PharmacoKinetic modelling for Antimicrobials in Paediatric Patients on Extracorporeal Therapies (APET)

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iii. SIGNATURE PAGE

iv. LIST OF ABBREVIATIONS

PICU	Paediatric Intensive Care Unit
QCH	Queensland Children's Hospital
PK	Pharmacokinetic
PD	Pharmacodynamic
ECMO	Extracorporeal membrane oxygenation
CRRT	Continuous renal replacement therapy
Cmax	Maximum concentration (mg/L)
Cmin	Minimum concentration (mg/L)
MIC	Minimum Inhibitory Concentration
CL	Clearance
Vd	Volume of distribution
$AUC_{(0-t)}$	Area under the curve for duration of 0-t, where t is time (h)
fT	Fraction of Time that concentrations of antimicrobials remains above the minimum inhibitory concentration
PT	Probability of Target attainment

1. ABSTRACT

Burden of disease: Sepsis is a dysregulated host response to an infection resulting in life threatening organ dysfunction. Annually, 1.2 million paediatric patient deaths are attributed to sepsis (1). Early, accurate identification of sepsis in paediatric patients is critical. Mortality associated with sepsis in Paediatric Intensive Care Units (PICUs) in Australia and New Zealand is approximately 6%(2). The healthcare burden in managing paediatric sepsis in Australian and New Zealand PICUs is estimated to cost over $30 million a year(2). Improvement strategies are required to decrease the burden on patients and the healthcare system. A key strategy is timely and effective antimicrobial therapy. Patients with refractory organ failure often require management with extracorporeal therapies. Extracorporeal therapies are extracorporeal membrane oxygenation (ECMO, heart/lung support) and continuous renal replacement therapy (CRRT, artificial kidney support) which provide support for reversible organ dysfunction for heart, lung and kidneys. Studies in adults have shown that extracorporeal therapies substantially alter pharmacokinetic parameters of antimicrobials, often resulting in suboptimal antimicrobial therapy. Little is known about the impacts of extracorporeal therapies on antimicrobials in paediatric patients.

The **aim** is to characterise the population pharmacokinetics of key antimicrobials (ampicillin, cefotaxime, ceftazidime, flucloxacillin, gentamicin, meropenem, piperacillin-tazobactam teicoplanin, vancomycin, anidulafungin, fluconazole, micafungin, voriconazole) in critically ill paediatric patients on extracorporeal therapies. A simulated antimicrobial model will be developed and assessed with the aim of optimising antimicrobial concentrations in paediatric patients on extracorporeal therapies.

Method: A prospective observational pharmacokinetic study for antimicrobials in critically ill paediatric patients on extracorporeal therapies (extracorporeal membrane oxygenation, and/or continuous renal replacement therapies) will be undertaken at two sites; the Queensland Children's Hospital in Brisbane and the University Children's Hospital in Zurich. The antimicrobial plasma samples will be assessed at various time points, for the antimicrobial pharmacokinetic analysis. The analysis will utilise a population pharmacokinetic modelling approach of P-Metrics 3.5.1 with gfortran complier (3). In building the antimicrobial pharmacokinetic models, the assessment will include correlations between the extracorporeal therapy settings, developmental factors and/or clinical factors for each paediatric patient.

SIGNIFICANCE STATEMENT

Sepsis is dysregulated host response to infection resulting in life threatening organ dysfunction. Extracorporeal therapies provide support for kidneys, heart and lungs when life threatening organ dysfunction presents. Antimicrobials are administered to critically ill patients for treatment of sepsis. Currently every year 1.2 million paediatric deaths are attributed to sepsis. Little is known about the effects of extracorporeal therapies on antimicrobials distribution or clearance in paediatric patients. However, in adult studies antimicrobial clearance and distribution is affected by extracorporeal therapies. This study aims to determine how extracorporeal therapies alter antimicrobials in paediatric patients. Assessment of current antimicrobial dosing regimens in the presence of extracorporeal therapies may lead to inadequate antimicrobial plasma concentrations in paediatric patients. Antimicrobial optimisation is essential for improved outcomes in paediatric patients. Development of pharmacokinetic model for antimicrobials for paediatric patients on extracorporeal therapies will assist with antimicrobial optimisation.

3. BACKGROUND INFORMATION

Infection is defined as the invasion and multiplication of microorganisms such as bacteria, virus, or fungus. Infection can lead to sepsis. Sepsis is a dysregulated host response to infection resulting in life threatening organ dysfunction (4, 5). Despite advances in prevention, diagnosis and treatments of invasive infections sepsis remains a leading cause of paediatric deaths (5-7). A paediatric patient is defined as a child from birth to 18 years of age. Mortality associated with paediatric sepsis is as high as 20% in the developing world (6, 7). The incidence of paediatric mortality in Australia and New Zealand is relatively low (8), however sepsis mortality in paediatric intensive care unit remains around 6% (2). Management of paediatric septic patients in Australia and New Zealand results in a direct healthcare cost of around $30 million a year (2). Improvement strategies for paediatric sepsis and decreasing the healthcare burden is required. A key strategy is appropriate, timely and optimal dose regimen for antimicrobials in paediatric sepsis.

Many children with sepsis develop multiple organ dysfunction (1, 9). When refractory multi-organ dysfunction presents paediatric extracorporeal therapies can be used to support the heart, lung and/or kidney function. Extracorporeal therapies are defined as extracorporeal membrane oxygenation (ECMO, heart-lung bypass) and continuous renal replacement therapies (CRRT, kidney bypass). ECMO provides a gas exchange, by pumping blood from the patient's body to an artificial lung (oxygenator) where oxygen is added, and carbon dioxide removed. This blood is sent back to the patient via a pump replacing or supporting the heart function (10). CRRT provides blood purification for the kidneys, regulating water, electrolytes, drugs, and toxins. Improving knowledge of the impact of paediatric extracorporeal therapies on antimicrobials, will assist with optimal dose regimens.

Optimal dosing of antimicrobials targets the concentration at which bactericidal or bacteriostatic effects are achieved – this is known as the minimum inhibitory concentration (MIC) and this target is specific for each antimicrobial class. Dosing studies, or 'pharmacokinetic studies', are performed to gain an understanding of the dose-concentration relationship in patient groups and how this relates to the targeted antimicrobial effect (11). Understanding the primary pharmacokinetic parameters (volume of distribution and clearance), of paediatric patients on extracorporeal therapies will provide data to better target effective antimicrobial dosing regimens for patients on extracorporeal therapies.

Studies in critically ill neonates and children receiving extracorporeal therapies are limited and current dosing is often extrapolated from adult critically ill patients. Critically ill paediatric

patients may have altered pharmacokinetics due to their disease state, alterations in renal and liver function, protein levels and tissue oedema, necessary medical interventions (extracorporeal therapies), as well as developmental changes and organ maturation. This means dosing regimens extrapolated from studies performed in adults may not be appropriate for paediatric patients and may lead to sub-therapeutic dosing and treatment failure, or super-therapeutic dosing and toxicity (12). The following parameters may impact on the suitability of antimicrobial dosing on critically ill paediatric patients:

Critical illness is associated with changes to protein binding and fluid shifts, leading to variability in tissue distribution, and organ dysfunction, leading to altered elimination of drugs (13). Additionally, pharmacokinetic differences exist between adults and paediatric patients, impacting on medication clearance and distribution. These differences are discussed below:

- Paediatric patients have immature kidney and liver function affecting medication elimination pharmacokinetics.
- Paediatric age dependent factors affect medication distribution through involvement of bilirubin and fatty acids.
- Infants albumin and α1-acid glycoprotein stores are lower affecting the binding affinity for medications. Therefore, infants may have more circulating medication due to a lower affinity to bind, impacting highly protein bound medications (14, 15).
- Infants have a higher water ratio compared to adults and this has implications for hydrophilic medications such as aminoglycosides and beta-lactams (15).
- Kidney development does not meet maturity with filtration, secretion and reabsorption until puberty.
- Infants require medications less frequently; this is especially important for the antimicrobial fluconazole, where neonates have an increased plasma half-life due to a greater volume distribution and decreased elimination renal clearance .

Studies in critically ill adults receiving extracorporeal therapies have shown that the pharmacokinetics of antimicrobials are often substantially altered (16, 17). In 2012, a study by Earnest et al. on the impact of CRRT on antimicrobial dosing found that hydrophilic antimicrobials, such as beta-lactams (e.g. cefotaxime, piperacillin/tazobactam, ampicillin), may concentrate intravascularly and are therefore more likely to be removed during CRRT (18). A study by Shekar et al. observed that critically ill adult patients receiving ECMO display altered pharmacokinetics consisting of an increased volume of distribution and decreased clearance for commonly used antimicrobials (19). A review by Cheng et al highlighted that ECMO alters pharmacokinetics of medications, through changes in volume distribution and clearance

especially in antimicrobials (20). The PHARMECMO study by Bougle et al. used therapeutic drug monitoring of antimicrobials in adult ECMO patients and found higher variability in the volume of distribution for beta-lactam antimicrobials, compared with non ECMO studies (21). A study by Abdul-Aziz et al concluded that due to high inter-patient variability therapeutic drug monitoring for medications may be the best approach when treating critically ill patients on extracorporeal therapies (22). Table 1 describes the ECMO factors that have altered antimicrobial distribution in adults, and propose how ECMO factors may affect paediatric patients (16, 23).

Table 1: Factors impacting on the distribution of medication for patients receiving ECMO and the possible impacts on paediatric patients

Factors affecting the distribution of antimicrobials while patients are receiving ECMO	**Proposed impact on paediatric patients**
Medication sequestration	*This will be same in all age groups however doses may vary in age groups. An example. gentamicin under 1-month dose 5mg/kg daily is used whilst greater than 1month 7.5mg/kg daily and >12 years 7mg/kg.*
Haemodilution from priming solution associated with frequent blood products transfusions and administration of crystalloid to maintain the circuit flows	*Priming volume will vary with age, it is higher percentage in infants, (volume per total body weight)so it is likely to alter the volume of distribution in this age group*.
Physiological changes associated with critically illness, altered blood pH affect medication ionisation and distribution into the tissues	*This should be similar in all age groups for paediatrics.*
Up-regulation of renin-angiotensin system alters handling of fluids and can change ratio of fluids in the body fluid compartment	*This is likely to be more significant in infants where the body fluid compartment is higher percentage*

Renal dysfunction is common in patients on ECMO with incidences reported to be as high as 60%, with greater than 30% of patients receiving both ECMO and CRRT (24). The cause of renal dysfunction is multifactorial, with hypoxia , poor organ perfusion prior to receiving ECMO, non-pulsatile flow and acute kidney injury as likely contributing to renal impairment. Renal dysfunction can increase the concentrations in the body of renally cleared medications and

therefore increase the risk of toxicity. The critically ill paediatric can also experience acute kidney injury, resulting in fluid overload, electrolyte imbalance and the accumulation of toxins.

Table 2: Factors impacting on the distribution of antimicrobials for patients receiving CRRT and the possible impacts on paediatric patients

Factors affecting the distribution of antimicrobials while patients are receiving CRRT	**Proposed impact on paediatric patients**
High protein binding antimicrobials	*No change in clearance (18)*
Antimicrobials with smaller volume distribution concentrate in plasma	*Rapid clearance expected with beta-lactams*
Physiological changes associated with critically illness, e.g. hypoproteinemia affect medication distribution into the tissues	*Increased clearance, especially with low protein binding antimicrobials*
Changes in blood or dialysate flow rate affects transmembrane pressure	*Altered antimicrobial clearance, need further assessment, at various flow rates.*
Modality type	*Increased clearance from haemodialysis mode compared to haemodiafiltration*
Dialysis membranes adsorption	*Some paediatric membranes shown adsorption for Meropenem – alters clearance(13)*
Filter changes saturation of filter	*Filter saturation expect change in clearance*

This study aims to develop optimised antimicrobial dosing regimens for common antimicrobials used to treat paediatric patients receiving extracorporeal therapies. This will be achieved by performing pharmacokinetic studies in this patient population for key antimicrobials. The results of this study will provide clinicians with optimal dosing regimens for antimicrobials in paediatric patients on extracorporeal therapy support.

4. STUDY AIM AND HYPOTHESIS

Our hypothesis is that current antimicrobial dosing in critically ill paediatrics on extracorporeal therapies produce sub-therapeutic antimicrobial concentrations.

Aim 1: Prospectively characterise the population pharmacokinetics of key antimicrobials; antibiotics (ampicillin, cefotaxime, ceftazidime, flucloxacillin, gentamicin,, meropenem, piperacillin-tazobactam, teicoplanin, and vancomycin) and key antifungals (anidulafungin, fluconazole, micafungin and voriconazole,) in critically ill paediatric patients on extracorporeal therapies.

Aim 2: Simulate novel antimicrobial dosing regimens that are optimised to achieve maximally effective antimicrobial exposure in paediatric patients on extracorporeal therapies.

Aim 3: Prospectively evaluate therapeutic concentrations for antimicrobials, using the antimicrobial dosing regimens (designed in Aim 2) in critically ill paediatric patients on extracorporeal therapies, to assess if target attainment achieved.

5. STUDY DESIGN

This is a prospective pharmacokinetic study of critically ill paediatric patients on extracorporeal therapies (extracorporeal membrane oxygenation, and/or continuous renal replacement therapies). This prospective study will be conducted in the two sites: the Queensland Children's Hospital, Brisbane and University Children's Hospital, Zurich. Patients are eligible to participate in the study if they are receiving either one or more of the antimicrobials listed in the below table.

Type of antimicrobial	Medication name ranked in frequency of use
Antibiotic	Ampicillin Cefotaxime Ceftazidime Gentamicin Flucloxacillin Meropenem Piperacillin/tazobactam Teicoplanin Vancomycin
Antifungals	Anidulafungin Fluconazole Voriconazole Micafungin

The antimicrobial medication and dose being administered is independent of the study and will be decided upon at the discretion of the paediatric intensivist senior medical officer (SMO).

As a default, where possible prospective consent will be sought. Due to the emergency nature of sepsis and extracorporeal therapies, it is anticipated that in certain situations timely informed consent may not be feasible in which case consent to continue will be used. A consent to continue process will be applied for the following reasons: Firstly, prospective recruitment with obtaining parental consent is likely to lead to delays in treatment which could expose paediatrics to risks associated with delayed treatment. Secondly the Australian National Statement Section 4.4.6 recognises that in emergency care research recruitment into a research study often has to be achieved quickly, and that a waiver of consent may be granted provided the conditions of National statement paragraph 2.3.6 are satisfied. The paediatric patient included in this study are acutely ill at risk of death and hence parental stress during extracorporeal therapy cannulation may interfere with their ability to consider and comprehend study information and therefore provide

consent. Acute care studies have demonstrated the difficulties for parents/guardian to make decisions at time of high stress, supporting deferred consent, opt out and consent to continue approaches (25). In cases where prospective consent cannot be obtained in a timely manner, we will utilise a consent to continue process for the study. Informed consent will be sought from the parent/guardian by the Study Co-ordinator, Registrar, Consultant or local nurse champion as soon as possible once the paediatric patient is stable and parent/guardian has time to adjust to the PICU environment. The parent/guardian will receive an information sheet about the study.

The default approach will be a face to face consent obtained within less than 24 hours after enrolment. As a default the team will seek consent whilst the patient is still in hospital allowing face to face consent.

It is unlikely, a child receiving extracorporeal therapies will be discharged from hospital within 24 hours. For most cases it is feasible to obtain a face to face consent whilst the patient is in PICU. There may be exceptional situations where paediatric patient is discharged early (over weekend), before the study team can get in touch with the family. On these rare occasions, consent from parents would be sought using phone consent, only after face to face contact has been attempted. Parents or guardians will be provided with the opportunity to revoke their consent and have the research blood samples securely disposed.

Sampling will be undertaken on any dose of the antimicrobial and this may before steady state has been achieved; however, patients may be enrolled up to 96 hours post administration of the first dose of the study antimicrobial.

i. INCLUSION CRITERIA

Paediatric patients from birth up to 18 years of age admitted to the Paediatric Intensive Care Unit at the Queensland Children's Hospital or University Children's Hospital Zurich are eligible if **ALL** of the following criteria are met.

- Consent to continue (Deferred consent will be followed up with written or phone consent obtained from the parent or carer).
- Admitted to PICU
- Age birth to <18 years
- Paediatric patients requiring extracorporeal therapies either extracorporeal membrane oxygenation and/or continuous renal replacement therapy.
- Arterial or venous access for sampling is present.

- Prescribed one or more of the following antimicrobials: ampicillin, cefotaxime, ceftazidime, flucloxacillin, gentamicin, meropenem, piperacillin/tazobactam, teicoplanin vancomycin, anidulafungin, fluconazole, micafungin and voriconazole. Note: Patients may receive multiple antimicrobials concurrently.

ii. EXCLUSION CRITERIA

Patients are excluded from the study if ONE OR MORE of the following criteria are met:

- No consent to continue
- Known allergy to study antimicrobial
- Pregnancy
- Ongoing massive blood transfusion requirements (>50% blood volume transfused in the previous 8 hours)
- Haemoglobin is less than 70 g/L
- Therapeutic plasma exchange in the preceding 24 hours
- No arterial or venous access for sampling
- Inability to get consent, due to the child's guardian is the department of child safety (DOCS).

iii. DURATION OF STUDY

It is expected that 24 months will be required to enrol paediatric extracorporeal membrane oxygenation and/or continuous renal replacement therapies patients and to analyse the data. This time period is based on the feasibility data from 2018/2019 (24-month period) for study antimicrobials: piperacillin/tazobactam, meropenem, vancomycin, gentamicin, ampicillin, flucloxacillin, cefotaxime, ceftazidime, tecioplain, anidulafungin, fluconazole, voriconazole and micafungin. There are approximately 2000 annual admissions to the PICU at Queensland Children's Hospital. In combined years 2018/19 there were 60 patents that received ECMO/CRRT and approximately 50 who received ECMO only support whilst CRRT numbers were lower with 30 patients received CRRT. Overall approximately 2000 or 50% of the paediatric patients admitted to the PICU in 2018/19 received an antimicrobial medication.

Table: Treatment incidence for QCH 2018/19 (24months) expressed as actual patient numbers over 24 months

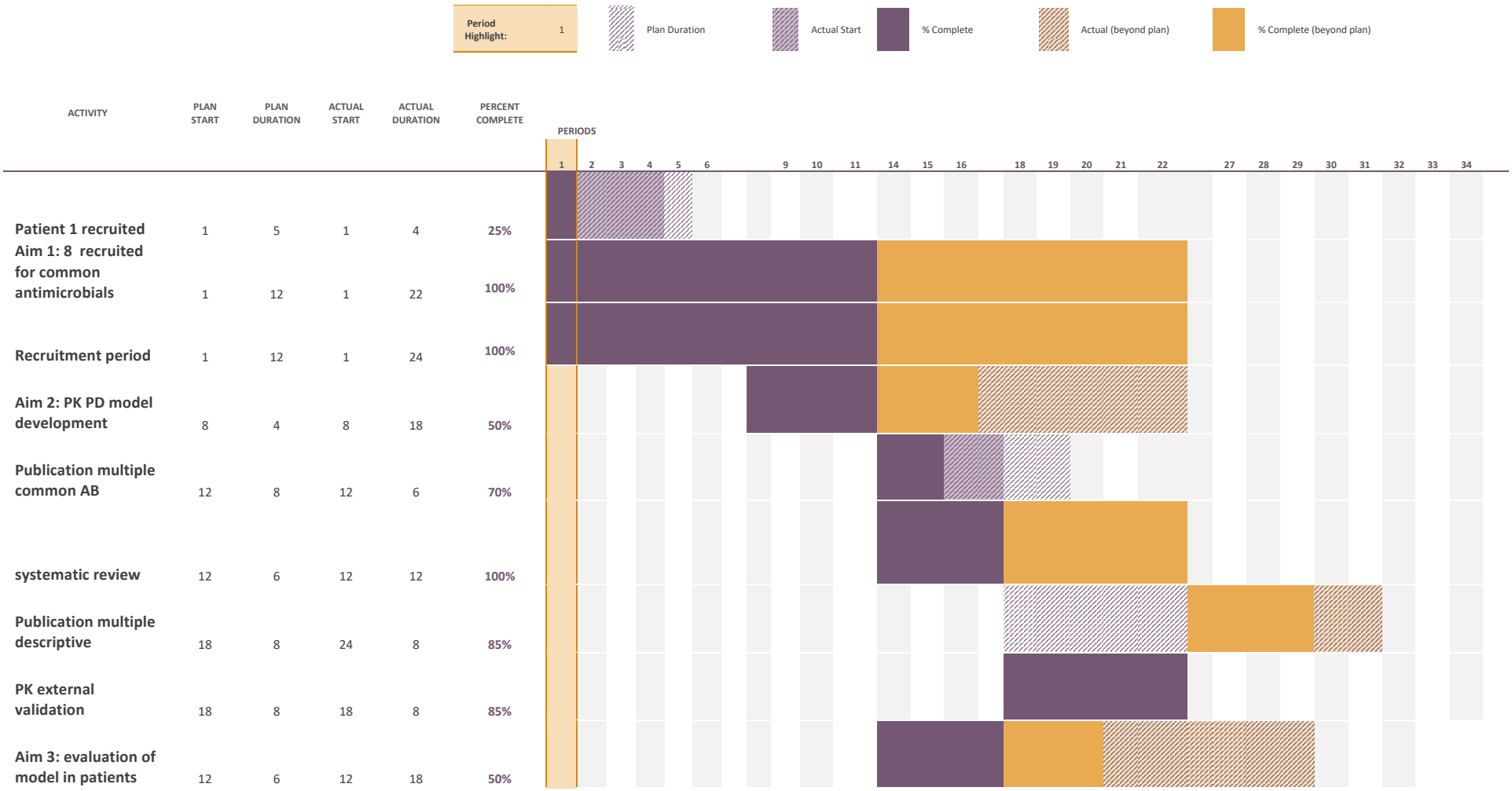
Study	Piperacillin-tazobactam	Meropenem	Ampicillin	Cefotaxime	Vancomycin	Gentamicin	Flucloxacillin	Fluconazole	Voriconazole	micafungin
ECMO&RRT	20	15	7	14	24	15	2	8	3	4
ECMO only	13	7	6	14	14	7	2	6	1	1
RRT only	13	7	6	13	13	7	2	6	1	1
ICU only	290	75	212	905	225	162	86	33	7	6

*It is expected due to the low numbers associated with ampicillin, flucloxacillin and the antifungals; fluconazole, voriconazole and micafungin, that the study will provide descriptive information for these antimicrobials. Teicoplanin, ceftazadime and anidulafungin have been included into the protocol, we do not have treatment incidence for these antimicrobials.

iv. WITHDRAWAL FROM THE STUDY

A parent or carer may withdraw their child from the study if they decide to do so at any time, irrespective of the reason. Patients may also be withdrawn from further sampling at the discretion of the investigator. The reason for withdrawal of a patient from further sampling will be recorded in the case report form.

v. APET Project Plan



6. METHODOLOGY

i. SAMPLE SIZE AND SELECTION

Recruitment in critically ill paediatric patients for antimicrobials studies have recruited less than ten patients (26, 27). For this study the sample size selected is similar to previous studies performed in paediatric patients in paediatric intensive care for example prospective studies Watt et al and Autmizguine et al of micafungin and fluconazole in critically ill paediatric patients on ECMO, consisted of 7 to 12 patients in their study cohort (26, 28). Whilst Rapp et al and Nehus et al prospective studies in CRRT in meropenem had 7 to 11 patients in their study (27, 29). The aim for this study is to have at least **eight** patients in each antimicrobial group, with each group stratified into the following:

	Cefotaxime	Meropenem	Flucloxacillin	Fluconazole	Voriconazole	Micafungin
ECMO/ CRRT	8	8	8*	8*	8*	8*
ECMO	8	8	8*	8*	8*	8*
CRRT	8	8	8*	8*	8*	8*

	Ceftazidime	Teicoplanin	Anidulafungin	Gentamicin	Vancomycin	Ampicillin	Piperacillin tazobactam
ECMO/ CRRT	8	8	8	8	8	8*	8
ECMO	8	8	8	8	8	8*	8
CRRT	8	8	8	8	8	8*	8

In the event a patient has multiple antimicrobial investigated medications, they may be assigned to multiple groups.

* It is anticipated from feasibility data that it is unlikely that eight patients will be recruited to these groups, however of the patients assigned to these groups, the information will be captured for descriptive analysis.

ii. SAMPLE COLLECTION

Patients receiving a study antimicrobial (piperacillin/tazobactam, cefotaxime, ceftazidime, teicoplanin, meropenem, ampicillin, vancomycin, gentamicin, flucloxacillin, anidulafungin, fluconazole, voriconazole and micafungin) intravenously through either central or peripheral access and extracorporeal therapy (extracorporeal membrane oxygenation and/or continuous renal replacement therapies) will have blood samples collected at different time points, depending on the frequency of the antimicrobial. Sampling will be allocated pre and post administration of one of the study antimicrobials. Sampling will use standard technique of whole

blood from the arterial sampling line that these paediatric intensive care patients already have present and no invasive procedure will be required for sampling. Collection times will be dependent on the antimicrobial frequency regimen and known pharmacokinetic parameters (see Table below). The collection times will be the same for all patients receiving the antimicrobial studied.

Detailed sample collection

1. Collect whole blood (0.1 to 0.5 mL) into a 0.6 mL lithium heparin paediatric collecting tube, it can be venous or arterial blood.
2. Immediately place collected blood sample into an ice bath and maintain the sample at a cooled temperature until centrifugation (within 6 h).
3. Centrifuge blood collection tube at 3000 rpm for 10 minutes at 4°C conducted in pathology
4. Manually aspirate plasma and transfer to polypropylene tubes, being careful to not transfer any red cells.
5. Store samples at -80°C until analysis
6. Samples will be protected from light when stored in the freezer

Each patient will have no more than 5 samples in 6 hours, or 8 in 12 hours The sample amount required is 0.5 mL, where several antimicrobials concentrations can be assessed. These paediatric patients require regular blood samples, and utilisation of existing samples may be assessed. Standard care for paediatric patients on extracorporeal therapies requires blood samples to be collected every four to six hours. World Health Organisation (WHO) guidelines for blood sample volume limits range from 1 to 5% of total blood volume within 24 hours and up to 10% of total blood volume over 8 weeks, or 3.8% of total blood volume for paediatric critically ill patient (30). In a 3 kg infant the estimated total blood volume ranges from 85 to 105 mL/kg which equates to a total blood volume of 255 mL to 315 mL. For the study if we took 8 samples in 12 hours this would equate to a total of 4.8m or 1.5 to 1.8 % of the total blood volume for a 3 kg infant, which is within the WHO recommendations for blood sampling.

Antimicrobial concentrations from the plasma samples will be at the Centre for Clinical Research, The University of Queensland (UQCCR). All samples will be assayed using validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) methodology already available at UQCCR. Samples will be batched for transport and subsequent analysis, with each batch conforming to the criteria of qualitative bioanalysis as prescribed by the Food and Drug Administration, Bioanalytical method validation, Guidance for industry.

Sample time points for each antimicrobial based on dosing frequency

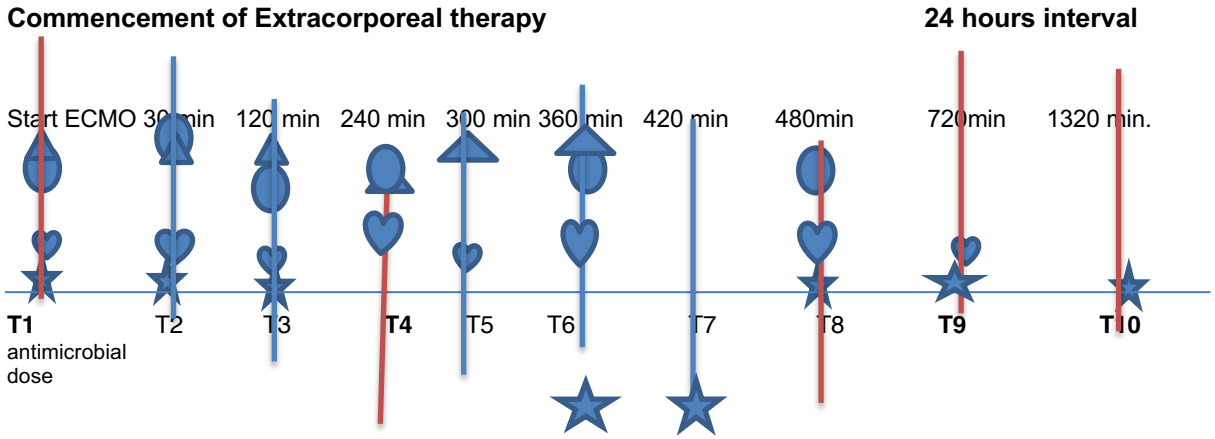
Dosing Interval	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
24 hourly	Pre-infusion	30 min	120 min	360 min	420 min	480	720 min	1320min
12 hourly				240 min	300 min	360	480min	720 min
8 hourly				240 min	360 min	480	NIL	NIL
6 hourly				240 min	300 min	360	NIL	NIL

Representation of the collection of samples over 24-hour interval

represents 6-hour intervals, represents 8-hour interval,

represents 12-hour interval and represents 24-hour interval

represents standard blood sampling for patient care



PATIENTS REQUIRING ANTIMICROBIAL ON EXTRACORPOREAL THERAPY

Point in time 1: pre infusion of initial antimicrobial dose, start of extracorporeal therapy, and ***standard blood sampling*** for patient care for extracorporeal therapies

Point in time 2: 30 minutes post antimicrobial dose (all intervals)

Point in time 3: 120 minutes post antimicrobial dose (all intervals)

Point in time 4: 240 minutes if 6, 8- or 12-hour antimicrobial interval dose, and ***standard blood sampling*** for patient care for extracorporeal therapies

Point in time 5: 300 minutes if 6 and 12 hourly antimicrobial interval

Point in time 6: 360 minutes post antimicrobial dose (all intervals)

Point in time 7: 420 minutes if 24 hourly antimicrobial interval

Point in time 8: 480 minutes if 8, 12 hourly and 24-hour antimicrobial interval, and ***standard blood sampling*** for patient care for extracorporeal therapies

Point in time 9: 720 minutes 12- and 24-hourly interval and ***standard blood sampling*** for patient care for extracorporeal therapies

Point in time 10: 1320 minutes if 24 hourly interval and ***standard blood sampling*** for patient care for extracorporeal therapies

Sample times are guides only. It is imperative that the time of administration, completion of administration of antimicrobial and the time of blood sample are accurately recorded on the patient data sheet (Appendix 10.1 and 10.2). Antimicrobial administration may include extended infusions.

iii. DATA COLLECTION:

Data collection will use paper-based case report form (CRF) including the following parameters:

- **Patient Demographics**: patient age (years), sex, gestational age (weeks), estimated height (cm), weight (kg).
- **Clinical information**: presenting admission, diagnostic related group (DRG), PIM-3 score (utilising Australian and New Zealand Paediatric Intensive Care registry), serum creatinine (mg/dL), blood urea nitrogen (mg/dL), albumin (g/dL), haematocrit (%), Liver function tests (aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT)), if urinary catheter is present urine output over the prior 12 h (mL).
- **Medication data**: concurrent vasoactive or diuretic medications, antimicrobial commencement date, dose and frequency, administration time (when commenced infusion and when stopped e.g. extended infusion versus bolus).
- **Blood culture** sensitivities if available will be assessed to determine if antimicrobial treatment is empiric, prophylaxis or treatment.
- **Antimicrobial sample**: time sample, after the antimicrobial where taken and storage of sample if appropriate.
- **Extracorporeal Membrane Oxygenation (ECMO**): when commenced, type of ECMO, ECMO flow rate, ECMO blood flow, shunt flow rate, type of pump, oxygenator, serum bilirubin, serum creatinine, blood urea, free plasma haemoglobulin, serum albumin, see attached appendix 10.1 for detailed data collection form
- **Continuous Renal Replacement (CRRT**): when commenced, type of CRRT, flow rate, serum creatinine, blood urea, serum bilirubin, serum albumin ultrafiltration rate (mL/h), post-filter replacement fluid (mL/h), pre-filter dilution flow rate (mL/ h),), fluid removal rate (mL/h), filtration fraction (%), blood flow rate (mL/min), dialysate flow rate (mL/h), and citrate flow rate (mL/h). see attached appendix 10.2 for detailed data collection form

The parameters will be later entered into a REDCap (Research Electronic Data Capture) database that will be held in a secure computer and routinely "backed up" in accordance with University of Queensland, and Centre for Children's Health Research procedures to ensure data preservation and safety.

7. ANALYSIS

i. PHARMACOKINETIC ANALYSIS

The pharmacokinetics analysis of piperacillin/tazobactam, vancomycin, cefotaxime, ceftazidime gentamicin, ampicillin, meropenem, flucloxacillin, teicoplanin, anidulafungin, fluconazole, voriconazole or micafungin will be performed using a population pharmacokinetic modelling approach using concentrations from the patient plasma samples. The pharmacokinetic model approach will use P-Metrics1.5.2 (Laboratory of Applied Pharmacokinetics and Bioinformatics, Los Angeles Ca, USA)44 in R studio (version 1.1.456) as a wrapper for R (version 3.5.1) and intel visula fortran complier XE 14.0. Antimicrobial concentrations from Aim 1 will be used to develop robust population pharmacokinetic models. These models will be used in simulations to develop novel dosing regimens that achieve therapeutic concentrations based on the relevant pharmacokinetic/pharmacodynamic target for each antimicrobial. Parameters targets of concentration maximum (C_{max}), area under the curve (AUC), fraction of time(fT) that concentrations of the antimicrobial remains above the minimum inhibitory concentration(MIC), clearance elimination and terminal volume of distribution will be quantified. The inclusion of clinical covariates (e.g. markers for renal function or albumin levels) in the model will characterise developmental and /or acute pathophysiological alterations of critically ill paediatric patients on extracorporeal therapies.

Building of the pharmacokinetic model will require selection and inclusion of clinical covariates based on the likelihood ratio test. Covariate inclusion will be based on biological plausibility and performed in a stepwise manner. Additionally, model diagnostics including an assessment of the 'goodness-of-fit' plots, precision of the parameter estimates, and a visual predictive check will be used for model evaluation. Probability of target attainment (PTA): Monte Carlo dosing simulations will be performed using the final model to determine the PTA using the appropriate pharmacokinetic/pharmacodynamic target for clinically relevant MICs for a variety of dose regimens and infusion durations (intermittent and extended). We will use the EUCAST database to guide the MIC for all relevant pathogens for the antimicrobials, rather than prospectively determining these values. The final model will derive novel dosing regimens targeted at

optimal bacterial killing. The antimicrobial plasma concentrations by these dose regimens will be assessed for attainment of pharmacodynamic targets.

ii. SAFETY CONSIDERATIONS

Adverse events are unlikely from this study. It is possible that adverse events may arise from blood collections and these will be reported to the principal investigator. The Human Research Ethics Committee will be notified of all serious adverse events.

8. QUALITY ASSURANCE

The chief investigator is responsible for the clinical trial to be performed in accordance with the protocol and the applicable regulatory requirements. The investigator is also responsible for ensuring compliance with all procedures required by the clinical trial protocol. The investigator is also required to provide reliable data in an accurate and legible manner.

The Queensland Children's Hospital Paediatric Intensive Care Unit is responsible for taking all reasonable steps to ensure the proper conduct of the clinical trial protocol.

Data management will be provided by the coordinating centre. The principal means of data collection and data processing will be via electronic paper CRFs. All forms will be signed and dated by the authorised study staff and all changes made following data submission will be recorded.

The coordinating centre will take all appropriate measures to safeguard and prevent access to this data by any unauthorised third party. The investigator(s) will also maintain confidentiality to all study documentation and ensure that accidental or premature destruction of these documents is prevented. The study documents will be stored at a secure facility for at least 15 years after the completion or discontinuation of the study.

9. ETHICS

The trial will be conducted in accordance with the principles laid down by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines for Good Clinical Practice and the applicable local regulatory requirement. Ethics Approval will be sought from Children's Health Queensland and University of Queensland.

10. FUNDING

Centre for Clinical research, University of Queensland will provide support if necessary, throughout the study

External funding has been sought for:

- A clinical nurse to collect blood samples for determining antimicrobial concentrations
- University of Queensland Centre for Clinical Research laboratory where laboratory staff will analyse the antimicrobial concentration data

11. APPENDIX: DETAILED COLLECTION FORM FOR EXTRACORPOREAL THERAPIES METHODS

i. Data collection form for Extracorporeal Membrane Oxygenation

Date			Time		
Age (Gestational)	Sex M/F		Weight(kg)		Height (cm)
ICU admission diagnosis					
Days/hours on ECMO					
ECMO flow rate (pump flow)					
ECMO Blood Flow (mL/min) (patient flow)					
Shunt flow rate					
Type of ECMO			VA	VV	Other
Pump			Rotaflow		
Oxygenator			Medtronic: Quadrox ID		
Serum bilirubin (micromol/L)			RRT: Yes/No If yes specify mode		
serum creatinine (micromol/L)			SCUF Rate	SCUF+ Counter-current (CC)	
Serum albumin (g/L)				SCUF rate	CC rate
Blood urea(mmol/L)			Total globulin (total protein)		
Free plasma Hb					
24-hour fluid balance *at 6am*					
Blood product transfusion details:					
Medications - co medications especially vasoactive medications					
Temperature	HR		Lactate(ABG)	CRP	Procalcitonin
Medication Name	Start time of infusion	End of infusion	Previous doses times and doses given if any		
		Level	Date and time started		
Time sample taken	sample source? e.g. Arterial line?		Dose and frequency		
Medication	Day started	Level	Dose		Frequency
Start time	End time				
Medication	Day started	Level	Dose		Frequency
Start time	End time				
Medication	Day started	Level	Dose		Frequency
Start time	End time				
Medication	Day started	Level	Dose		Frequency
Start time	End time				
Date	Time	Organism identified	Sensitive		Culture positive (hrs)
			Yes	No	

ii. Data collection form for Continuous Renal Replacement therapy

Date			Time		
Age (gestational)	Sex M/F		Weight(kg)	Height (cm)	
ICU admission diagnosis					
Days/hours on CRRT					
CRRT flow rate (pump flow)					
CRRT Blood Flow (mL/min) (patient flow)					
Shunt flow rate					
Type of CRRT			Citrate	Heparin	Other
serum creatinine (micromol/L)			FO%	Urine Output (mL/kg/h)	
Serum albumin (g/L)			Fluid removal		
Blood urea(mmol/L)			Total globulin (total protein)		
Free plasma Hb					
24-hour fluid balance *at 6am*					
Blood product transfusion details:					
Medications					
Temperature	HR		Lactate(ABG)	CRP	Procalcitonin
Medication	Day started	Level	Dose	Frequency	
Start time	End time				
Medication	Day started	Level	Dose	Frequency	
Start time	End time				
Medication	Day started	Level	Dose	Frequency	
Start time	End time				
Medication	Day started	Level	Dose	Frequency	
Start time	End time				
Medication	Day started	Level	Dose	Frequency	
Start time	End time				
Date	Time	Organism identified	Sensitive		Culture positive (hrs)
			Yes	No	

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