Sub Study:

Optimizing antibiotic dosing in the Staphylococcus aureus Network Adaptive Platform trial (O-SNAP)

Summary

Target antimicrobial concentrations associated with efficacy (pharmacodynamic targets) of first-line antibiotics for *Staphylococcus aureus* bacteraemia are informed only by *in vitro* or animal studies. To date, there have been no studies that have identified a pharmacodynamic (PD) target for anti-staphylococcal β-lactam antibiotics in humans and it is unclear whether achievement of the commonly cited PD targets correlates with improved clinical outcomes in children and adults with *S. aureus* bacteraemia. In addition, data on how frequently these targets can be achieved with oral antibiotic treatment are limited.

This multicentre, prospective pharmacokinetic-pharmacodynamic (PKPD) study of anti-staphylococcal β-lactam antibiotics will be the first to define the therapeutic target for serum antibiotic concentrations to achieve treatment success in children and adults with SAB (i.e. PD target). This study is embedded within the SNAP trial, and aims to recruit 550 participants comprising 400 adults, 150 children from paediatric and adult hospitals involved in the SNAP trial in Australia and New Zealand over a 3-year study duration. The primary objective of the study is to determine in patients receiving treatment with a β-lactam for methicillin-sensitive *S. aureus* bacteraemia (MSSA), if there is a difference in treatment success at day 90 between those in whom the published PD target (Ft>MIC >50%) are achieved and those in whom they are not achieved.

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1. ABBREVIATIONS

cfDNA	Cell free DNA
CFU	colony-forming unit
CTCAE	Common Terminology Criteria for Adverse Events
DSA	Domain-Specific Appendix
ft	Free Drug Concentration
ICU	Intensive Care Unit
KDIGO	Kidney Disease Improving Global Outcomes
LFT	Liver Function Test
MSSA	Methicillin-susceptible *Staphylococcus aureus*
MIC	Minimum Inhibitory Concentration
NZ	New Zealand
O-SNAP	Optimizing antibiotic dosing in the *Staphylococcus aureus* Network Adaptive Platform trial
PCR	Polymerase Chain Reaction
PD	Pharmacodynamic
PK	Pharmacokinetics
PSSA	Penicillin-susceptible *Staphylococcus aureus*
SAB	*Staphylococcus aureus* bloodstream infections
SNAP	*Staphylococcus aureus* Network Adaptive Platform trial
TDM	therapeutic drug monitoring
qPCR	Quantitative Polymerase Chain Reaction
UEC	Urea, Electrolytes & Creatinine
VPC	Visual predictive checks.

2. SUB STUDY GOVERNANCE

2.1. Sub Study Investigators

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Prof Jason Roberts

Investigators:

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3. BACKGROUND AND RATIONALE

3.1. Sub study definition

The primary aims of this sub study is to determine in patients receiving treatment with either intravenous flucloxacillin, cloxacillin or cefazolin for methicillin-sensitive *S. aureus* bacteraemia, if there is a difference in the proportion of patients with treatment success at day 90 between those in whom the conventional PKPD target are achieved and those in whom they are not achieved.

Null hypothesis: There is no difference in the proportion of patients with treatment success between those who achieve the conventional PKPD target and those who do not.

Definitions:

• *conventional PKPD target for β-lactam antibiotics: Fraction of time that the free drug concentration exceeds the bacterial MIC (fT>MIC) for at least 50% of the dosing interval.*

• *treatment success:*

- *patients being alive without any of the following occurring between 14-90 days after **platform or registry entry**: (i) new metastatic focus of infection and (ii) isolation of S. aureus from a new sterile site.*

Secondary aims:

1. To define the population PKPD targets of antimicrobials in *S. aureus* bacteraemia (including PSSA and MSSA) focusing on the first line intravenous β-lactam antibiotics (benzylpenicillin/penicillin G, cefazolin, flucloxacillin, cloxacillin) and oral antibiotics (cefalexin, amoxycillin, flucloxacillin).
2. Use pharmacometrics to develop a novel PKPD model to determine the relationship between drug exposure and:
    a. treatment success (as defined above)
    b. *in vivo* bactericidal activity defined by a reduction in bacterial load by 3 $\log_{10}$ CFU/ml (subset of 50 children and 50 adults)
    c. drug-related adverse effects
3. To describe the PK of the other study medications in SNAP including:
    a. Intravenous antibiotics directed against all silos (PSSA, MSSA and MRSA) - (cloxacillin, vancomycin, clindamycin, lincomycin and daptomycin)
    b. Oral antibiotics (cefadroxil, ciprofloxacin, rifampicin, cloxacillin, dicloxacillin, doxycycline, clindamycin, fusidic acid, levofloxacin, linezolid, moxifloxacin, tedizolid, trimethoprim-

sulfamethoxazole)

4. To determine the relationship between drug exposure and immunological response to antibiotic treatment in those who achieve treatment success versus failure (subset of 50 adults)
5. To collect PK data for other antimicrobials and non-steroidal anti-inflammatories to contribute to future PK models.

3.2. Sub study background

Staphylococcus aureus bloodstream infections (SAB) are the most common, serious bacterial infections in both children and adults. The first-line treatment for a β-lactam-susceptible *S. aureus* bacteraemia is anti-staphylococcal penicillin or cephalosporins, however there is an alarming paucity of data to inform antibiotic dosing for SAB, with no published studies in children or adults identifying the target serum drug concentrations required to achieve treatment success (the PD target). The conventional PKPD target for β-lactam antibiotics is the time that the free drug concentration (fT) exceeds the bacterial minimum inhibitory concentration (MIC) of more than 50% of the dosing interval (i.e. fT>MIC>50%) and is informed only by *in vitro* and animal studies [1-6,10]. It is unknown whether achievement of this widely cited PKPD target on which most dosing guidelines are based, truly correlates with improved clinical outcomes in SAB. This multicentre, prospective study will address a major research gap in children and adults by determining whether achievement of this conventional PKPD target translates to improved SAB outcomes. The clinical benefit of appropriate antibiotic dosing for bacterial sepsis is well described - mortality is more than threefold higher when high-risk patients do not receive prompt antibiotic treatment at the right dose.

4. SUB STUDY DESIGN

This sub study will be conducted as part of the SNAP trial. The sub study design will be:

- We will recruit 400 adults and 150 children from major paediatric and adult hospitals with biobanking facilities in Australia and New Zealand that are already involved in the SNAP trial.
- Patients who are enrolled in the platform or the registry of the SNAP trial that are currently receiving either IV or oral antibiotic treatment AND consent to O-SNAP over the 3-year study period will be included in the study.
- A desired minimum of 30 patients receiving each studied antibiotic alone; will be enrolled.
- Antibiotics will be administered as per the SNAP trial protocol or according to standard hospital guidelines as determined by the treating clinicians.
- To collect study blood samples, convenience sampling method will be used where study blood will be taken with each clinically indicated blood test to determine antibiotic concentrations. Cytokines will be measured in a subset of 50 adults, while bacterial load will be quantified in a subset of 50 adults and 50 children. Renal and liver function will be assessed as part of routine clinical care.
- Blood samples will be collected from a vascular access device or peripheral cannula, or venipuncture or finger prick. . Refer to section 4.4 for sample collection.

4.1. Population

Adults and children aged ≥1 year with SAB who are enrolled in the platform or registry of the SNAP trial and are currently receiving either IV or oral antibiotic treatment, will be approached for consent for O-SNAP sub-study if at a participating site, at the time of consent for the SNAP platform or registry.

4.2. Eligibility criteria

Patients are eligible for this sub study if they meet core inclusion criteria set out in section 6.5.1 of the Core Protocol (i.e., *Staphylococcus aureus* complex grown from ≥1 blood culture, and admitted to a participating hospital at the time of eligibility assessment) and meet all the sub study inclusion and none of the sub study exclusion criteria outlined below.

4.2.1. Sub study inclusion criteria

Adults and children (aged ≥1 year) enrolled in the platform or the registry of the SNAP trial at a site participating in the O-SNAP sub-study.

4.2.2 Sub study exclusion criteria

Nil.

4.3. Interventions

4.3.1. Sub Study Interventions

There are no randomised interventions in this sub study. Participants will receive either randomised SNAP trial allocated domain interventions (if eligible for domains), or treatment as per standard care (if ineligible for domains or enrolled in registry only).

4.4. Sample Collection

4.4.1. Blood samples:

i) <u>For all O-SNAP participants (aged ≥1 year who are enrolled in either SNAP Platform or Registry) :</u>

Blood samples will be collected from patients during their acute care hospital admission using a 'convenience method' i.e. where possible, study samples will be taken at the same time as other clinically indicated blood tests to prevent the need for additional invasive procedures. Blood samples will be taken either via venipuncture, finger prick, or central venous line (after an appropriate blood discard).

The maximum number of blood draw for antibiotic concentration for each participant is 10 across the study period. From our previous PK studies that have used similar methodology, the median number of study blood samples taken was 5 in those patients aged >3 months. Refer to Table 1 for more information on the blood sample volume and collection tubes.

These following types of blood samples will be collected:

A. Antibiotic concentrations:

- *<u>Children (1 to <18 years):</u>* A convenience sampling method will be used where an additional 0.5ml of blood will be taken with each clinically indicated blood test within 21 days from the commencement of IV or oral antibiotic treatment. The maximum number of samples/patient/day is 5 in children >2 years, 3 in children 1-2 years old.

- *<u>Adults (≥18 years):</u>* A convenience sampling method will be used where an additional 3-6ml of blood (volume dependent on size of serum gel tubes available at each study site) will be taken with each clinically indicated blood test between day 2 and 21 from the commencement of IV antibiotic

treatment (Samples to be taken after 24 hours of IV antibiotic treatment, and after 48 hours of oral antibiotic treatment). The maximum number of samples /patient/day is 5.

B. Renal and liver function:

Children and Adults (>1 year): Data will be collected from standard clinical testing of blood for renal function and albumin from day 1+/-1 day and day 5+/- 1 day from the commencement of antibiotic treatment. Acute renal injury will be defined as per Backbone Domain Specific Protocol section 8.5.2. [7]. Grades of LFT abnormalities will be classified according to definitions from the Common Terminology Criteria for Adverse Events (CTCAE).

ii) For O-SNAP participants who have a confirmed MSSA or PSSA and receiving one of the following IV antibiotics (benzylpenicillin/penicillin G, cefazolin, flucloxacillin, cloxacillin) OR oral antibiotics (cefalexin, amoxycillin, flucloxacillin):

Refer to Table 1 for more information on the blood sample volume and collection tubes. In addition to the *4.4.1 i) A. Antibiotic Concentrations* (convenience samples) and *4.4.1 i) B. the renal and liver function* blood samples. These following types of blood samples will be collected:

A. Antibiotic concentrations:

- *Adults (≥18 years):* For intravenous (IV) antibiotic administration, a maximum of **three additional sub study-specific (3-6ml) blood** samples will be collected via venipuncture or central venous line, at a specific time-points between days 2 and 21 from the commencement of antibiotic treatment. Samples must be taken at least 24 hours after starting the IV antibiotic (from day 2). The sampling regimen depends on whether the IV antibiotic is administered as a bolus/intermittent/prolonged infusion or continuous infusion:

Bolus/intermittent/prolonged infusion

(i) **Two samples between days 2 and 16** from the commencement of antibiotic treatment at the following time points:

- 2 hours after starting the infusion
- 30 minutes prior to the next dose (i.e. trough concentration)

(ii) **A third sample** taken 30 minutes prior to a dose (trough) at least 5 days after sample 2.

Continuous infusion

(i) **One sample at any time between days 2 and 16** from the commencement of antibiotic treatment to measure the steady-state concentration (Css).

(ii) **A second sample** taken at any time at least 5 days after the first sample, to measure the steady-state concentration (Css).

IV to Oral

If the patient has changed from IV therapy to one of the three oral antibiotics of interest (cefalexin, amoxycillin, flucloxacillin) before day 21 from the commencement of antibiotic treatment; a further two blood samples will be taken at least 48 hours from the start of the oral therapy at:

(i) 2 hours post dose; and

(ii) 30 minutes prior to the next dose (i.e. trough concentration)

Oral only

If the patient is enrolled in O-SNAP after IV therapy (late registry patient) and is receiving one of the three oral antibiotics of interest (cefalexin, amoxycillin, flucloxacillin) before day 21 from the commencement of antibiotic treatment; two blood samples will be taken at least 48 hours from the start of the oral therapy at:

(i) 2 hours post dose; and

(ii) 30 minutes prior to the next dose (i.e. trough concentration)

B. Bacterial load:

- *Children (1 to <18 years)*: Two 0.5ml bloods samples will be collected in a subset of 50 children via convenience sampling method at any two timepoints separated by >24 hours within the first 72 hours from the commencement of antibiotic treatment. These samples will be taken only from the first 50 children enrolled in the sub-study who have a confirmed MSSA or PSSA and receiving one of the IV antibiotics (benzylpenicillin/penicillin G, cefazolin, flucloxacillin, cloxacillin) or oral antibiotics (cefalexin, amoxycillin, flucloxacillin) and when the time of sub-study entry is within 72 hours from the start of antibiotic treatment. If the time of sub-study entry has exceeded the first 72 hours from the start of antibiotic treatment, bacterial load samples will not be collected from the participant.

- *Adults (≥18 years):* In a subset of 50 adults, two 2ml bloods samples will be collected via venipuncture or central venous line at any two timepoints separated by >24 hours within the first 72 hours of from the commencement of antibiotic treatment; preferably align with the convenience sampling time or at the same time when other study blood samples are taken to minimize invasive procedures. These samples will be taken only from the first 50 adults enrolled in this sub-study who have a confirmed MSSA or PSSA and receiving one of the IV antibiotics (benzylpenicillin/penicillin G, cefazolin, flucloxacillin, cloxacillin) or oral antibiotics (cefalexin, amoxycillin, flucloxacillin) and when the time of sub-study entry is within 72 hours from the start of antibiotic treatment. If the time of sub-study entry has exceeded the first 72 hours from the start of antibiotic treatment, bacterial load samples will not be collected from the participant.

The bacterial load will be quantified using quantitative 16sPCR and/or high-throughput metagenomic sequencing of cell free DNA (cfDNA). This will inform the secondary outcome where the PD target for a 3 log10 CFU/ml reduction in bacterial load will be determined.

C. Immunological Testing:

- *Adults (≥18 years) only*: In a subset of 50 adults, two additional 2-6 ml blood samples (volume dependent on size of serum gel tubes available at each study site) will be taken at any two timepoints separated by > 24 hours within the first 96 hours from the start of antibiotic treatment for cytokine testing, preferably align with the convenience sampling time or at the same time when other study blood samples are taken to minimize invasive procedures. These samples will be taken from the first 50 adults enrolled in this sub-study who have a confirmed MSSA or PSSA and receiving one of the IV antibiotics (benzylpenicillin/penicillin G, cefazolin, flucloxacillin, cloxacillin) or oral antibiotics (cefalexin, amoxycillin, flucloxacillin) and when the time of sub-study entry is within 96 hours from the start of antibiotic treatment. If the time of sub-study entry has exceeded the first 96 hours from the commencement of antibiotic treatment, bacterial load samples will not be collected from the participant.

Table 1 - Blood sample, volume and tube required for testing

Test	**Antibiotic concentration**	**Bacterial load (Subset of 50 adults and 50 children)**	**Immunology (Subset of 50 adults)**	**Renal and Liver Function**
Tube	Serum gel tube	Zymo tube	Serum gel tube	Serum gel tube
Minimum blood volume	0.5ml – Children 3ml to 6ml – Adults	0.5 ml – Children 3ml – Adults	2ml to 6ml – Adults only	As per standard practice
Testing	Free (serum) and total (serum) antibiotic concentrations determined by mass spectrometry	Bacterial load will be determined by quantification of cfDNA and /or16s qPCR	27-plex human cytokine panels	Creatinine and Albumin level
Processing *	Centre For Clinical Research-University of Queensland	South Australian Health and Medical Research Institute	MCRI	Site pathology as per standard practice

*Stored at each study site at -80°C and shipped in batches

4.4.2 Microbiological samples:

Bacterial MIC testing: All *S. aureus* isolates from blood will be stored at -80°C. Bacterial isolates will be centrally collated from O-SNAP sub-study participants as per SNAP Protocol. Broth microdilution MIC to the antibiotics received will be determined by the central lab.

4.5. Concomitant care

As per SNAP core protocol

4.6. Endpoints

4.6.1. Primary sub study endpoint

In patients with MSSA SAB treated with IV flucloxacillin, cloxacillin, or cefazolin, the difference in “treatment success” between those who do or do not achieve the pre-defined pharmacodynamic target for β-lactam antibiotics.

4.6.2. Secondary sub study endpoints

- Population PKPD models for each of the three first line IV and oral β-lactam antibiotics for SAB will be developed.
- New PD targets will be defined for the antibiotics against *S. aureus* and optimized dosing regimens simulated using PKPD modelling.
- The PK of other study antibiotics randomized in SNAP platform will be described.
- The relationship between antibiotic exposure and immunological response to infection will be determined using population PKPD modelling to inform an optimized antibiotic dosing schedule and explore the potential benefit of adjunctive immunomodulatory therapy in *S. aureus* bacteremia.

5. SUB STUDY CONDUCT

5.1. Sub study-specific data collection

5.1.1. Microbiology

Broth microdilution MIC for the *S. aureus* isolates to the relevant antibiotics will be determined as per core SNAP Trial.

5.1.2. Clinical data collection

Additional sub study-specific data will be collected as follows:

- Height and Weight at the start of antibiotic treatment
- Start and end date of relevant concomitant medications administered during the antibiotic treatment course which affect the PKPD of the studied antibiotics; concomitant medications that will be evaluated as covariates in the PKPD model include:
    - Other antimicrobials that are listed as per SNAP baseline CRF- “Antibiotics”.
    - Non-steroidal anti-inflammatories
    - Radiocontrast material
- Antibiotic Dosing details:
    - Date/time of two previous antibiotic dose prior to antibiotic concentration sample collection
    - Medication infusion times, dosage, frequencies, and administration method
- Laboratory data:
    - UEC and Albumin on Day 1 +/- 1 day and Day 5 +/-1 day from the start date of antibiotic treatment, as well as any other results available
    - Antibiotic levels: Date/time/method of sample collection, processing and storage
- Other:
    - Start and end date if patients are in ICU +/- on inotropic support during the antibiotic treatment course

Patient receiving renal replacement therapy or extracorporeal membrane oxygenation, including modality and start and end data.

5.1.3 Sub study specific study timeline

Table 2: Sub study-specific schedule of visits, data collection and follow-up

Detailed Sample/ Procedure/ Visit Description	**O-SNAP SUB-STUDY** **Days of antibiotic treatment course (D1 = First day of Antibiotic Treatment)**																
Days of Antibiotic Course	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**21**
Substudy screening	Screening for O-SNAP Participants that have entered SNAP Platform/Registry																
ALL O-SNAP STUDY PARTICIPANTS (aged >1y, ENROLLED IN SNAP PLATFORM OR REGISTRY, and CURRENTY RECEIVING ANTIBIOTIC TREATMENT FOR SAB)																	
CONVENIENCE SAMPLING																	
Antibiotic Concentration Serum Blood samples: *All substudy participants (Adults and Children) ; Collected through convenience sampling during treatment course.*	Children: 0.5ml of blood taken with each clinically indicated blood collection. Maximum 10 samples drawn for antibiotic concentrations across the study, max. 5 samples/day is 5 in children >2 years, 3 in children 1-2 years old.																
	Adults: 2 to 6 mL of blood taken with each clinically indicated blood collection. Samples must be taken after 24 hours of IV antibiotic treatment and 48 hours from oral antibiotic treatment . Maximum 10 samples drawn for antibiotic concentrations across the study, max. 5 samples/day/participant.																
STANDARD CLINICAL CARE SAMPLING																	
Renal and Liver Function Test: UEC and Albumin *All Substudy participants (Adults & Children); Collected from tests performed as per standard clinical practice.*	+/- 1 day				+/- 1 day												
FOR O-SNAP STUDY PARTICIPANTS WITH (1) CONFIRMED PSSA/MSSA and **(2) RECEIVING ANY OF THIS ANTIBIOTICS –** IV (benzylpenicillin/penicillin G, cefazolin, flucloxacillin, cloxacillin) OR oral (cefalexin, amoxycillin, flucloxacillin)																	
Bacterial load Blood samples: *Subset of 50 adults and 50 children: 2 blood samples collected anytime separated by >24 hours within the first 72 hours of antibiotic treatment* ****Do Not collect samples if the time of O-SNAP entry is after the first 72hrs from the start of antibiotic treatment***	Children: **TWO** 0.5ml samples (> 24 h apart)																
	Adults: **TWO** 3ml samples (> 24 h apart)																

Detailed Sample/ Procedure/ Visit Description	O-SNAP SUB-STUDY Days of antibiotic treatment course (D1 = First day of Antibiotic Treatment)																
Days of Antibiotic Course	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**21**
Immunology Testing Blood samples: *Subset of 50 adults only 2 blood samples collected anytime separated by >24 hours within the first 96 hoursof antibiotic treatment.* ***Do Not take these samples if the time of O-SNAP entry is after the first 96hrs from the start of antibiotic treatment.***	Adults: **TWO** x 2 -6 mL samples (> 24 h apart)																
ADDITIONAL SAMPLING AT SPECIFIC TIMEPOINTS																	
Antibiotic Concentration Serum Blood samples (adults only): *For bolus/intermittent infusion (II), and prolonged infusion (PI) - peak and trough samples, taken at specific timepoints (3 samples in total).*		Adults (bolus/II/PI): Collect **TWO** 3 to 6 mL samples between days 2 – 16 at (1) 2 h after starting infusion, (2) 30 mins prior to next dose (trough)						Adults (bolus/II/PI): Collect a **3rd** sample taken 30 mins prior to a dose (trough) ≥ 5 days after sample 2									
For continuous infusions (CI) - steady-state concentration (Css) samples, taken at specific timepoints (2 samples in total).						Adults (CI): Collect **ONE** 2-6 mL sample anytime between days 2 – 16 (Css)											
								Adults (CI): Collect a **2nd** sample taken ≥ 5 days after the 1st sample (Css)									
For oral therapy (cefalexin, amoxycillin, flucloxacillin) including IV to oral and enrolled on oral therapy – peak and trough samples, taken at specific timepoints (2 samples in total).																	
								Adults: If changed from IV to oral / enrolled on orals (cefalexin, amoxycillin, flucloxacillin), collect **TWO** x 2 to 6 mL samples at (1) 2 h post dose (2) 30 mins prior to next dose (trough)									
DATA COLLECTION																	
Collect data as per substudy CRF	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

**** All samples will be collected during the participant's acute care hospital admission (including Hospital in the Home (HITH) if sample collection is feasible). If the participant is discharged from the SNAP or Sub-study enrolling hospital, sampling will be ceased.***

5.2. Blinding

5.2.1. Blinding

Not relevant.

5.2.2. Unblinding

Not relevant.

6. Data Analysis Plan

PK modelling will be used to determine the fT>MIC for each participant for the primary and secondary outcomes analysis.

1. Logistic regression will be used to adjust outcomes for other comorbid and predictive factors that influence treatment success.

2. A first-order conditional estimation method will be used to estimate pharmacokinetic parameters and their variability. Model evaluation will be based on graphical and statistical criteria, including goodness-of-fit plots and visual predictive checks.

The final PKPD model for each drug will be used to determine optimised dosing regimens for adults and children (i.e. a dose that maximizes benefit for minimal risk) by developing a utility curve that combines the risk-benefit profile.

7. STATISTICAL CONSIDERATIONS

7.1. Statistical modelling

7.1.1. Primary model

Data from the 400 adults and 150 children will be analysed separately given the significant differences in treatment success and attainment of the conventional PKPD target between the two groups: treatment success for MSSA bacteraemia is between 77-82% in adults, and 83-97% in children[11, 12]; and probability of attaining the conventional PKPD target is 71-91% in adults[16], and 65% in children. Data for all the studied antibiotics (benzylpenicillin, cefazolin, flucloxacillin) will be analysed together as the PKPD target is expected to be the same for all β-lactam antibiotics. Sample size calculations are based on detecting a 15% difference in treatment success between those who achieve the conventional PKPD target and those who do not, using

a two-sided 5% significance level for a Pearson's Chi-squared test. This 15% difference has been determined to be a clinically meaningful outcome measure by the SNAP clinical pharmacology working group and trial steering committee. *For adults*, assuming that 80% of adults attain the conventional PKPD target and that 82% of this group achieves treatment success, a total of 400 adults will have 80% power to detect a difference of 15% in treatment success. *For children,* assuming that 65% of children attain the conventional PKPD target and that 96% in this group achieves treatment success, a total of 150 children will have 80% power to detect a difference of 15% in treatment success.

7.1.2. Secondary analysis

Drug-specific PKPD models: A minimum of 30 patients, each with ≥2 concentrations per studied antibiotic will be enrolled. This sample size will estimate the 95% confidence interval for population PK model parameters within a 20% precision level with a power of 90%.[13]

Population PKPD analysis will use non-linear mixed effects modelling (NONMEM). Development of the population PKPD model will involve:

1. characterizing the relationship between drug concentrations and clinical effects;
2. evaluating biologically plausible covariates (e.g. age, weight, creatinine level, body surface area, body mass index) linking the dose-concentration-effect. Therapeutic effects include treatment success, reduction in bacterial load by $3\log_{10}$ CFU/ml, and adverse effects; and
3. evaluating time-dependency of the PKPD covariates and parameters using time-course profiles.

Correlation between bacterial burden and outcome: The correlation between bacterial burden and treatment success will be determined using logistic regression.

7.2. Interactions with interventions in the SNAP trial domains

Nil

7.3. Potential impact on trial integrity if findings released prior to overall platform conclusions are reached

Post-randomisation endpoints to be used in this sub-study will only be available after the main clinical trial findings have been published.

8. ETHICAL CONSIDERATIONS

8.1. Potential sub study-specific adverse events

This study does not involve any additional procedures (i.e. no additional lines, needle sticks) in children and only 4 additional procedures in adults outside those that are clinically indicated. The volume of blood taken is minimal (<2% total blood volume in line with research guidelines) and poses no significant risk to study participants.

8.2. Sub study-specific consent issues

At the involved sites, patients approached for consent for enrolment into the SNAP platform, will have the option to opt-in to this sub study. The protocol will be submitted as an appendix to the main trial protocol.

9. GOVERNANCE ISSUES

9.1. Proposed budget

This budget estimation is based on total of 550 participants for a study duration of 3 years.

Descriptions	Total $
Personnel Cost	
Research Assistant – 550 participants x 10 hours x $35/hr	$192,500
Consumables	
Biobanking – 550 participants x 10 samples x $20/sample	$110,000
Free antibiotic level (serum samples) – 550 participants x 10 samples x $15/sample	$82,500
Bacterial Load -100 participants x 2 samples x $25/sample	$5,000
Luminex – 50 participants x 1 assay = 50 assays run in duplicate = 100 assays / 30 test per kit = 4kits	$19,520
Bacterial MIC Broth Microdilution – 550 Isolates x $10 run in triplicates	$5,500
Sample Shipping – 16 sites x 2 batches x 300/batch x 3 labs	$28,800
Total Consumable Cost	$263,320
Total	**$443,820**

9.2. Funding of sub study

This sub study has not received any additional sub study-specific funding. The study will be piloted with in-kind support from Prof Jason Roberts and A/Prof Amanda Gwee's NHMRC investigator grants.

9.3. Funding of sub study interventions and outcome measures

As Above

9.4. Proposed timeline

September 2023 – July 2026

9.5. Sub study-specific declarations of interest

All investigators involved in SNAP maintain a registry of interests. These are updated periodically and publicly accessible on the study website.

10. REFERENCES

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