinary tract
   7. Skin
   8. Catheter-related
   9. Central nervous system
   10. Other (please state)

[2] Only enter medication used during or immediately prior to this hospital admission. Include concurrent treatment the patient is given during the duration of the study.

[3] Recent medication list – Please record use of any of the following medication during one month prior to hospital admission or between admission and inclusion in this study:

- Amikacin
- Amoxicillin
- Amoxicillin/clavulanate (Augmentin)
- Azithromycin
- Cefepime
- Cefotaxime
- Ceftazidime
- Ceftriaxone
- Cefuroxime
- Ciprofloxacin
- Clarithromycin
- Clindamycin
- Erythromycin
- Gentamicin
- Imipenem/cilastatin
- Levofloxacin
- Moxifloxacin
- Piperacillin/tazobactam
- Ticarcillin/clavulanic acid
- Tobramycin
- Trimethoprim/Sulfamethoxazole (septrin)
- Vancomycin
## Indication for Piperacillin/Tazobactam

1. **Indication for pip/taz [1]**  
   - If other please state below:

## Recent and Concurrent Anti-infective Therapy [2]

2. **Recent Medications [3]**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Start Date</th>
<th>End Date</th>
<th>Dose</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>
[1] COPD (airflow limitation due to chronic bronchitis or emphysema)

[2] Congestive cardiac failure - (NYA class III and IV) [NYHA class III - Marked limitation in activity due to symptoms, even during less-than-ordinary activity, e.g. walking short distances (20–100 m). NYHA class IV - Severe limitations. Experiences symptoms even while at rest. Mostly bedbound patients.]

[3] Chronic liver disease (biopsy-proven cirrhosis, documented portal hypertension, episodes of past upper-GI bleeding attributed to portal hypertension, or previous episodes of hepatic encephalopathy)

[4] Immunosuppression - (primary immunodeficiency or immunodeficiency secondary to radiation treatment, use of cytotoxic drugs or steroids (see below), or malignancy)

[5] Smoking Status
   Please categorise as:-
   Current smoker - within past 3 months
   Previous smoker - stopped smoking for more than 3 months
   Never Smoker – Never smoked
### Previous And Current Medical History

<p>| | | | | | | | | | |</p>
<table>
<thead>
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</tr>
</thead>
<tbody>
<tr>
<td>1. Diabetes</td>
<td>Yes</td>
<td>If yes, specify type below</td>
<td>No</td>
<td>Type 1</td>
<td>Type 2</td>
<td>Type 2</td>
<td>Type 2</td>
<td>Type 2</td>
<td></td>
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<tr>
<td></td>
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<td></td>
<td>(diet)</td>
<td>(tablets)</td>
<td>(Insulin)</td>
<td></td>
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</tr>
<tr>
<td>2. Respiratory Disease [1]</td>
<td>Yes</td>
<td>No</td>
<td>COPD</td>
<td>Yes</td>
<td>No</td>
<td>Other (specify)</td>
<td></td>
<td></td>
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<tr>
<td>3. Cardiac Disease [2]</td>
<td>Yes</td>
<td>No</td>
<td>Congestive cardiac failure</td>
<td>Yes</td>
<td>No</td>
<td>Other (specify)</td>
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<tr>
<td>4. Neurological Disease</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td>Specify</td>
<td></td>
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<tr>
<td>5. Hepatic Disease</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td>Specify</td>
<td></td>
<td></td>
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<tr>
<td>6. Gastrointestinal Disease</td>
<td>Yes</td>
<td>No</td>
<td></td>
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<td>Specify</td>
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<tr>
<td>9. Other Disease [5]</td>
<td>Specify</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
[1] Collected at the time of administration of the first dose of piperacillin/tazobactam

[2] Temp
Please record the patients’ temperature

A. No secretions   B. Non-purulent   C. Purulent

A. No infiltrate
B. Localised infiltrate/1 quadrant
C. Patchy, diffuse infiltrate - 2 quadrants (same side)
D. Patchy, diffuse infiltrate - 2 quadrants (opposite sides)
E. Patchy, diffuse infiltrate - 3 quadrants
F. Patchy, diffuse infiltrate - 4 quadrants

[5] To convert noradrenaline mg/hr to µg/kg/min
CTICU 0.08mg/ml; AICU 0.1mg/ml

\[(\text{Noradrenaline rate (mg/hr)})*1000/(\text{[patients weight (kg)]}* 60)= \text{[Noradrenaline rate µg/kg/min]}\]

[6] Blood leukocytes (x mm$^{-3}$)
A. > 4000 to < 11000
B. < 4000 or > 11000

[7] Urine output [last 24hours]
A. <200 mL/24h
B. 200-500 mL/24h
C. >500 mL/24h
### Episode 1 (Prior to first dose of piperacillin/tazobactam) [1]

<table>
<thead>
<tr>
<th><strong>Date/Time first dose administered</strong></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Temp (°C) [2]</strong></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Airway secretions [3]</strong></th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Chest radiograph [4]</strong></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Oxygen saturation</strong></th>
<th></th>
<th>%</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>PaO₂ [record from most recent blood gas]</strong></th>
<th></th>
<th>kPa</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>PaCO₂ [record from most recent blood gas]</strong></th>
<th></th>
<th>kPa</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>FiO₂ (%) [record from most recent blood gas]</strong></th>
<th></th>
<th>%</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>PEEP (cm H₂O) [at time of last blood gas]</strong></th>
<th>≤5</th>
<th>6-8</th>
<th>9-11</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Mean arterial pressure (mmHg)</strong></th>
<th>0-70</th>
<th>&gt;70 to &lt;90</th>
<th>≤90</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Dobutamine (any dose)</strong></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Noradrenaline or adrenaline (for at least 1 hr (doses given are in µg/kg/min)) [5]</strong></th>
<th>≤0.1</th>
<th>&gt;0.1</th>
<th>None</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Platelets (x10⁹/L )</strong></th>
<th>&gt;150</th>
<th>101-150</th>
<th>51-100</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Bilirubin (µmol/l)</strong></th>
<th>0-19</th>
<th>20-32</th>
<th>33-101</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Blood leukocytes (x mm⁻³) [6]</strong></th>
<th>A</th>
<th>B</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Serum/Plasma</strong>&lt;br&gt;Creatinine</th>
<th>µmol/l</th>
<th></th>
<th></th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Protein</strong>&lt;br&gt;µmol/l</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Urea</strong>&lt;br&gt;µmol/l</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Urine output [last 24hours] [7]</strong></th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Continuous veno-venous haemofiltration</strong></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>ECMO</strong></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>
ONLY PATIENTS HAVING FIRST DOSE PHARMACOKINETIC SAMPLES COLLECTED REQUIRE ANY SAMPLES TO BE COLLECTED DURING THIS EPISODE.

[1] The Glasgow Coma Score is preferably calculated by the patients nurse, and is scored conservatively (for the patient receiving sedation or muscle relaxants, normal function is assumed unless there is evidence of intrinsically altered mentation

[2] from arterial line or by venepuncture

[3] from heamofiltration circuit, ONLY for patient undergoing renal replacement therapy

<table>
<thead>
<tr>
<th>Hours post-dose</th>
<th>Volume of tube required</th>
<th>Sample code</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEFORE DRUG ADMINISTERED</td>
<td>Blood [1]</td>
<td>6ml</td>
</tr>
<tr>
<td></td>
<td>Blood (RRT) [2]</td>
<td>1ml</td>
</tr>
<tr>
<td></td>
<td>Non-directed bronchial lavage</td>
<td></td>
</tr>
<tr>
<td>15 mins</td>
<td>Blood [1]</td>
<td>4ml</td>
</tr>
<tr>
<td></td>
<td>Blood (RRT) [2]</td>
<td>1ml</td>
</tr>
<tr>
<td>1 hour</td>
<td>Blood [1]</td>
<td>4ml</td>
</tr>
<tr>
<td></td>
<td>Blood (RRT) [2]</td>
<td>1ml</td>
</tr>
<tr>
<td>2 hours; 15 mins</td>
<td>Blood [1]</td>
<td>4ml</td>
</tr>
<tr>
<td></td>
<td>Blood (RRT) [2]</td>
<td>1ml</td>
</tr>
<tr>
<td>3 hours; 45 mins</td>
<td>Blood [1]</td>
<td>4ml</td>
</tr>
<tr>
<td></td>
<td>Blood (RRT) [2]</td>
<td>1ml</td>
</tr>
<tr>
<td>5 hours; 15 mins</td>
<td>Blood [1]</td>
<td>4ml</td>
</tr>
<tr>
<td></td>
<td>Blood (RRT) [2]</td>
<td>1ml</td>
</tr>
<tr>
<td>6 hours; 45 mins</td>
<td>Blood [1]</td>
<td>4ml</td>
</tr>
<tr>
<td></td>
<td>Blood (RRT) [2]</td>
<td>1ml</td>
</tr>
</tbody>
</table>

[4] To convert noradrenaline ml/hr to µg/kg/min

\[
\frac{(\text{Noradrenaline rate(mg/hr)}\times 1000)}{\text{([patients weight(kg)]\times 60)}} = \text{Noradrenaline rate(µg/kg/min)}
\]
### Episode 1 CONTINUED

<table>
<thead>
<tr>
<th>Glasgow Coma Score [1]</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td>13-14</td>
<td>10-12</td>
</tr>
<tr>
<td></td>
<td>6-11</td>
<td>&lt;6</td>
<td></td>
</tr>
</tbody>
</table>

Patients in steady state only arm of the study do not have samples for episode one collected

Sign and print name ______________________ Date DD/MM/YYYY

### Safety Criteria for NBL

1. Requiring FIO > 80  
   - Yes [ ]  
   - No [ ]

2. Requiring PEEP > 12 cmH20  
   - Yes [ ]  
   - No [ ]

3. Severe bronchospasm  
   - Yes [ ]  
   - No [ ]

4. Uncontrolled intracranial pressure  
   - Yes [ ]  
   - No [ ]

*Please Note: If you have answered “YES” to any of the above, the patient is not eligible for NBL sampling*

### Sampling Schedule

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-dose</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>HH:MM</td>
</tr>
<tr>
<td>15 mins</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>HH:MM</td>
</tr>
<tr>
<td>1 hour</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>HH:MM</td>
</tr>
<tr>
<td>2 hours; 15 mins</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>HH:MM</td>
</tr>
<tr>
<td>3 hours; 45 mins</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>HH:MM</td>
</tr>
<tr>
<td>5 hours; 15 mins</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>HH:MM</td>
</tr>
<tr>
<td>6 hours; 45 mins</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>HH:MM</td>
</tr>
</tbody>
</table>

### Observation following NBL

<table>
<thead>
<tr>
<th>Timing post-dose</th>
<th>Target time</th>
<th>Oxygen saturation (%)</th>
<th>FiO₂ (%)</th>
<th>PEEP (cm/h²0)</th>
<th>Norad (µg/kg/min) [4]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-NBL</td>
<td>HH:MM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 mins</td>
<td>HH:MM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 mins</td>
<td>HH:MM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 hour</td>
<td>HH:MM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 hours</td>
<td>HH:MM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
[1] Collected on day 3

[2] Temp
Please record the patients’ temperature

A. No secretions  B. Non-purulent  C. Purulent

A. No infiltrate
B. Localised infiltrate/1 quadrant
C. Patchy, diffuse infiltrate - 2 quadrants (same side)
D. Patchy, diffuse infiltrate - 2 quadrants (opposite sides)
E. Patchy, diffuse infiltrate - 3 quadrants
F. Patchy, diffuse infiltrate - 4 quadrants

[5] To convert noradrenaline mg/hr to µg/kg/min
CTICU 0.08mg/ml; AICU 0.1mg/ml

\[(\text{Noradrenaline rate(mg/hr)}*1000/([\text{patients weight(kg)}]*60)= \text{Noradrenaline rate µg/kg/min}\]\n
[6] Blood leukocytes (x mm\(^{-3}\))
A. > 4000 to < 11000
B. < 4000 or > 11000

[7] urine output [last 24hours]
A. <200 mL/24h
B. 200-500 mL/24h
C. >500 mL/24h
### Episode 2 [1]

<table>
<thead>
<tr>
<th>Date/Time first dose administered</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temp (°C) [2]</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Airway secretions [3]</strong></td>
<td>A</td>
</tr>
<tr>
<td><strong>Chest radiograph [4]</strong></td>
<td>A</td>
</tr>
<tr>
<td><strong>Oxygen saturation</strong></td>
<td></td>
</tr>
<tr>
<td><strong>PaO₂ [record from most recent blood gas]</strong></td>
<td></td>
</tr>
<tr>
<td><strong>PaCO₂ [record from most recent blood gas]</strong></td>
<td></td>
</tr>
<tr>
<td><strong>FiO₂ (%) [record from most recent blood gas]</strong></td>
<td></td>
</tr>
<tr>
<td><strong>PEEP (cm H₂O) [at time of last blood gas]</strong></td>
<td>≤5</td>
</tr>
<tr>
<td><strong>Mean arterial pressure (mmHg)</strong></td>
<td>0-70</td>
</tr>
<tr>
<td><strong>Dobutamine (any dose)</strong></td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Noradrenaline or adrenaline (for at least 1 hr (doses given are in µg/kg/min)) [5]</strong></td>
<td>≤0.1</td>
</tr>
<tr>
<td><strong>Platelets (x10⁹/L )</strong></td>
<td>&gt;150</td>
</tr>
<tr>
<td><strong>Bilirubin (µmol/l)</strong></td>
<td>0-19</td>
</tr>
<tr>
<td><strong>Blood leukocytes (x mm⁻³) [6]</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Serum/Plasma</strong></td>
<td>Creatinine</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
</tr>
<tr>
<td><strong>Urine output [last 24hours] [7]</strong></td>
<td>A</td>
</tr>
<tr>
<td><strong>Continuous veno-venous</strong></td>
<td>Yes</td>
</tr>
<tr>
<td><strong>ECMO</strong></td>
<td>Yes</td>
</tr>
</tbody>
</table>
[3] The Glasgow Coma Score is preferably calculated by the patient's nurse, and is scored conservatively (for the patient receiving sedation or muscle relaxants, normal function is assumed unless there is evidence of intrinsically altered mentation

[1] from arterial line or by venepuncture

[2] from haemofiltration circuit, ONLY for patient undergoing renal replacement therapy

<table>
<thead>
<tr>
<th>Sample timing</th>
<th>Volume of tube required</th>
<th>Sample code</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 mins PRE-DOSE</td>
<td>Blood [1] 4ml</td>
<td>Pl 8</td>
</tr>
<tr>
<td>Blood (RRT) [2] 1ml</td>
<td>Hf8</td>
<td></td>
</tr>
<tr>
<td>15 mins POST-DOSE</td>
<td>Blood [1] 6ml</td>
<td>Pl 9</td>
</tr>
<tr>
<td>Blood (RRT) [2] 1ml</td>
<td>Hf 11</td>
<td></td>
</tr>
<tr>
<td>45 mins</td>
<td>Blood [1] 6ml</td>
<td>Pl 10</td>
</tr>
<tr>
<td>Blood (RRT) [2] 1ml</td>
<td>Hf 11</td>
<td></td>
</tr>
<tr>
<td>Non-directed bronchial lavage</td>
<td>NBL 2</td>
<td></td>
</tr>
<tr>
<td>2 hours</td>
<td>Blood [1] 6ml</td>
<td>Pl 11</td>
</tr>
<tr>
<td>Blood (RRT) [2] 1ml</td>
<td>Hf 11</td>
<td></td>
</tr>
<tr>
<td>3 hours; 45 mins</td>
<td>Blood [1] 4ml</td>
<td>Pl 12</td>
</tr>
<tr>
<td>Blood (RRT) [2] 1ml</td>
<td>Hf 12</td>
<td></td>
</tr>
<tr>
<td>Non-directed bronchial lavage</td>
<td>NBL 3</td>
<td></td>
</tr>
<tr>
<td>4 hours; 30 mins</td>
<td>Blood [1] 4ml</td>
<td>Pl 13</td>
</tr>
<tr>
<td>Blood (RRT) [2] 1ml</td>
<td>Hf 13</td>
<td></td>
</tr>
</tbody>
</table>
### Episode 2 CONTINUED

| Glasgow Coma Score [1] | 15 | 13-14 | 10-12 | 6-11 | <6 |

### Safety Criteria for NBL

1. Requiring FiO > 80
   - Yes
   - No
2. Requiring PEEP > 12 cmH20
   - Yes
   - No
3. Severe bronchospasm
   - Yes
   - No
4. Uncontrolled intracranial pressure
   - Yes
   - No

*Please Note: If you have answered “YES” to any of the above, the patient is not eligible for NBL sampling*

### Sampling Schedule

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>15 mins PRE-DOSE</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>N/A</td>
</tr>
<tr>
<td>15 mins POST-DOSE</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>HH:MM</td>
</tr>
<tr>
<td>45 mins</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>HH:MM</td>
</tr>
<tr>
<td>2 hours</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>HH:MM</td>
</tr>
<tr>
<td>3 hours; 45 mins</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>HH:MM</td>
</tr>
<tr>
<td>4 hours; 30 mins</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td>HH:MM</td>
<td></td>
</tr>
</tbody>
</table>

### Observation following NBL

<table>
<thead>
<tr>
<th>Timing post-dose</th>
<th>Target time</th>
<th>Oxygen saturation (%)</th>
<th>FiO₂ (%)</th>
<th>PEEP (cm/H²0)</th>
<th>Norad (µg/kg/min) [4]</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 mins (prior to NBL)</td>
<td>HH:MM</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
</tr>
<tr>
<td>30 mins</td>
<td>HH:MM</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
</tr>
<tr>
<td>45 mins</td>
<td>HH:MM</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
</tr>
<tr>
<td>1 hours; 15 mins</td>
<td>HH:MM</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
</tr>
<tr>
<td>2 hours (prior to NBL)</td>
<td>HH:MM</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
</tr>
<tr>
<td>2 hours; 15 mins</td>
<td>HH:MM</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
</tr>
<tr>
<td>2 hours; 30 mins</td>
<td>HH:MM</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
</tr>
<tr>
<td>3 hours</td>
<td>HH:MM</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
</tr>
<tr>
<td>4 hours; 15 mins</td>
<td>HH:MM</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
</tr>
<tr>
<td>6 hours</td>
<td>HH:MM</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
</tr>
</tbody>
</table>
**Episode 3 - Notes**

**ONLY PATIENTS WHO HAD FIRST DOSE PHARMACOKINETIC SAMPLES COLLECTED REQUIRE ANY SAMPLES TO BE COLLECTED DURING THIS EPISODE.**

[1] Collected 8 hours after the final study dose piperacillin/tazobactam

[2] Temp
Please record the patients’ temperature

A. No secretions  
B. Non-purulent  
C. Purulent

A. No infiltrate
B. Localised infiltrate/1 quadrant
C. Patchy, diffuse infiltrate - 2 quadrants (same side)
D. Patchy, diffuse infiltrate - 2 quadrants (opposite sides)
E. Patchy, diffuse infiltrate - 3 quadrants
F. Patchy, diffuse infiltrate - 4 quadrants

[5] To convert noradrenaline ml/hr to µg/kg/min

\[ \frac{([\text{Noradrenaline rate(mg/hr)}] \times 1000)}{([\text{patients weight(kg)}] \times 60)} = \text{Noradrenaline rate (µg/kg/min)} \]

[6] Blood leukocytes (x mm\(^{-3}\))
A. > 4000 to < 11000
B. < 4000 or > 11000

[7] urine output [last 24hours]
A. <200 mL/24h
B. 200-500 mL/24h
C. >500 mL/24h
### Episode 3 [1]

Patients in steady state only arm of the study do not have data or samples for episode three collected

<table>
<thead>
<tr>
<th>Sign and print name</th>
<th>Date DD/MM/YYYY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Date/Time last dose administered

<table>
<thead>
<tr>
<th>DD/MM/YYYY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

#### Temp (°C) [2]

<table>
<thead>
<tr>
<th>XX.X</th>
</tr>
</thead>
</table>

#### Airway secretions [3]

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
</table>

#### Chest radiograph [4]

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>No X-ray</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>E</td>
<td>F</td>
<td></td>
</tr>
</tbody>
</table>

#### Oxygen saturation

<table>
<thead>
<tr>
<th>XX.X %</th>
</tr>
</thead>
</table>

#### PaO₂ [record from most recent blood gas]

<table>
<thead>
<tr>
<th>XX.X kPa</th>
</tr>
</thead>
</table>

#### PaCO₂ [record from most recent blood gas]

<table>
<thead>
<tr>
<th>XX.X kPa</th>
</tr>
</thead>
</table>

#### FiO₂ (%) [record from most recent blood gas]

<table>
<thead>
<tr>
<th>XX.X %</th>
</tr>
</thead>
</table>

#### PEEP (cm H₂O) [at time of last blood gas]

<table>
<thead>
<tr>
<th>≤5</th>
<th>6-8</th>
<th>9-11</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-14</td>
<td>.</td>
<td>≥15</td>
</tr>
</tbody>
</table>

#### Mean arterial pressure (mmHg)

<table>
<thead>
<tr>
<th>0-70</th>
<th>&gt;70 to &lt;90</th>
<th>≤90</th>
</tr>
</thead>
</table>

#### Dobutamine (any dose)

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

#### Noradrenaline or adrenaline (for at least 1 hr (doses given are in µg/kg/min)) [5]

<table>
<thead>
<tr>
<th>≤0.1</th>
<th>&gt;0.1</th>
<th>None</th>
</tr>
</thead>
</table>

#### Platelets (x10⁹/L)

<table>
<thead>
<tr>
<th>&gt;150</th>
<th>101-150</th>
<th>51-100</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-50</td>
<td>0-20</td>
<td></td>
</tr>
</tbody>
</table>

#### Bilirubin (µmol/l)

<table>
<thead>
<tr>
<th>0-19</th>
<th>20-32</th>
<th>33-101</th>
</tr>
</thead>
<tbody>
<tr>
<td>102-204</td>
<td>&gt;204</td>
<td></td>
</tr>
</tbody>
</table>

#### Blood leukocytes (x mm⁻³) [6]

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
</tr>
</thead>
</table>

#### Serum/Plasma

<table>
<thead>
<tr>
<th>Creatinine</th>
<th>µmol/l</th>
<th>Albumin</th>
<th>µmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>µmol/l</td>
<td>Urea</td>
<td>µmol/l</td>
</tr>
</tbody>
</table>

#### Urine output [last 24hours] [7]

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
</table>
ONLY PATIENTS WHO HAD FIRST DOSE PHARMACOKINETIC SAMPLES COLLECTED REQUIRE ANY SAMPLES TO BE COLLECTED DURING THIS EPISODE.

[1] from arterial line or by venepuncture

<table>
<thead>
<tr>
<th>Hours post-dose</th>
<th>Volume of tube required</th>
<th>Sample code</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Blood [1]</td>
<td>Pl 14</td>
</tr>
<tr>
<td></td>
<td>Non-directed bronchial lavage</td>
<td>NBL 4</td>
</tr>
</tbody>
</table>

Check microbiology results for any blood cultures taken during or 1 week before 5 days of study drug. Options are none/negative/positive. If blood culture collected record date/time. If positive culture need free text for species

[3] The Glasgow Coma Score is preferably calculated by the patients nurse, and is scored conservatively (for the patient receiving sedation or muscle relaxants, normal function is assumed unless there is evidence of intrinsically altered mentation

[4] To convert noradrenaline ml/hr to µg/kg/min

$$\frac{([\text{Noradrenaline rate(mg/hr)}] \times 1000)}{([\text{patients weight(kg)}] \times 60)} = \text{Noradrenaline rate (µg/kg/min)}$$
### Episode 3 CONTINUED

<table>
<thead>
<tr>
<th>Glasgow Coma Score [3]</th>
<th>15</th>
<th>13-14</th>
<th>10-12</th>
<th>6-11</th>
<th>&lt;6</th>
</tr>
</thead>
</table>

### Safety Criteria for NBL

1. Requiring FiO > 80  
   - Yes | No
2. Requiring PEEP > 12 cmH20  
   - Yes | No
3. Severe bronchospasm  
   - Yes | No
4. Uncontrolled intracranial pressure  
   - Yes | No

*Please Note: if you have answered “YES” to any of the above, the patient is not eligible for NBL sampling*

### Sampling Schedule

<table>
<thead>
<tr>
<th>Hours post-dose</th>
<th>Blood [1]</th>
<th>Non-directed bronchial lavage</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 hours</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Observation following NBL

<table>
<thead>
<tr>
<th>Hours post-dose</th>
<th>Oxygen saturation (%)</th>
<th>FiO₂ (%)</th>
<th>PEEP (cm/H₂O)</th>
<th>Norad (µg/kg/min) [4]</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 hours (prior to NBL)</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
</tr>
<tr>
<td>8 hours; 15 mins</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
</tr>
<tr>
<td>8 hours; 30 mins</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
</tr>
<tr>
<td>9 hours</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
</tr>
<tr>
<td>12 hours</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
</tr>
</tbody>
</table>
Episode 4 - notes

ONLY PATIENTS WHO HAD FIRST DOSE PHARMACOKINETIC SAMPLES COLLECTED REQUIRE ANY SAMPLES TO BE COLLECTED DURING THIS EPISODE.

[1] At extubation

<table>
<thead>
<tr>
<th>Sample code</th>
</tr>
</thead>
<tbody>
<tr>
<td>endotracheal tube ETT1</td>
</tr>
</tbody>
</table>

[2] If the patient already has a tracheostomy please tick; otherwise please state reason tracheostomy not collected

Episode 5 - notes

ONLY PATIENTS WHO HAD FIRST DOSE PHARMACOKINETIC SAMPLES COLLECTED REQUIRE ANY SAMPLES TO BE COLLECTED DURING THIS EPISODE.

[3] Days 14 to 17 after first dose administration

<table>
<thead>
<tr>
<th>Volume of tube required</th>
<th>Sample code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood 2ml</td>
<td>PI 15</td>
</tr>
</tbody>
</table>

[4] Record time and date of blood sample
### Episode 4 [1]

Patients in steady state only arm of the study do not have data or samples for episode four collected

<table>
<thead>
<tr>
<th>Sign and print name</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DD/MM/YYYY</td>
</tr>
</tbody>
</table>

#### Sampling Schedule

<table>
<thead>
<tr>
<th>Endotracheal tube</th>
<th>Tube not collected? [2]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Already has tracheostomy</td>
</tr>
<tr>
<td></td>
<td>Other:</td>
</tr>
</tbody>
</table>

### Episode 5 [3]

Patients in steady state only arm of the study do not have data or samples for episode five collected

<table>
<thead>
<tr>
<th>Sign and print name</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DD/MM/YYYY</td>
</tr>
</tbody>
</table>

#### Sampling Schedule

<table>
<thead>
<tr>
<th>Blood</th>
<th>Serum/Plasma results [4]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DD/MM/YYYY HH:MM</td>
</tr>
<tr>
<td>Creatinine</td>
<td>XXX.X µmol/l</td>
</tr>
<tr>
<td>Albumin</td>
<td>XXX.X µmol/l</td>
</tr>
<tr>
<td>Protein</td>
<td>XXX.X µmol/l</td>
</tr>
<tr>
<td>Urea</td>
<td>XXX.X µmol/l</td>
</tr>
</tbody>
</table>
Additional Cardiovascular and Renal Support - Notes

[1] Patients not on ECMO should have a tick entered in the N/A box.

[2] Patients not on renal replacement therapy at the time of the sample being taken should have a tick entered in the N/A.
## Additional Cardiovascular and Renal Support

### Extra Corporeal Membrane Oxygenation [1]

<table>
<thead>
<tr>
<th>Type</th>
<th>Veno-venous</th>
<th>Veno-arterial</th>
<th>Start date and time</th>
<th>Stop date and time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>DD/MM/YYYY HH:MM</td>
<td>DD/MM/YYYY HH:MM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

### Renal replacement therapy [2]

<table>
<thead>
<tr>
<th>Type</th>
<th>Prisma</th>
<th>Prisma Flex 4</th>
<th>Episode 1</th>
<th>Episode 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample No.</td>
<td>Episode</td>
<td></td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td></td>
<td>Sample time</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td>Fluid removed</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1</td>
<td>7</td>
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</tr>
<tr>
<td>1</td>
<td>8</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
[1] Record any other NBLs taken on the patient between 3 days prior to starting pip/taz and episode 2.
### Microbiology

<table>
<thead>
<tr>
<th></th>
<th>None</th>
<th>Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-directed lavage from episode 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>If positive – which organism?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIC to pip/tax</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date and time of sample</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>If positive – which organism?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIC to pip/tax</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-directed lavage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date and time of sample</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>If positive – which organism?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIC to pip/tax</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Cultures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date and time of sample</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>If positive – which organism?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIC to pip/tax</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
[1] The APACHE II score is recorded in the Critical Care Minimum Data Set.
### Sign Off

<table>
<thead>
<tr>
<th>Item</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photocopy of RRT records attached</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug Card photocopied and attached</td>
<td></td>
<td></td>
</tr>
<tr>
<td>APACHE II score [1]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient withdrawn early for study?</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>If yes:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Reason?</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### CRF completed

<table>
<thead>
<tr>
<th>Sign and print name</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix 3: PROPEL Pat Info Sheet (first dose and steady state) v2.1
Patient information sheet – Key facts

PROPEL

A study to determine antibiotic levels in the blood and lungs of patients in intensive care

Please read this carefully and refer to the full information sheet

- Piperacillin/tazobactam is an antibiotic that is to be given to treat a possible chest infection as part of your standard care – it is not a study drug and has been licensed for 15 years.
- You are being invited to take part in a research study which will look at the amount of piperacillin/tazobactam in the blood and lungs.
- The study is being carried out by Dr Tim Felton (Clinical Research Fellow), Dr William Hope (Consultant in Infectious Diseases) and Dr Andrew Bentley (Intensive Care Consultant) and members of the research staff.
- This research study forms part of a PhD thesis for a research student (Dr Tim Felton).
- If you agree to take part in the study you will have:
  - Assessments of your health at pre-determined time points during the study.
  - A number of blood samples taken at pre-determined time points over the next three weeks. In total we will collect approximately 6 tablespoons of blood. Most blood samples will be taken through a drip needle that is already in place.
  - 4 samples of fluid collected from the lungs while you are asleep.
- Members of the research team will have access to your medical records during and after study participation.
- You will not benefit directly from taking part in the study and potential risks are explained.
- You do not have to agree to being included in the study if you do not want to. You are free to withdraw from the study at any time and this will not affect the care you receive.
- We ask permission that you give your specimens as a gift.

Please refer to the Information Sheet - Thank you for your time
PATIENT INFORMATION SHEET
(first dose and steady state)

PROPEL

Plasma and intrapulmonary population pharmacokinetics of piperacillin/tazobactam in critically ill patients

A study to determine antibiotic levels in the blood and lungs of patients in intensive care

We would like to invite you to take part in our research study. Before you decide on whether to participate it is important for you to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully and discuss it with others if you wish. One of our team will go through the information sheet with you. Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part in the study.

If you have further questions either now or in the future, please feel free to contact Dr Tim Felton (Principal Investigator) (details at the end of the Information Sheet) or visit the study website (sites.mhs.manchester.ac.uk/aip)

Part 1 tells you the purpose of this study and what will happen to you if you take part. Part 2 gives you more detailed information about the conduct of the study.
Part 1.

1. What is the purpose of the study?
Piperacillin/tazobactam (Pip/taz) is an antibiotic that is very commonly used for treating infection in patients in intensive care. Pip/taz has been used for over 15 years and is licensed to treat seriously unwell patients. Previous studies of patients in intensive care show that although patients received the same amount of an antibiotic this led to very different amounts of the antibiotic in their blood and lungs. By comparison when given to patients who are not seriously unwell the same antibiotic will produce very similar amounts of antibiotics in the blood. This research study will look at the amount of pip/taz in the blood and lungs of patients in intensive care with the aim of allowing us to use pip/taz more effectively in the future.

2. Why have I been chosen?
The intensive care doctors believe that you require treatment with pip/taz. The research team is looking for 40 patients to participate in this study.

3. Do I have to take part?
No. It is up to you to decide whether or not to take part. If you do take part you will be given this information sheet to keep and be asked to sign a consent form. You are free to withdraw at any time and without giving a reason. If you decide not to take part or withdraw from the study at any time this will not affect the standard of care you receive or your relationship with your doctor.

4. What will happen to me if I take part?
This study involves 5 separate episodes. There will be no further involvement in the study after you have been discharged home.

First episode: Up to 7 blood samples will be collected (via a drip needle) starting just before the first dose of pip/taz to just before the next dose (8 hours later). Additionally just before the antibiotic is given a sample of fluid will be taken from the lung by putting injecting approximately 1½ tablespoons of water through the tube into your lungs, connected to the ventilator, and then immediately sucking the water back out again.

Second episode: Up to 7 further blood samples will be taken (via a drip needle) between 2 doses of pip/taz, on day 3 or 4 of treatment. Additionally 2 further samples of fluid from the lungs will be collected during this episode.

Third episode: After the final dose of study antibiotic, a single blood sample will be taken.
Fourth episode: As your breathing improves the tube into your windpipe may no longer be needed. When the tube is removed it will be collected for analysis.

Fifth episode: Between 2 and 4 weeks after starting the course of pip/taz a final blood sample will be collected.

The level of pip/taz, your kidney function and markers of inflammation will be measured in each of the blood samples. The fluid samples from the lungs will be used to measure either the level of pip/taz or to measure the amount of “bug” in the lung causing infection. From the tube we will look for evidence of the bugs that cause infection.

**a) What does taking the blood tests involve?**

The blood samples will be taken through drip lines that are used as part of routine care of intensive care patients. Therefore we will not need to use a needle to take each blood sample. The final blood test (episode 5) may be taken while you are no longer in intensive care and may require use of a small needle to take the sample. Occasionally patients notice a small bruise at
the point where the needle has pierced the skin. In total we will take approximately 6 tablespoons of your blood.

b) **What is involved in taking the fluid samples from the lung?**
The fluid sample from the lung is taken by putting a fine tube through the tube in your windpipe that is connected to the ventilator. We flush 20mls (approximately 1 ½ tablespoons) of water through the tube, into your lung and then suck it straight out again. Studies have shown that this test is safe. This test is performed 3 times a week on every patient in intensive care as part of routine care. These fluid samples will be collected in addition to those collected as part of routine care. If the tube in your windpipe has been removed before the final sample of fluid has been collected (i.e. before episode 3) we will collect a sputum sample instead.

5. **Will taking part affect my current treatment?**
If you agree to take part in this study you will not need to change any other aspect of your treatment. We ask you not to be involved in any other drug studies while you are involved in this study.

6. **What is the drug that is being tested?**
This research project is studying the combination of antibiotic drugs piperacillin and tazobactam. Pip/taz has been used for over 15 years and is licensed to treat seriously unwell patients. **Piperacillin is a penicillin so it is important you tell us if you are allergic to penicillin antibiotics.** This study will use the standard licensed dose which is 4.5g either twice or three times a day.

7. **What are the alternatives for diagnosis or treatment?**
You have been asked to be involved in this study because you are due to start pip/taz as prescribed by the clinical team. During this study the clinical team is allowed to prescribe any other drugs that you may require. If the clinical team think that pip/taz needs to be changed to another antibiotic they are free to do so, however we may stop collecting any further blood samples.

8. **What are the side effects of any treatment received when taking part?**
Serious side effects of pip/taz include:

- Severe allergic reaction (anaphylaxis)

Common side effects include:

- Rash
Nausea and vomiting
Diarrhoea
Thrush (Candida infection)

Less common side effects include:
- Abnormal blood count
- Abnormal liver tests
- Abnormal kidney test
- Headache
- Fits

If a serious side effect occurs, pip/taz will be stopped immediately and emergency treatment given if needed. If you suffer from or are concerned about any of these symptoms please contact either the clinical ICU team or research team on the numbers given in section 16.

9. What are the other possible disadvantages and risks of taking part?

During the study a number of blood samples will be taken. The majority of these samples will be taken via a plastic tube into the vein that is used routinely in patients in intensive care. If you do not have a drip needle then blood samples will be collected using a needle. Blood tests are very safe. You might feel a slight discomfort and occasionally a little bruising occurs afterwards at the needle site.

A maximum of 4 fluid samples will be collected from your lungs. This test is performed regularly (3 times a week) for patients in intensive care. Problems arise approximately every 1 or 2 times every 100 times this test is performed. These problems are usually minor with the most common being a drop in the oxygen level in the blood. Extra oxygen is given until the oxygen levels return to level it was before the test. In most people this is temporary, lasting less that 1 hour. To minimise the risk of this occurring extra oxygen is given to all patients for 5 minutes before the procedure.
10. **What are the possible benefits of taking part?**

Taking part in this study will not directly benefit you as an individual but the information we get from this study may help improve the treatment of future intensive care patients in the future who need antibiotics.

11. **What happens when the research study stops?**

During the study the antibiotic will be given for 5 days. At the end of the fifth day the medical team on the intensive care unit may chose to continue, change or stop the antibiotic treatment.

12. **Contact Details**

If you have any questions or concerns about this research study or you develop any medical problems that may be related to pip/taz you may contact:

Dr Tim Felton  
Education and Research Centre  
University Hospital of South Manchester (Wythenshawe Hospital)  
Southmoor Road  
Manchester  
M23 9LT UK  
Telephone: (0161) 2915811

If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.
Part 2

13. **What if relevant new information becomes available?**
If new information regarding pip/taz becomes available during the course of the study a member of the research team will discuss this with you. If you decide not to continue the study your clinical care will continue on the intensive care unit. If you decide to continue in the study we may ask you to sign an agreement outlining the discussion.

14. **What will happen if I don’t want to carry on with the study?**
You are free to withdraw from the study at any time. If you do chose to withdraw from the study we will process all samples and data collected up until your withdrawal.

15. **What if there is a problem?**
   a) **Complaints:**
   If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions (0161 291 5811). If you remain unhappy and wish to complain formally, you can do this through the Patient Advice and Liaison Service (P.A.L.S) [0161 291 5600].
   b) **Harm:**
   In the event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone’s negligence then you may have grounds for a legal action for compensation against University Hospital of South Manchester NHS trust but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate).

16. **Will my taking part in this study be kept confidential?**
All data relating to this study will be kept strictly confidential and handled, processed, stored and destroyed in compliance with the Data Protection Act 1998. Your name will be coded and separated from all your research records. All research data will be kept for fifteen years following completion of the study.

17. **What will happen to any samples I give?**
During the study samples of blood and fluid from the lungs will be collected. These samples are in addition to the samples required for your clinical care. Samples used will be labeled with coded identifying numbers and not with information that directly identifies you. Many of the samples will be stored in secure freezers at either the Clinical Research Facility at University Hospital of South Manchester (Wythenshawe Hospital) or at the Stopford Building at The
University of Manchester. Samples will be tested in laboratories within the University Hospital of South Manchester, The University of Manchester and The University of Liverpool.

We will ask that you donate all samples as a gift so that the samples can be stored for future analyses relating to this research programme and for possible use in future studies. All future studies will require approval from a Research Ethics Committee before we can use your samples. This will enable us to make the most of any new developments in knowledge and techniques.

18. Will any genetic tests be done?
No.

19. What will happen to the results of the research study?
It is intended that the results of this research project will be presented at medical conferences and published in a medical journal. Your data will not be identifiable in the manuscript. A copy of the research findings will be available on the study website (sites.mhs.manchester.ac.uk/aip) and as a newsletter which will be available on request. This research study also forms part of a PhD thesis for a research student. You will not be identified in the written thesis that is submitted.

20. Who is organising and funding the research?
This research project is jointly being funded by the Medical Research Council and the University Hospital of South Manchester. The research team will receive no payments or incentives to include you in this study.

21. Who has reviewed the study?
This research study has been reviewed and given approval by the NRES Committee North West - Greater Manchester South Research Ethics Committee.

You will be given a copy of the information sheet and a signed consent form to keep.

Thank you for considering to take part in this research study and for taking the time to read this information sheet.
Appendix 4: PROPEL consent (first dose and steady state) v2.0
PATIENT CONSENT FORM
(first dose and steady state)

PROPEL

Plasma and intrapulmonary population pharmacokinetics of piperacillin/tazobactam in critically ill patients

A study to determine antibiotic levels in the blood and lungs of patients in intensive care

Please initial in each box to confirm that you have read and understood the study information document.

1. I confirm that I have read and understood the Patient Information Sheet version…., dated……………. and I have had time to consider it and a chance to ask questions.

2. I understand that my taking part is voluntary and that I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected.

3. I understand that my medical notes might be inspected for regulatory reasons by the Local Health Authority, Hospital Trust, Research Ethics Committee or other regulatory bodies and by the study team (including a PhD student). This will be done only where it is relevant to my taking part in the research. I give permission for these individuals to have access to my records.

4. I consent to the collection of personal and health related data about me, including medical information for the purpose of the study.

5. I consent to the collection of blood samples and collection of samples from the lung.

6. I agree to gift my samples for future research which has received a favorable opinion from a Research Ethics Committee.

7. I agree to take part in the above study.

8. I would like to have a copy of the research findings newsletter at the end of the study. Yes/No

Name of Patient __________________________ Signature __________________________ Date (dd/mm/yy) ____________

Name of Person Taking Consent __________________________ Signature __________________________ Date (dd/mm/yy) ____________