Study Title	CAMERA 2 - Combination Antibiotic Therapy for Methicillin Resistant Staphylococcus Aureus infection – An investigator-initiated, multi-centre, parallel group, open labelled randomised controlled trial
Abbreviated Title	CAMERA2
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Contents

CAMERA2 Study Synopsis

TITLE	CAMERA2 – **C**ombination **A**ntibiotic therapy for **ME**thicillin **R**esistant Staphylococcus **A**ureus. A multi-centre RCT to determine if 7 days of intravenous β-lactam in combination with standard therapy will lead to better 90-day complication-free survival, compared to standard therapy alone in adult participants with methicillin-resistant *S. aureus* (MRSA) bloodstream infection.
BACKGROUND	MRSA bacteraemia has a mortality of approximately 25%, exceeding that of methicillin-sensitive *S. aureus* primarily due to the shortcomings of vancomycin, which is used in the majority of participants with MRSA bacteraemia. Whilst several new antibiotics have become available for MRSA, none have been shown to be clearly superior to vancomycin. Although MRSA is by definition resistant to β-lactams, multiple *in-vitro* and animal studies have demonstrated synergy of vancomycin or daptomycin with β-lactams against MRSA.
PRIMARY OUTCOME MEASURE	Composite outcome at 90 days – any of: 1. All-cause mortality 2. Persistent bacteraemia at day 5 or beyond 3. Microbiological relapse - positive blood culture for MRSA at least 72 hours after a preceding negative culture 4. Microbiological treatment failure. Positive sterile site culture for MRSA at least 14 days after randomisation.
SECONDARY OUTCOME MEASURES	1. All-cause mortality 14, 42 and 90 days 2. Proportion with persistent bacteraemia at day 2 3. Persistent bacteraemia at day 5 or beyond 4. Proportion with acute kidney injury defined as ≥stage 1 modified RIFLE criteria (1.5-fold increase in the serum creatinine, or glomerular filtration rate (GFR) decrease by 25 percent) at any time within the first 7 days OR new need for any form of renal replacement therapy at any time in the first 90 days. 5. Microbiological relapse 6. Microbiological treatment failure 7. Duration of intravenous antibiotic treatment 8. Direct health care costs
STUDY DESIGN	Open label, parallel-group, multi-centre, randomized controlled trial (RCT)

Standard therapy group	Intravenous vancomycin dosed in accordance with the Australian Therapeutic Guidelines or Infectious Diseases Society of America (IDSA) guidelines, with subsequent adjustment to maintain trough levels at 15-20 mg/dL OR Intravenous daptomycin 6-10 mg/kg per day, adjusted for renal function (details of renally adjusted dosing provided in full protocol). The choice of daptomycin or vancomycin is clinician-determined and may be influenced by such factors as local practice, the vancomycin MIC of the isolate and evidence emerging during the course of the study
Combination therapy group	As above plus intravenous β-lactam for seven days. The β-lactam will be flucloxacillin 2g QID in Australia, and cloxacillin 2g QID in Singapore; for those on haemodialysis, the β-lactam will be cefazolin 2g post-dialysis 3 times per week; for those with minor penicillin allergy, cefazolin 2g TDS IVI.
STUDY DURATION	Jan 2015-Dec 2019
NUMBER OF PARTICIPANTS	440 participants.
INCLUSION CRITERIA	1. Age ≥ 18 years. 2. ≥1 set of blood cultures positive for MRSA 3. Able to be randomized within 72 hours of blood cultures being collected. 4. Likely to remain as inpatient for 7 days following randomization (or an outpatient receiving haemodialysis and is accessible for follow up by the site PI).
EXCLUSION CRITERIA	1. Previous type 1 hypersensitivity reaction to ß-lactams 2. Polymicrobial bacteraemia (not counting contaminants) 3. Previous participation in the trial 4. Known pregnancy 5. Current β-lactam antibiotic therapy which cannot be ceased or substituted 6. Participant's primary clinician unwilling to enrol patient 7. Moribund (expected to die in next 48 hours with or without treatment) 8. Treatment limitations that preclude the use of antibiotics Note that we are NOT planning to exclude participants with renal failure.
RANDOMISATION	Eligible participants will be randomized 1:1. Randomization will be stratified by site and chronic intermittent haemodialysis status, in permuted blocks of

	variable size.
BLINDING	This will be an open-label study, but the investigators assessing the primary outcomes and performing the statistical analysis will be blinded to treatment allocation.
SAMPLE SIZE CALCULATION	The recruitment target of 440 is based on an expected failure rate for the primary outcome of 30% in the control arm (based on data from CAMERA1) and the ability to detect a clinically meaningful absolute decrease of 12.5% (the midpoint of expert clinicians' estimates of 10-15%), with a two sided alpha of 0.05, a power of 80%, and assuming ~10% drop out.
ANALYSIS	Analysis of the primary outcome will be by modified intention to treat (all participants with data available for the primary endpoint will be analysed according to the treatment allocation, regardless of what treatment they received). A per protocol analysis will also be performed. The per protocol population is defined as 1) for the combination group: received at least 75% of β-lactam doses; 2) for the standard treatment group: received ≤1 defined daily dose of β-lactam after randomisation; 3) has available day 90 data. We also plan pre-specified subgroup analyses for the following groups: i) Main treatment was daptomycin vs. vancomycin; ii) Vancomycin MIC of primary isolate ≥1.5μg/ml, or 〈1.5 μg/ml; iii) Participants receiving haemodialysis compared with those who are not; iv) Those who received >24 hours of β–lactam antibiotics prior to randomization compared with those who did not; v) uncomplicated vs complicated SAB; vi) Participants recruited in Singapore vs Australasia; vii) Participants with baseline immunosuppression versus those without; viii) Participants with left-sided endocarditis vs those without; ix) Participants with community-associated MRSA vs healthcare associated MRSA (defined either genotypically or by non-multi vs multidrug-resistant phenotype).

1. Introduction

1.1 Abbreviations

ADR	Adverse drug reaction
AE	Adverse event
AKTN	Australian Kidney Trials Network
ANZCOSS	Australian and New Zealand Co-operative Outcomes of Staphylococcal Sepsis
ASID CRN	The Australian Society for Infectious Diseases Clinical Research Network
ß-lactams	a family of related antibiotics which includes the penicillins cephalosporins and carbepenems
CI	Chief Investigator – A researcher who contributes to the funding, planning, and running of the entire study
CK	Creatinine kinase
CNS	Coagulase negative Staphylococcus
Co-I	Co-investigator (a clinician or research assistant who aids the PI at a site)
CRP	C-reactive protein
CTN	Clinical trial notification (to the Therapeutic Goods Administration)
DNA	Deoxyribonucleic acid
DOB	Date of Birth
DSMB	Data safety monitoring board
EDC	Electronic data capture
EDTA	Ethylenediaminetetraacetic acid (an anticoagulant)
EUC	Electrolytes, urea & creatinine
eCRF	Electronic case report forms
FBC	Full blood count

GCP	Good clinical practice
GFR	glomerular filtration rate
GP	General practitioner
HRN	Hospital record number
HREC	Human research ethics committee
hVISA	heteroresistant Vancomycin intermediate *Staphylococcus aureus*
ICH	International Conference on Harmonisation
ICU	Intensive care unit
ID	identification
ID physician	Infectious disease physician
IDSA	Infectious disease society of America
Index blood culture	The 1st positive MRSA blood culture taken from the patient for that clinical episode.
IRB	Institutional review board
IV	Intravenous
IVI	Intravenous infusion
LFT	Liver function test
LiHep	Lithum heparin
Menzies	Menzies School of Health Research
MIC	Minimum inhibitory concentration
MRSA	Methicillin Resistant *Staphylococcus aureus*
MRSA-B	MRSA bacteraemia
MSSA	Methicillin Sensitive *Staphylococcus aureus*

MSSA-B	MSSA bacteraemia
NHMRC	National Health and Medical Research Council
NMRC	National Medical Research Council, Singapore
NOK	Next of kin
PI	Principal Investigator (a clinician responsible for one site)
QALY	Quality-adjusted life years
QID	4 times per day
RC	research co-ordinator (responsible for multiple sites)
RCT	Randomised control trial
RIFLE criteria	Acronym for Risk, Injury, and Failure; and Loss; and End-stage kidney disease (classification system for Acute Kidney Injury)
SAB	*Staphylococcus aureus* bacteraemia
SAE	Serious adverse events
SCRN	The Singapore Infectious Diseases Clinical Research Network
SOP	Standard Operating Procedure
SUSAR	Suspected unexpected serious adverse reaction
TDS	3 times per day
TGA	Therapeutic Goods Administration
VISA	Vancomycin intermediate *Staphylococcus aureus*
VRSA	Vancomycin resistant *Staphylococcus aureus*
IXRS	interactive voice/Web response system

1.2 Background and Rationale

1.2.1 Overview *Staphylococcus aureus* is one of the most important bacterial pathogen of humans, and causes a broad range of infections, ranging from superficial skin infections, deep skin and tissue abscesses and bone infections, to invasive bloodstream infections. Methicillin-resistant *S. aureus* (MRSA) is resistant to the mainstay of *S. aureus* therapy, the anti-staphylococcal penicillins (such as flucloxacillin) and is hence more difficult to treat. Therapies for MRSA are either less effective (e.g., vancomycin) or much more expensive (e.g., daptomycin) than the anti-staphylococcal penicillins are for methicillin-susceptible *S. aureus*. Alternative therapies, including novel combinations of existing agents, are therefore urgently required to treat invasive MRSA infections, each episode of which results in a mortality of 20-50% [1].

Invasive MRSA infection causes a substantial burden of disease. The Australian and New Zealand Co-operative Outcomes of Staphylococcal Sepsis (ANZCOSS) study included data from 33 hospitals and found that of 10,085 SAB cases in 6 years (2007-2012), 2,881 (22%) were MRSA with an average of 480 MRSA-B cases per year [1]. Although data are lacking, the disease burden is likely to be even higher in large population centres in Asia as indicated by high case numbers in hospitals in Singapore (Table 1). Although hospital-acquired MRSA infections have decreased in the US, UK and Australia with improved infection control practices, community-associated strains of MRSA have emerged in the past 10–15 years and the majority of invasive MRSA infections are now community-onset rather than nosocomial [2]. This is reflected in ANZCOSS data, where community-onset cases of MRSA-B (index blood culture taken <72 hours following admission) increased from 51% in 2007 to 69% in 2012. Attempts to prevent MRSA infections outside the hospital system are unlikely to be effective, and further reductions in the incidence of MRSA infections are not expected.

MRSA is associated with poor outcomes. Bloodstream infections with MRSA have a higher mortality than those caused by MSSA [3]. The ANZCOSS dataset demonstrates that 30 day mortality is higher at 24% for MRSA compared to 17% for MSSA (P<0.001). In a Thai hospital, mortality rates were 67% and 46% for MRSA and MSSA respectively [4]. This high mortality, not only in Australia, Singapore, New Zealand and Israel, but also in resource limited settings where SAB is common and infection control practices are suboptimal, is a key reason for the currently described randomised controlled trial (RCT).

Current therapies for MRSA-B are limited and associated with poor outcomes: A significant factor contributing to poorer outcomes with MRSA-B compared to methicillin-susceptible *S. aureus* bacteraemia (MSSA-B) is the limitations of vancomycin (the current standard antibiotic therapy for invasive MRSA infections). Compared with anti-staphylococcal ß-lactams such as oxacillin and its

derivatives (flucloxacillin, cloxacillin, and naficillin), vancomycin demonstrates slower bacterial killing [5], poorer tissue penetration [6], slower clearance of bacteraemia [7,8] and higher mortality [9,10]. For MSSA bacteraemia in ANZCOSS, 30 day mortality was 21% (133/638) and 12% (937/6950) for those treated with vancomycin or β–lactams respectively. Furthermore, treatment with vancomycin compared to β–lactams was a risk factor for 30-day mortality among all participants with SAB, independent of MRSA vs MSSA status (P<0.001) [1]. In addition, strains of MRSA with decreased susceptibility to vancomycin (heterogenous vancomycin intermediate resistance *S. aureus* [hVISA]) are beginning to emerge worldwide [11]. In recent years, several alternative agents to vancomycin have become available for the treatment of MRSA bacteraemia, including linezolid, daptomycin, tigecycline and ceftaroline. Each of these has been found to be non-inferior to vancomycin for MRSA infections, but none have been shown to be superior [12] and all are associated with a high cost and a substantial risk of adverse effects [13]. Thus, vancomycin continues to be recommended as the first-line agent for severe MRSA infections by both the Infectious Diseases Society of America [14] and the Australian Therapeutic Guidelines: Antibiotic [15]. Ceftaroline has only recently become available for MRSA pneumonia and skin infections, but no trials have yet been completed comparing it with vancomycin for MRSA bacteraemia. However, even if ceftaroline were to eventually prove more effective, its cost far exceeds that of vancomycin (estimated drug cost for a 4 week course of ceftaroline=A$8,680, compared with vancomycin=A$260). Ceftaroline resistance is an additional concern with a recent Australian study finding overall resistance rates of 17% amongst MRSA and 41% in sequence type 239 MRSA [16]. An alternative strategy to improve outcomes from MRSA bacteraemia is to combine vancomycin with a second agent, aiming for synergistic bacterial killing [17,18]. Neither linezolid nor daptomycin demonstrate synergy with vancomycin against MRSA [18]. However, β–lactam antibiotics, to which MRSA is inherently resistant, demonstrate an unexpected but consistent synergy with vancomycin and daptomycin respectively against MRSA. Given that β–lactams are cheap (e.g., 7 days of flucloxacillin costs $47), safe and widely available, they are an attractive alternative to more expensive drugs as second agents to combine with vancomycin.

1.2.2 Previous studies of β-lactam combination therapy

Due to poor outcomes with vancomycin monotherapy, and the emerging problem of decreased vancomycin susceptibility in MRSA [19], multiple research teams have investigated the combination of vancomycin or daptomycin with various β-lactam antibiotics (reviewed in detail in Davis et al. [20]).

In vitro studies of vancomycin and β-lactam combinations

At least 16 *in-vitro* studies have explored synergy between vancomycin and β-lactams against MRSA isolates [21-36], all but one of which found evidence of synergy in some or all of the tested strains. These studies varied in their methodology (checkerboard synergy testing or time-kill curves), types of strains tested (MRSA vs hVISA vs VISA) and the β-lactams used, but a consistent finding across nearly all the studies was synergistic bacterial killing in *most* but not *all* strains tested. There was a general tendency across these studies (and *within* some studies [23,33]) to an increasing degree of synergy with increasing vancomycin MICs. Synergy has been reported with all ß-lactams tested (including cefazolin), but the largest effect has been observed with oxacillin and naficillin. Flucloxacillin is also considered in the same antibiotic class "antistaphylococcal semi-synthetic penicillins".

Animal studies of vancomycin and β-lactam combinations. The few studies that have assessed combinations of vancomycin with β-lactams in animal models have all found evidence of synergy [23,28,32]. Climo found faster sterilisation of infection with vancomycin plus nafcillin in MRSA rabbit endocarditis and renal abscess models [23]. Ribes tested various combinations of linezolid, vancomycin and impienem in a murine peritonitis VISA model using time-kill curves, and found faster bacterial killing with vancomycin plus imipenem compared with vancomycin alone, in both strains tested [28]. Finally, Fernandez investigated the anti-MRSA cephalosporin ceftobiprole against an MRSA and a VISA strain in a rat endocarditis model. They found good activity of ceftobiprole against both strains in terms of sterilising vegetations and preventing mortality; the combination of vancomycin plus cefobiprole led to faster killing on time-kill curves, but similar rates of mortality and of sterilisation of vegetations compared with ceftobiprole alone [32].

Human studies of vancomycin and β-lactam combinations.

There are few published data on β-lactam based combination therapy for MRSA in humans. In a single-centre retrospective cohort study, Dilworth and colleagues described the outcomes of 50 participants with MRSA bacteraemia who received combination therapy with vancomycin and at least 24 hours of β-lactam (at their clinicians' discretion), and compared them with 30 participants

treated at the same hospital, during the same time period with vancomycin alone [35]. They found a higher rate of microbiological eradication in the combination therapy group (96% vs 80%, p=0.02), which persisted on a multivariate model attempting to control for potential confounders (adjusted odds ratio for achieving microbiological eradication in the combination group=11.24, p=0.01).
In the only prospective clinical trial to date (CAMERA1), Davis et al. [37] randomised 60 patients with MRSA bacteraemia to standard therapy with vancomycin alone, or to combination therapy with vancomycin and flucloxacillin. The study was conducted in seven centres in Australia and was open-label in design. Patients receiving combination therapy cleared bacteraemia at a mean of two days compared to three days with standard therapy (P=0.06).

In-Vitro studies of daptomycin and β-lactam combinations

At least 10 *in vitro* studies have examined the combination of daptomycin with various β-lactams against MRSA and VISA strains [38-48]. The findings of these studies are remarkably similar to the vancomycin/β-lactam synergy papers cited above: synergy for most but not all strains tested, and an increasing degree of synergy with increasing MICs to both vancomycin and daptomycin. No studies have found evidence of antagonism with this combination.

Animal studies of daptomycin and β-lactam combinations

A recently published animal study mirrored the findings of the *in-vitro* studies. Garrigos used a rat tissue cage model of MRSA infection to study the combination of daptomycin with cloxacillin, and found superior cure rates with the combination than with daptomycin alone [49].

Human studies of daptomycin and β-lactam combinations

As for the vancomycin/β-lactam combination, there are no clinical trials of daptomycin with β-lactams either published or in trials registries. However limited observational data suggest this combination may be effective, particularly MRSA with poor response to daptomycin. In a case series of 7 participants with persistent MRSA bacteraemia for more than 1 week despite high-dose daptomycin, all cleared their bacteraemia within 48 hours once naficillin or oxacillin was added to their therapy [50]. In a second case series of 22 participants with persistent MRSA bacteraemia despite daptomycin for a median of 10 days, the addition of ceftaroline lead to clearance of bacteraemia in all cases, in a median of 2 days [51].

A key question that emerges from these data is: what is the mechanism of the observed synergy? The mechanisms have not been entirely elucidated, but are becoming clearer over time. Increasing vancomycin resistance in *S. aureus* is paradoxically associated with decreasing MICs to oxacillin, and

this so-called "see-saw effect" [35,52] is at least in part due to deletion of the MecA gene in some strains of VISA and VRSA [53,54], and possibly to other structural changes in penicillin binding proteins and cell wall thickness. β-lactams have been shown to enhance binding of daptomycin to the bacterial cell wall [48]. Finally, Sakoulas et al. recently reported novel data derived from *ex-vivo* study of human blood which adds another potential advantage for the use of ß-lactams for MRSA – they lead to increased activity of innate host defence peptides such as cathelicidin LL-37[55], which in turn allow more efficient bacterial killing.

Thus there is considerable *in-vitro*,*in-vivo* and growing clinical evidence that the combination of vancomycin or daptomycin with a β-lactam may be more effective than vancomycin or daptomycin alone for improving outcomes of this common and devastating infection.

1.2.3 Pilot data from the CAMERA1 pilot study

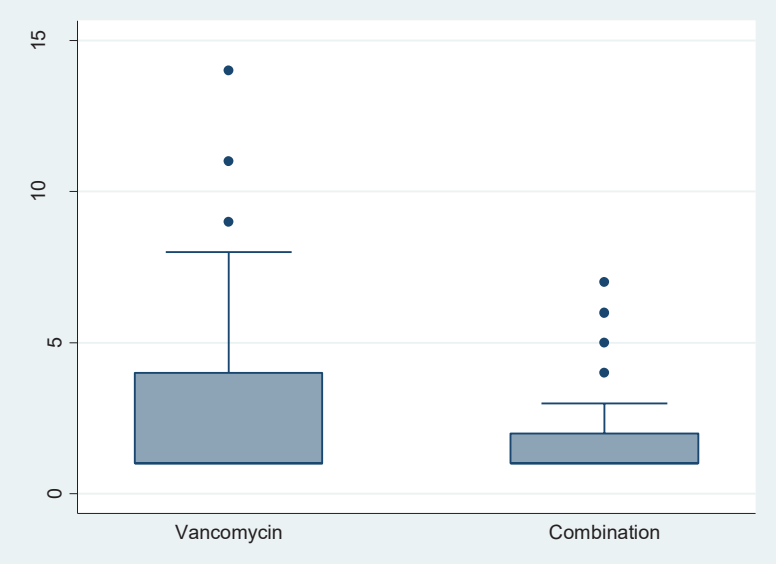


Figure 1 – Duration of bacteraemia (days) in both treatment groups in the CAMERA1 study

In order to refine assumptions, determine feasibility and look for a signal of efficacy, we designed a pilot RCT, called CAMERA – Combination Antibiotic treatment for MEthicillin Resistant staphylococcus Aureus. CAMERA1 was an open-label RCT of vancomycin alone compared with vancomycin plus flucloxacillin for adults with MRSA bacteraemia, recruited within 48 hours of blood draw, with duration of bacteraemia as the primary end point.

Between January 2011 and May 2014, 60 patients from seven hospitals were randomly assigned to receive vancomycin (n=29), or vancomycin plus flucloxacillin (n=31). The mean duration of bacteremia was 3.0 days in the vancomycin group and 1.9 days in the combination group. The rate ratio of means was 0.65 (95% confidence interval [CI] 0.41, 1.02; P=0.06), indicating that the mean

time to resolution of bacteraemia in the combination group was 65% of the vancomycin group. Bacteremia for >3 days occurred in 8/29 (28%) and 4/31 (13%) in the vancomycin and combination groups respectively (P=0.20); and bacteremia for >7 days occurred in 4/29 (14%) and 1/31 (3%) in the vancomycin and combination groups respectively (P=0.19). There was no difference in the secondary outcomes of 28 and 90 day mortality, relapsed infection, nephrotoxicity, or hepatotoxicity.

1.3 Objectives and hypotheses

We hypothesise that the addition of β-lactams to standard therapy in adults with MRSA bacteraemia will lead to synergistic bacterial killing and hence faster clearance of bacteria from the bloodstream and other infected foci, thereby reducing the risk of disseminated infection and death.

Primary Objective: To determine if 7 days of intravenous β-lactam in combination with standard therapy will lead to better 90 day complication-free survival, compared to standard therapy alone in adult participants with methicillin-resistant *S. aureus* (MRSA) bloodstream infection.

1.4 Trial design

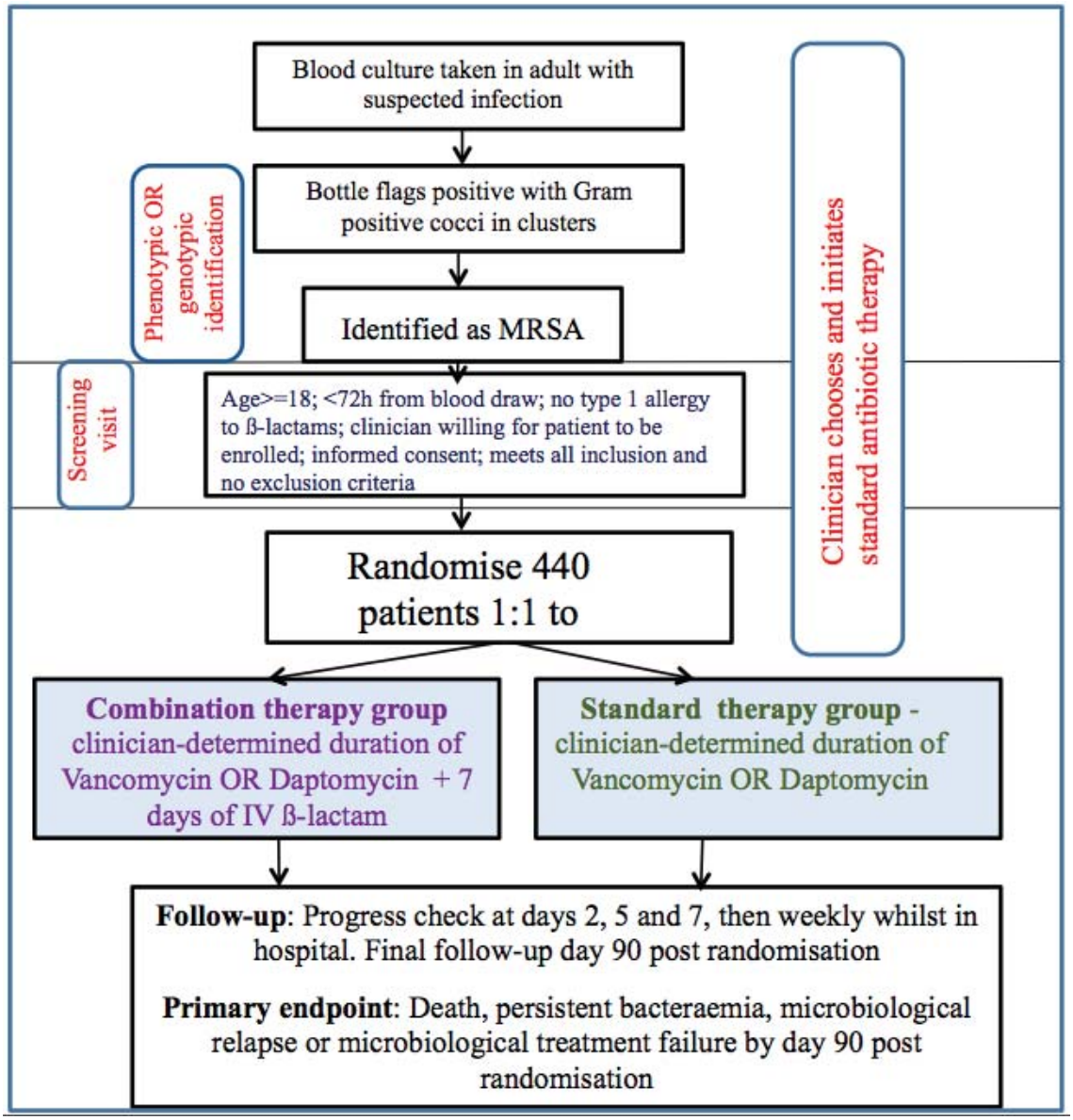
CAMERA is an investigator-initiated, multi-centre, parallel group, open-label, randomised controlled trial powered for superiority, which compares combination antibiotic therapy with standard antibiotic therapy in adults with MRSA bacteraemia.

We will enrol 440 participants into the study over a period of 4 years. The duration of study participation for each participant is 90 days. During their initial hospitalisation period, data will be prospectively collected on day 1 (the day of randomisation), then progressively until day 90 post randomisation

Consented participants will be randomised on day 1 to receive standard therapy alone, or standard therapy plus 7 days of IV β-lactam. Data will be captured for the 90 days post randomisation from the participant's medical records and medication charts (electronic and paper). As long as the participant remains an inpatient, their medical records will be reviewed at least weekly until discharge or the 90 day time point whichever occurs first. Information that will be collected includes any SAE's that have occurred, any further blood test results (FBC, EUC, LFTs, CRP, blood cultures and vancomycin levels), administration of any antibiotics, and evidence of relapse or microbiological treatment failure. If discharged the participant will be contacted via telephone if required at day 90, a medical record review will also occur at day 90 for any other admissions, positive blood cultures, antibiotic administration, and any relevant data that was not recorded at the previous medical record reviews. If phone contact at the 90 day time point is unsuccessful for any study participant

and a vital status has not been determined by medical record review then the site investigator will repeat the medical record review at 6 month time intervals until either the vital status is determined or data collection for the study has ceased.

Figure 2 – Overview of trial design



2. Methods

2.1 Study setting

We are planning to recruit from 24 Australian, 1 New Zealand, 3 Singaporean and 2 Israeli acute care hospitals. Other sites may be added during the course of the study. Sites have been selected on the

basis of i) Their prevalence of MRSA bacteraemia (at least 10 cases per year and ideally 20); ii) The availability of an experienced and committed site principal investigator; and iii) The availability of a suitably qualified research nurse OR senior registrar to assist study related activities.

2.2. Eligibility criteria

2.2.1 Participant Inclusion criteria

1. Age ≥ 18 years
2. ≥1 set of blood cultures positive for MRSA
3. Able to be randomized within 72 hours of blood culture being collected
4. Likely to remain as an inpatient for 7 days following randomization (or an outpatient receiving haemodialysis and is accessible for follow up by the site PI).

2.2.2 Participant Exclusion criteria

1. Previous type 1 hypersensitivity reaction to β-lactams
2. Mixed blood culture with more than one pathogen (contaminants are not counted here – i.e., a mixed growth of MRSA and coagulase negative Staphylococcus [CNS] is eligible, as long as the CNS is clinically judged to be a likely contaminant)
3. Previous participation in the trial
4. Known pregnancy
5. Current β-lactam antibiotic therapy which cannot be ceased or substituted
6. Patient's primary clinician unwilling to enrol patient
7. Moribund (expected to die in next 48 hours with or without treatment)
8. Treatment limitations that preclude the use of antibiotics. Participants who are "not for resuscitation" or "not for ICU admission" may still be enrolled if they are for active management of infection including the use of all necessary antibiotics and intravenous fluids.

2.3 Treatment of Study Participants

Participants will be randomised to either the standard care arm (2.3.1) or the combination therapy arm (2.3.2). The standard treatment arm will receive intravenous vancomycin or daptomycin whilst the combination therapy arm will receive 7 days of IV β-lactam in addition to the intravenous vancomycin or daptomycin. The day of randomisation is considered day 1 of treatment and the last dose of β-lactam to be given is the dose next due prior to 23:59hrs on day 7 (i.e., the last scheduled dose prior to midnight). The β-lactam used in the combination therapy arm will be prescribed by a doctor from the treating team and will be supplied from the hospital's pharmacy. Storage conditions of the β-lactam will be as per hospital pharmacy policy and will not be monitored for the study purposes. The participant's drug charts (electronic and/or paper) will be reviewed for compliance

with study treatment (including vancomycin, daptomycin and study β-lactam). Any missed dose/s and non-study β-lactams administered will be recorded on the CRFs.

2.3.1 Standard care arm - Either intravenous vancomycin dosed in accordance with the Australian Therapeutic Guidelines: Antibiotic version 15, 2014 (15-20mg/kg q12h, preceded by a loading dose of 20-35mg/kg if considered appropriate by the treating clinician) or the Infectious Diseases Society of America (IDSA) guidelines [57] with subsequent adjustment to maintain trough levels at 15-20 mg/dL OR Daptomycin 6-10mg/kg per day IVI (both drugs will be adjusted for renal function, **Tables 1–5**). Dosing of vancomycin may follow local guidelines if broadly in line with the Australian Therapeutic Guidelines: Antibiotic and the IDSA guidelines [56]. The choice of vancomycin or daptomycin will be at the clinician's discretion. Similarly, dosing in patients with renal impairment may follow local guidelines as long as they are broadly in line with the below recommendations. As there may be some variation between sites in vancomycin dosing strategies, vancomcyin trough levels will be collected for all patients. Randomisation will also be stratified by site. The choice of vancomycin or daptomycin will be at the clinician's discretion. Continuous infusion of vancomycin will be discouraged during the first 7 days, but if a clinician considers this necessary, this will be allowed and will be recorded in the CRFs. The non-antibiotic management and duration of the intravenous vancomycin or daptomycin will be at the clinicians' discretion, but will be in line with Australian Therapeutic Guidelines and IDSA guidelines [14]. These recommend from 14–42 days of intravenous treatment, depending on factors such as the result of a blood culture at 2–4 days after index blood culture, result of echocardiogram, and the presence and removal of a focus of infection.

2.3.2 Combination therapy arm - In addition to standard treatment, an intravenous β-lactam will be added for the first 7 calendar days following randomisation (day 1 being the day of randomisation – hence patients will receive 6–7 days of β-lactam). This β-lactam will be flucloxacillin 2g q6h IVI in Australia and New Zealand, and cloxacillin 2g q6h IVI in Singapore and Israel (where flucloxacillin is not generally available). For those with a history of minor allergy to any penicillin (rash or unclear history, but not anaphylaxis or angiooedema), it will be cefazolin 2g q8h IVI. For haemodialysis patients, it will be cefazolin 2g three times per week post dialysis. If flu/cloxacillin is temporarily unavailable at a study site, then cefazolin can be used in its place, even in the absence of β-lactam allergy or of haemodialysis. However, this is not the preferred option, so should only occur if there are genuine supply issues.

2.3.3 Criteria for discontinuing or modifying allocated interventions

2.3.3.1 Adjusting for renal function

The starting maintenance vancomycin dose will be dosed as per the following table from TG15 Antibiotic:

Table 1 - Adjustment of starting maintenance vancomycin doses according to renal function (for a 70kg adult)

Creatinine clearance (mL/min)	**Starting maintenance dosage**	**Timing of trough (predose) plasma concentration measurement**
more than 90	1.5 g 12-hourly	before the fourth dose
60 to 90	1 g 12-hourly	before the fourth dose
20 to less than 60	1 g 24-hourly	before the third dose
less than 20	1 g 48-hourly	48 hours after the first dose
On haemodialysis [57]	25mg/kg	Immediately prior to next haemodialysis session

For those on haemodialysis, blood is to be taken at the commencement of each dialysis session and sent for an urgent vancomycin level. The dose as per the nomogram (Table 2) is then administered and timed for the vancomycin infusion to be completed simultaneously with the completion of dialysis.

Table 2 – Adjustment of ongoing vancomycin doses for those on haemodialysis [57]

Vancomycin level (mg/L)	**Next vancomycin dose (mg)**
<5	2000
5–15	1500
15–20	1000
20–25	500
>25	0

Other antibiotic doses will be adjusted for renal function as per Table 3 ([flu]cloxacillin), Table 4 (cefazolin), and Table 5 (daptomycin).

Table 3 – Adjustment of (flu)cloxacillin doses according to renal function

GFR	**Flucoxacillin Dose**	**Cloxacillin dose**

>50ml/min	2g q6h IVI	2g q6h IVI
11-50 ml/min	2g q6h IVI	2g q6h IVI
≤10 but not on haemodialysis	1g q8h IVI	2g q6h IVI
On continuous renal replacement therapy	2g q6h IVI	2g q6h IVI
On haemodialysis	Not for flucloxacillin (Cefazolin 2g 3x/week)	Not for cloxacillin (Cefazolin 2g 3x/week)

Table 4 – Adjustment of cefazolin doses according to renal function

GFR	**Cefazolin Dose**
>40ml/min	2g q8h IVI
21-40 ml/min	1g q8h IVI
≤20 but not on haemodialysis	1g q12h IVI
On continuous renal replacement therapy	2g q12h IVI
On haemodialysis	2g 3x/week post dialysis

Table 5 – Adjustment of daptomycin doses according to renal function

GFR	**Daptomycin Dose**
>50ml/min	6-10mg/kg IVI q24h
11-50 ml/min	6-8mg/kg q24h IVI
≤10 but not on haemodialysis	8mg/kg q48h IVI
On continuous renal replacement therapy	8mg/kg q48h IVI
On haemodialysis	8mg/kg q48h IVI, dose after dialysis

2.3.3.2 Change of "backbone drug" (vancomycin or daptomycin) after randomisation

Whilst unnecessary changes will be discouraged, it will be left to the treating clinician's discretion to switch these drugs if needed. The most likely situation where a switch might occur is if a patient is commenced on vancomycin, but the vancomycin MIC of the MRSA isolate is later determined to be ≥1.5 µg/ml. It is controversial in this situation if one should switch to daptomycin or continue with vancomycin. If a patient develops a suspected adverse drug reaction to daptomycin (e.g., raised serum creatine kinase CK) or vancomycin (e.g., rash), then clinicians may also choose to switch.

If a patient's backbone drug is switched, they will still be analysed in the group to which they were randomised (standard or combination). In the subgroup analysis (Vancomycin vs Daptomycin), they will be counted as the drug which they received the majority of doses of in the first 7 days post randomisation. For example, if a patient switches from vancomycin to daptomycin on day 3, they will be counted in the daptomycin group.

Switching to a backbone drug other than vancomycin or daptomycin will be discouraged. If a participant is switched to another non-β-lactam backbone drug (e.g., linezolid, cotrimoxazole, clindamycin, tigecycline, quinupristin-dalfopristin) this will be a protocol deviation, but they will continue on the study and will still be analysed in the group to which they were randomised (standard or combination). Switching the backbone drug to ceftaroline (a β-lactam with anti-MRSA activity) at any time in the first 90 days will be a protocol violation, but the participant will remain in the study and be analysed in the group to which they were randomised, but will likely be excluded from the per-protocol analysis (in accordance with criteria in section 2.10)

2.3.3.3 β-lactam use after randomisation

Standard therapy group: The use of all β-lactams will be prohibited in participants allocated to the standard therapy group for the first 14 days after randomisation, and will be discouraged for the entire duration of IV vancomycin/daptomycin. If a patient develops an indication for broadening of antibiotic therapy, the site principal investigator should recommend a non-β-lactam agent (e.g., clindamycin, quinolones). If a patient allocated to the standard therapy group receives a β-lactam within the first 14 days post randomisation in spite of this, this will be recorded as a protocol violation, but the patient will remain in the study.

Combination therapy group: The β-lactam may be switched (within the limits of flucloxacillin, cloxacillin and cefazolin) by the patient's clinician if there is a serious clinical need to do so (e.g., suspected allergy or toxicity). The β-lactam must be ceased at the end of day 7. The β-lactam should not be switched to a broader spectrum agent (such as piperacillin/tazobactam or meropenem) during the first 7 days of randomisation. If a patient allocated to the combination therapy group

receives a β-lactam other than flu/cloxacillin or cephazolin within the first 7 days post randomisation, this will be recorded as a protocol violation, but the patient will remain in the study.

2.3.4 Strategies to improve adherence to protocol

2.3.4.1 Training of site PIs

All site PIs will be trained in the study protocol, SOPs and their reporting requirements by the project manager, a study chief investigator or delegate, prior to the site being opened for recruitment. All site PIs will complete a computer-based training course in Good Clinical Practice.

The project manager or delegate will have regular phone contact with all enrolling site investigators.

2.3.4.2 Documentation in patient's medical record and bedside chart

A sticker will be placed in the patient's medical record (one on the progress notes on the day of randomisation, and one in the front inside cover of the medical record ["old notes"] if one exists). This sticker will alert clinicians that the patient has been randomised to the CAMERA2 study, with a brief explanation of the study, and confirmation that the participant (or the person responsible) has provided written informed consent.

A copy of the study synopsis will be placed in the bedside chart (observations and drug chart) of the patient. A checklist of study procedures will also be placed in the bedside chart.

For sites with electronic medical records and/or prescribing, an electronic "sticker" will be used, and appropriate annotations will be made to the electronic drug chart.

2.3.4.3 Checking of drug charts

The medication chart (be it paper or electronic) will be checked regularly by the site PI or their delegate (registrar or research nurse) for the first 14 days whilst an inpatient to ensure adherence to the study protocol.

2.4 Outcomes

2.4.1 Primary outcome – Complication-free 90 day survival

The primary outcome is a composite outcome measure with 4 components, to be assessed 90 days after randomisation (randomisation = day 1). These are any of:

1. All-cause mortality
2. Persistent bacteraemia at day 5 or beyond
3. Microbiological relapse - positive blood culture for MRSA at least 72 hours after a preceding negative culture

4. Microbiological treatment failure. Positive sterile site culture for MRSA at least 14 days after randomisation. This includes pus from deep tissue or organ abscesses, synovial fluid, blood or other normally sterile sites. It does not include urine, sputum or superficial swabs.

2.4.2 Secondary outcomes

All outcomes below refer to the time period from randomisation to day 90

1. All-cause mortality at days 14, 42 and 90 days
2. Persistent bacteraemia at day 2
3. Persistent bacteraemia at day 5 or beyond
4. Acute kidney injury defined as ≥stage 1 modified RIFLE criteria (1.5-fold increase in the serum creatinine, or glomerular filtration rate (GFR) decrease by 25 percent) at any time within the first 7 days, OR new need for renal replacement therapy at any time from days 1 to 90. This endpoint does not apply to participants who were already on haemodialysis at randomisation.
5. Microbiological relapse - positive blood culture for MRSA at least 72 hours after a preceding negative culture
6. Microbiological treatment failure. Positive sterile site culture for MRSA at least 14 days after randomisation
7. Duration of intravenous antibiotic treatment
8. Direct health care costs

2.4.3 Rationale for these outcome measures

2.4.3.1 Primary outcome measure

Whilst the key outcome of interest is all-cause mortality, a study powered to detect a clinically meaningful 5% absolute mortality reduction would require over 2000 participants, which is beyond the capacity of this study, and would be used as the primary outcome of a possible future trial depending on the results of the current study. Hence a composite outcome measure incorporating mortality and microbiological measures of treatment failure has been chosen. Clinical assessments of treatment failure have been avoided due to their subjective nature. Since there exists no generally agreed upon outcome measure for *S. aureus* bacteraemia trials, we generated the primary outcome measure according to the following principles – we chose an outcome that was:

- patient-centered and clinically meaningful
- as objective as possible
- simple to measure with as small a departure as possible from usual clinical processes
- consensus from a group of experts (the chief investigators) following repeated cycles of

assessment, discussion and reassessment.

- consistent with outcomes used in contemporary RCTs (e.g. the ARREST trial of adjunctive rifampicin for SAB [58]).

The 90-day post randomisation time point was chosen because the majority of participants will have completed their initial course of intravenous and oral antibiotic treatment by this time; using 28-day mortality may miss an important proportion of infection-related mortality and hence later time points are increasingly used [58].

2.4.3.2 Secondary outcome measures

Each component of the composite primary outcome measure has been included as a secondary outcome measure. In addition, we have included acute kidney injury (defined according to the validated RIFLE criteria [59]). This is because several small studies have raised the possibility of vancomycin plus β-lactam combinations being nephrotoxic [60,61], although both the cited studies involved piperacillin-tazobactam as the β-lactam.

2.5 Trial Procedures

2.5.1 Participant timeline

See figure 2 and table 6

Table 6. Schedule of visits, data collection and follow-up.

Visit Day	Pre-Screen	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Days 8-13	Day 14	Day 15-41	Day 42	Days 43-89	Day 90
Check eligibility	x													
Informed consent		x												
Demographic data		x												
Clinical details		x												
Randomise		x												
Ensure Blood cultures are ordered by treating clinicians	x		x			x		(x)[a]	(x)[a]	(x)[a]	(x)[a]			
Ensure FBC, EUC, LFTs, CRP and Vancomycin levels are ordered by treating clinicians			x			x		x	As clinically indicated					
Vancomycin OR Daptomycin doses		x	x	x	x	x	x	x	x	x	(x)[b]	(x)[b]	(x)[b]	
Combination therapy group: B-lactam doses		x	x	x	x	x	x	x						
Clinical progress assessment			x			x		x		x	Weekly whilst in hospital			x
Vital status (alive)		x		x		x		x		x		x		x
Additional data review									x	x	x	x		x

a. If blood cultures are still positive at day 5, they should be recollected on day 7 and then every 48h until negative.

If they are negative on day 5, they should be recollected if there is any clinical suspicion of relapse (eg. recurrent fever)

b. Minimum recommended duration of vancomycin or daptomycin is 14 days – clinicians may choose to give longer courses, typically up to 42 days but sometimes longer

2.5.2 Screening

Study visit window: Within 72 hours of the positive blood culture collection.

All patients with a positive blood culture for MRSA will be referred by the microbiology laboratory to the site investigator or their delegate (sub-investigator or properly qualified research nurse), as soon as MRSA is identified. This includes identification by rapid methods, including but not limited to PCR-based methods and latex-agglutination methods, in addition to traditional phenotypic methods. Direct identification from positive blood culture bottles is acceptable. The following information will be transcribed onto a screening log by a member of the study team at the time of referral: date and time the blood culture was collected, the hospital record number (HRN), name & date of birth (DOB) of the patient and date and time the referral was received. The site investigator or their delegate will approach the doctors of the treating team and ask permission to approach the patient or their surrogate decision maker (next of kin, NOK) for potential recruitment onto the study and record their response in the screening log. The screening CRF (CRF1) will be filled in for all patients with a blood culture positive for MRSA, but only for the index blood culture (as defined in 2.5.2 below). The site investigator will do this using information gathered from the medical record and the patient's treating clinician. If the patient is clearly not eligible (e.g., age < 18 years), they will not be approached for consent. If the patient appears to be possibly eligible, they will be approached for an informed consent discussion.

The "index" blood culture will be used to assess eligibility (i.e., whether the patient is within 72h of collection). This means the first positive blood culture for the current clinical episode. A clinical episode begins when a patient develops signs or symptoms of infection and ends when all treatment has been ceased, all symptoms and signs have resolved and at least 90 days have passed.

2.5.3 Informed Consent

An informed consent discussion will be held with each participant or, for those not competent to make their own decisions (e.g., unconscious), their person responsible. "Person responsible" consent will only be used in jurisdictions where it is allowed, and where the site has research governance approval to do so. The consent process will be carried out by a site investigator or their suitably trained delegate. The information for the discussion will be provided in written and oral formats that have been approved by the HREC and in a language comprehensible to the potential participant or their person responsible, using interpreters if necessary. In the case where the person responsible is not physically present within the recruitment time frame, the consent discussion can take place over the telephone, and the person responsible can give verbal consent via the telephone. The person conducting the consent discussion should then document this in the medical record. The person responsible will then need to sign the consent form as soon as possible afterwards.

The information presented will detail the exact nature of the trial and what is expected of the participant including any risks or benefits in taking part. It will be clearly stated that the participant is free to withdraw from the trial at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal. The participant or person responsible will be allowed time to ask questions. In the event that a participant who was not competent when initially recruited into the study becomes competent to make their own decisions, the participant will have the study explained to them and an opportunity to consent to remaining in the study or to withdraw. The site investigator or delegate will regularly check to see if the participant becomes competent.

The participant or person responsible will personally sign and date the latest approved version of the consent form, as will the site investigator or their delegate who conducted the consent discussion. If one was used, an interpreter will also sign and date the consent form. Where the participant is illiterate, they can sign the consent form with their mark rather than their signature, as long as a witness is able to sign also. A copy of the information statement and consent form will be provided to the participant. No trial related procedure will be undertaken before documented informed consent is obtained. An original copy of the consent form will be retained at the recruitment site by the site investigator.

2.5.4 Randomisation and blinding

Prior to proceeding with randomisation, the site investigator or their delegate will ensure that documented informed consent has been obtained and that the participant is eligible to be enrolled. To randomise the participant the site investigator or their delegate will log onto the web-based interactive randomisation system (IXRS) and enter the details required to obtain the treatment allocation assigned for that participant. The participant will be assigned a randomisation number and group allocation. Compulsory fields required prior to randomisation are screening number, confirmation of eligibility, age, hospital record number, confirmation of consent, recruitment site and haemodialysis status.

Participants will be randomised in a 1:1 ratio to the standard or combination treatment arms, using a web-based IXRS, available 24 hours per day, 7 days per week. Randomisation will be stratified by site, and by receipt of haemodialysis, and will be in permuted blocks of variable block size. The randomised sequence allocation will be stored on the secure server of the web-based IXRS provider, and will not be available to any investigators or member of study staff. This will be an open-label study, but the investigators assessing the primary outcomes (see below – blinded outcomes

assessment committee) will be blinded to treatment allocation. As this study is open-label, in the event of medical emergency the treating clinicians of all study participants will already know whether or not the participant is receiving the study drug (β-lactam) and hence there is no unblinding procedure necessary.

2.5.5. Day 2–90

The site PI or their delegate will make contact (either by phone or preferably in person on the ward) with the treating team on at least days 2, 5 and 7, then weekly while the patient remains an inpatient, with the exception of weekends and public holidays. The purpose of this contact is to check compliance with the protocol in terms of study drug prescribing and ordering of routine clinical blood tests.

Standard Operating Procedures (SOPs) will contain step by step details on how to recruit patients and collect data.

2.5.6 Endpoint assessment

The composite primary endpoint will be assessed by a blinded endpoint adjudication committee. This committee will consist of three infectious diseases physicians (IDPs), to be appointed by the trial management committee. This committee will be provided with an extract of study data that does not contain patient identifiers, and does not contain any mention of treatment allocation or any detail about antibiotic treatment of any kind, but does contain:

1) Demographic details (such as age and sex)

2) Comorbidities

3) Clinical details (including focus of infection, SOFA scores, echocardiography results)

4) Date and result of all blood cultures taken during days 1–90

5) Date and result of all other available clinical cultures taken from days 1–90 (e.g., cultures of aspirated pleural fluid or pus).

6) Vital status at day 90 and date of death if applicable.

The members of the committee may request more information if needed, but this will only be provided if it is available and does not provide direct or indirect evidence of treatment allocation. Each of the three members of the committee will then independently determine if, in their view the patient has met the primary endpoint. If there is a discrepancy between the three assessments, the majority will determine the endpoint.

2.5.7 Discontinuation/Withdrawal of participants from trial treatment

The participants or NOK have the right to choose to withdraw from the study at any time and the investigator may discontinue a participant from the study or from treatment if deemed appropriate at any time. Reasons why a participant may be withdrawn from the study include, but are not limited to, participant or person responsible request, primary treating clinician's request, participant was enrolled and is ineligible (either arising during the study or was overlooked at time of screening and enrolment). Participants will not be withdrawn due to adverse events. The decision to withdraw a participant from the study must be discussed with the coordinating investigators.

If the participant or person responsible withdraws consent from participating in the study and also withdraws consent for collection of future information, no further evaluations will be performed, and no additional data will be collected. The sponsors may retain and continue to use any data or samples collected before such withdrawal of consent. Participants that abscond will continue to be followed, if possible, until the end of the trial to avoid missing data. Participants withdrawn from the treatment by the treating clinicians will continue to be followed up to the end of the trial to avoid missing data and will be used in the intention-to-treat analysis. This study has allowed for 10% of participant withdrawal. Withdrawn participants will not be replaced.

If a participant is withdrawn the reason will be recorded in the database.

2.5.8 End of trial

The end of trial will be the date the last participant has had the day 90 assessment CRF completed or the study window has concluded and the participant is lost to follow up.

2.6 Study timeline

This project will run for 5 years, with a predicted recruitment period of 3.5-4 years.

Table 7 – Study timelines

	2014		2015		2016		2017		2018		2019	
Finalisation of protocol												
Development of eCRFs												
Ethics applications												
Site preparations												
Recruitment												
Data cleaning and analysis												
Writing of paper(s)												

Justification of timeline

In our pilot CAMERA1 study, 380 MRSA-B participants were assessed for eligibility, of whom 106 were eligible (28%) and 60 were randomised (57% consent rate, 16% enrolment rate). The most common reasons for exclusion were (not mutually exclusive): i) >48h since index blood culture collection (127); ii) End stage renal failure (56); Treating team declined to enrol (41); Allergy to β-lactam or vancomycin (19); receiving a β-lactam which cannot be ceased or substituted (22); polymicrobial bacteraemia (12); age<18 years (15); immunosuppression (4) and previous enrolment in CAMERA (1). Based on our pilot data, extending the enrolment deadline to 72 hours and including renal failure would increase the expected proportion of eligible participants from 29% to 40%, which at 60% consent rate, would increase the overall enrolment rate from all MRSA-B to 24%. On this basis, our estimated 20% enrolment rate is conservative. Hence we propose to include 27 sites, with a total mean annual number of MRSA-B cases of ~653 (see table 1), of whom we would expect 261 (40%) to be eligible and 130 (50% of these) to be enrolled. Hence to achieve the target sample size of 440, we will need a minimum recruitment period of 3.4 years (assuming all sites start at the same time), and we have allowed up to 4 years for recruitment.

2.7 Sample size

We have estimated that the failure rate for the primary outcome in the control group will be 30% (as per data from the CAMERA1 study). We are aiming to detect a clinically meaningful absolute reduction in failure by 12.5%. The absolute risk reduction we want to detect is based on what is considered clinically significant – which is a subjective quantity, based on expert opinion. When CIs of CAMERA2 were asked about this, the answers ranged from 10 to 15%. Hence we have arbitrarily taken the midpoint of 12.5%, resulting in a sample size required of 438 (including 11.1% inflation for 10% drop out). A trial of 394 participants with complete data for the primary outcome will have 83% power to detect a statistically significant difference at the two-sided 5% level. We will therefore aim to randomise 440 participants to allow for ~10% drop out and have at least 394 participants for analysis.

2.8 Assignment of interventions

2.8.1 Allocation

Participants will be randomised in a 1:1 ratio to the standard or combination treatment arms, using a web-based interactive randomisation system, available 24 hours per day, 7 days per week (Spiral Software, Wellington, New Zealand).

Randomisation will be stratified by site, and by receipt of haemodialysis, and will be in permuted blocks of variable block size.

2.8.2 Allocation concealment

The randomised sequence allocation will be stored on the secure server, and will not be available to any investigators or member of study staff.

2.8.3 Implementation

A commercial provider of randomisation services (Spinnaker software) will generate the allocation sequence and store it on their secure servers. Participants will be enrolled by site principal investigators or their delegates (research nurse or co-investigator). The person enrolling the patient will, following obtaining written informed consent, obtain the treatment allocation by logging onto the web-based database and will then assign the allocated treatment to the patient.

2.8.4 Blinding

This will be an open-label study, but the investigators assessing the primary outcomes will be blinded to treatment allocation.

2.9 Data Management and Quality Assurance

Source data

Source documents are those where data are first recorded, and from which participants' case report form (CRF) data are obtained. These include but are not limited to hospital records both electronic and paper (which will include medical history, previous and current medications, any relevant radiography test, blood test results, haemodynamic parameters and medical correspondence) and paper or electronic clinic records (which will include vital status, recent medical history & relevant blood culture results). A further potential data source will be through telephone conversations with the study participant or person responsible or GP.

Storage and archiving of study documents (CRF's and consent forms) will be the responsibility of the site principal investigator and these will remain at the site of recruitment. All study participants will be allocated a unique number at time of screening (screening number), this screening number will be added to all the CRF's for that participant. The participants will also have their HRN recorded on the CRF's as this information will be required to ensure the correct medical record is accessed during medical record reviews.

Data Recording and Record Keeping

Data for this study will be recorded via a secure, Electronic Data Capture (EDC) web-based system. It will be transcribed by the site PI or their delegate from the paper CRFs onto the EDC. Data will be stored in a re-identifiable manner in the database, using a unique screening number for each patient. The database will contain validation ranges for each variable to minimise the chance of data entry errors. An audit trail will maintain a record of initial entries and changes made; reasons for change; time and date of entry; and user name of person who made the change. Data queries will be raised by the project manager and study monitor, and missing data or suspected errors will be raised as data queries and resolved prior to database lock and analysis. The database will contain in-line capability so that these queries and answers are logged as part of the audit trail.

For each potential participant screened (including those who are not eligible), the screening eCRF will be completed by the site PI or their delegate. For each participant enrolled, eCRFs must be completed. This also applies to records for those patients who fail to complete the study. The site PI should ensure the accuracy, completeness and timeliness of the data reported to the sponsor in the eCRFs and in all required reports. A comprehensive validation check program will verify the data and automatically generate discrepancies for resolution by the investigator. Manual discrepancies can also be raised if necessary.

In addition, for selected data fields, accurate and reliable data collection will be assured by verification of the eCRFs against the investigator's records by the study monitor (source document verification), and the maintenance of medication compliance will be captured in the CRF's from the participant's medication chart (source document) by the investigator.

2.10 Statistical methods

Statistical analysis plan

Data will be reported in accordance with the CONSORT guidelines for reporting of randomised trials. Proportions will be compared between treatment groups with Fisher's exact or χ^2 tests, and the absolute difference in proportions reported with corresponding 95% confidence intervals. All-cause mortality will be presented in a Kaplan-Meier graph.

The ***primary analysis*** of both primary and secondary endpoints will be according to modified intention to treat principles (all participants with data available for the endpoint will be analysed according to the treatment allocation, regardless of what treatment they received).

A ***secondary per-protocol analysis*** of all endpoints will be conducted. The per-protocol population is defined as 1) for the combination group: received at least 75% of β-lactam doses; 2) for the standard treatment group: received ≤1 defined daily dose of β-lactam; 3) has available day 90 data. For example, a patient who was allocated to flucloxacillin 2g QID for 7 days (28 doses in total), must receive at least 21 doses during the first 7 days to be included in the per-protocol population. A patient on haemodialysis three times per week who is prescribed cefazolin 2g post-dialysis, must have received at least 3 doses in the first 7 days (i.e., have missed no doses if dialysed 3 times on days 1-7 or a maximum of 1 dose if dialysed 4 times on days 1-7). We will perform the following subgroup analyses:

i) ***Standard treatment was daptomycin vs vancomycin***. This is because it is possible that daptomycin and vancomycin are not equivalent in terms of the primary outcome. Even though at least one previous RCT has directly compared them and found daptomycin to be non-inferior to vancomycin, there was a trend towards improved success with daptomycin for the MRSA subgroup [62]. Similarly, the synergistic effect of a β-lactam may differ depending on the backbone drug.

ii) ***Vancomycin MIC of primary isolate ≥1.5µg/ml, or <1.5 µg/ml***. Synergy between β-lactams and vancomycin or daptomycin appears to be more pronounced in isolates with higher vancomycin MICs. Conversely, higher vancomycin MICs have been associated with worse outcomes, including higher mortality [63]. The difference between the combination therapy group and the standard therapy group is likely to be larger (in the direction of benefit) in those with a higher vancomycin MIC.

iii) ***Participants receiving intermittent chronic haemodialysis compared with those who are not***. Haemodialysis participants may have worse outcomes from MRSA bacteraemia than those not on haemodialysis, and they will be receiving a different β-lactam regimen than others (Cefazolin 3 times per week rather than (flu)-cloxacillin 4 times daily). Hence the benefit of combination therapy may be smaller in those on haemodialysis.

iv) **Those who received >24 hours of β–lactam antibiotics within the 72 hours prior to randomization compared with those who did not**. The effect of any intervention for MRSA bacteraemia is likely to be greatest within the first 24–48 hours after onset. The benefit of combination therapy is likely to be smaller in those who have received β-lactams prior to randomisation, because of a dilution of effect (the control group having received the intervention for a time).

v) **Uncomplicated vs complicated SAB** (uncomplicated SAB defined as per IDSA guidelines: exclusion of endocarditis; no implanted prostheses; follow-up blood cultures performed on specimens obtained 2–4 days after the initial set that do not grow MRSA; defervescence

within 72 hours of initiating effective therapy; and no evidence of metastatic sites of infection)[14]. Complicated SAB participants have worse outcomes and longer durations of bacteraemia. The effect of combination therapy is likely to be larger in this group. Because we expect the combination therapy arm to result in a shorter duration of bacteraemia and thus fewer patients to have positive blood cultures at days 2–4, we will also use an *a priori* definition of uncomplicated SAB that does not include the day 2–4 blood culture criteria.

vi) **Participants recruited in Australia/New Zealand vs Singapore vs Israel.** We expect that ~50% of patients will be recruited from Singapore.

vii) **Those with baseline immunosuppression vs. those without.** These are different patient groups with regards to underlying comorbidities and risk for severe sepsis.

viii) **Participants with left-sided endocarditis vs. those without.** Those with left-sided endocarditis generally have a poorer prognosis than those without.

ix) **Participants with community-associated MRSA vs healthcare associated MRSA** (defined either genotypically or by non-multi (nmMRSA) vs multidrug-resistant (mMRSA) phenotype; nmMRSA defined as resistant to <3 classes of non β-lactam antibiotics, and mMRSA as ≥3 classes of non β-lactam antibiotics).

A simple ***health economic analysis*** will also be carried out, using the primary outcome measures for the trial to inform a modelling study. We will borrow cost and quality of life estimates from other studies/data sources.

Interim analyses and stopping guidelines

The DSMB will conduct an interim analysis after 220 patients have been randomised and followed for 90 days, OR 2 years following the date of the first patient randomised, whichever comes first.

The interim analysis will review outcome data and answer the following questions:

1. Are there any significant safety issues that may present an ethical issue in continuing the study? This may include adverse events, but also study conduct and protocol violations
2. Is there overwhelming data suggesting the superiority of one arm that may present an ethical issue in continuing the study?
    a) Using the Haybittle-Peto rule, and 90 day all-cause mortality as the outcome of interest, the study will be stopped early if there is a difference in 90-day mortality rate with a p-value of less than or equal to 0.001.
3. Are there any other factors that may impact on the feasibility / usefulness of the study? E.g., rate of enrolment, unexpected low rate of outcomes, unable to fund, protocol violations etc.

2.11 Monitoring and trial co-ordination

2.11.1 Trial co-ordination

This trial will be co-ordinated from the Menzies School of Health Research in Darwin (CIs Davis, Tong and Chatfield, and study co-ordinator#1), in collaboration with the Singapore Infectious Diseases Clinical Research Network (CI Lye and study co-ordinator#2). The study will also have input from the Australasian Society for Infectious Diseases (ASID) Clinical Research Network (CRN) and the Australian Kidney Trials Network (AKTN).

2.11.2 Data safety and monitoring board (DSMB)

An independent DSMB will be established to review the progress of the study and monitor adherence to the protocol, participant recruitment, outcomes, complications, and other issues related to participant safety. They will also monitor the assumptions underlying sample size calculations for the study and alert the investigators if they see substantial departures as the data accumulate.

The DSMB will be composed of experts in infectious diseases, biostatistics and clinical trials. The DSMB members will all be independent of the investigators (none of them will be chief investigators or site investigators).

The DSMB will make recommendations as to whether the study should continue or be terminated, consider participant safety or other circumstances as grounds for early termination, including either compelling internal or external evidence of treatment differences or feasibility of addressing the study hypotheses (e.g., poor participant enrolment, poor adherence).

2.11.3 Study monitoring

Study monitoring will be provided by the responsible monitor(s) at the Menzies School of Health Research (or designee) in accordance with the Monitoring Plan and "International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use" Good Clinical Practice.

The responsible monitor will visit each study site at least once per year and will be allowed, on request, to inspect the various records (source documents, paper CRFs, eCRFs and other pertinent data) provided that subject confidentiality is maintained in accord with local requirements.

It will be the monitor's responsibility to inspect the eCRFs throughout the study, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them. The monitor must verify that the subject received the study drug as randomised. The monitor should have access to laboratory test reports and other subject records needed to verify the entries on the eCRF. The site PIs agree to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved in a timely manner.

2.12 Safety

All trial medications are licensed for use in Australia, Singapore, New Zealand and Israel with established safety profiles.

2.12.1 Serious adverse events (SAEs)

A SAE is defined as any experience that:

- Results in death
- Is life-threatening
    - The term “life threatening” refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically may have caused death, if it were more serious.
- Results in unexpected prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity
- Is a medically important event or reaction

In this trial, expedited reporting of SAEs to HREC will only be required if they are thought by the reporting clinician (the site PI or their delegate) to be related to the intervention arm study drugs (possibly, probably or definitely as defined in 2.12.3). Such SAEs will be reported on the SAE Reporting Form by the site PI or delegate to the sponsor or delegate within 24 hours of the site study team becoming aware of it. The site PI will also report the SAE to the lead HREC for their site within 72 hours. If it is also an unexpected drug reaction, the Sponsor or delegate will report to the TGA Therapeutic Goods Administration (see 2.12.2).

2.12.2 Adverse drug reactions (ADRs)

Investigators will be asked to report all suspected adverse drug reactions (regardless of severity or seriousness) which are thought to be related to study drugs in both intervention and control arms (including vancomycin, daptmoycin, flucloxacillin, cloxacillin and cefazolin). These data will be collected routinely on CRF5.

SUSARs (Suspected Unexpected Serious Adverse Drug Reactions)

ADRs which are serious (as defined for SAEs above) AND are unexpected (as defined by not being listed as an adverse effect in the approved product information) AND are related to the intervention arm study drug (i.e., the β-lactam) will qualify for expedited reporting to the sponsor. As for SAEs, the site PI or their delegate will also report the SUSAR to the HREC within 72 hours. In addition, the sponsor will report the SUSAR to the TGA within 7 calendar days for fatal and life-threatening unexpected serious adverse drug reactions, and within 15 calendar days for other serious adverse drug reactions. All overseas sites must adhere to their country's regulatory reporting guidelines, any reports or actions they receive from their country's regulatory agency in response to a report involving a participant on the CAMERA2 trial should be copied and forward to the sponsor within 72 hours of receipt.

2.12.3 Causality

The principle site investigator will make a judgement regarding whether an adverse event is clinically significant and whether or not it is related to the allocated treatment. The degree of certainty with which an adverse event is attributable to treatment or an alternative cause will be determined by how well the event can be understood in terms of:

- Temporal relationship with the administration of the treatment or cessation of treatment
- Reactions of a similar nature previously observed in the individual or others following treatment

The relationship of the adverse event to treatment will be specified as follows:

Not related	In the PI's opinion, there is not a causal relationship
Unlikely	The temporal association between treatment and the adverse event is such that treatment is not likely to have any reasonable association.
Possibly	The adverse event could have been caused by treatment.
Probably	The adverse event follows a temporal sequence from the time of treatment and cannot be reasonably explained by the known characteristics of the subject's clinical presentation/history.
Definitely	The adverse event follows a reasonable temporal sequence from the time of treatment or reappears when the treatment is repeated.

2.12.4 Non-expedited reporting of adverse events and adverse drug reactions

In addition to the expedited reporting described above, a summary of all adverse drug reactions (to any of the study drugs including vancomycin, daptomycin or the beta-lactams), including SAEs and SUSARs, will be provided to the HREC and DSMB on a regular basis for review, with the frequency determined by each HREC's policy.2.12.5

2.12.5 Summary of expedited reporting of adverse events and adverse drug reactions:

In summary: SAEs and SUSARs <u>not</u> thought to be related to the study drug (e.g. death from overwhelming S. aureus sepsis) do not need to be reported in this trial. SAEs thought to be possibly, probably or definitely related to beta-lactam study drug (e.g. an anaphylactic reaction to flucloxacillin) must be reported by the site PI or their delegate to the sponsor (in this case the project manager and CIs at Menzies) within 24 hours, and to the HREC within 72 hours of their becoming aware of it. If it is an expected side effect (i.e., one listed in the product information, such as allergic reaction or diarrhoea), it does not need to be reported to the TGA. If it is both unexpected and serious, it needs to be reported to the TGA within 7 days (fatal or life threatening) or 15 days (other).

2.13. Ethical considerations

2.13.1 General ethical considerations

The study will be conducted according to the declaration of Helsinki, the Australian National Health and Medical Research Council (NHMRC) criteria for the ethical conduct of research in humans and the principles of Good Clinical Practice [64].

All antimicrobials in this study are registered for use in Australia, Singapore, NZ and Israel. The intervention (the addition of β-lactam to standard therapy) is unlikely to cause harm, and has proven safe both in published human studies and in our own pilot RCT. Furthermore this combination is routinely used in participants with SAB prior to the availability of antibiotic susceptibility results. MRSA-B is a common condition whose outcomes remain unacceptable with current therapies and this fact along with the strong *in-vitro* and *in-vivo* signals justify the conduct of this RCT. Written informed consent will be sought from all participants; in some jurisdictions, consent will be sought from a surrogate decision maker if the patient is not competent to consent. Approval will be sought from relevant human research ethics committees (HRECs) for all sites.

The study protocol, information statements, consent forms, and any other documents required for ethics approval will be submitted to the relevant Human Research Ethics Committees for approval before the study commences. Each HREC reviewing the protocol must be properly constituted as per the country's regulatory requirements (according to NHMRC requirements for Australian sites) and have the capacity to review the study. Approvals must specify the study title, version numbers, and

identify all documents reviewed and state the date of review. No amendments to, or deviations from, the protocol must be initiated without prior written approval from the relevant HREC. The exceptions to this are:

- administrative aspects that have no bearing on subjects;
- the need to address regulatory requirements; and/or,
- the need to eliminate immediate hazards to the subjects.

The investigator will inform the HREC of the following:

- all protocol amendments, informed consent changes or revisions of other documents originally submitted for review
- serious and/or unexpected adverse events attributable to study beta-lactams
- new information that may affect the safety of the subjects or the proper conduct of the trial
- annual updates of study progress
- termination of the study including provision of a final study report.

2.13.2 Informed consent. See section 2.5.3.

2.14 Regulatory approvals

Even though all products are licensed for use in Australia and Singapore, the β-lactams will be used outside their approved indication. Hence a Clinical Trials Notification (CTN) will be lodged with the Therapeutic Goods Administration (TGA) for all Australian sites. For Singapore sites, this protocol and the associated informed consent documents will be reviewed and approved by the National Healthcare Group DSRB (for National University and Tan Tock Seng Hospitals), Singhealth CIRB for Singapore General Hospital, and the Singapore Health Science Authority prior to initiation of study procedures.

2.15 Access to data

The trial steering committee will be the custodians of the final trial dataset. No-one outside the trial steering committee will be given access to the data without the permission of the trial steering committee. No identifying data will be given to any third parties at any stage. Following study close out and locking of the database, it will be stored on the servers of the Menzies School of Health Research.

2.16 Dissemination policy

The trial results will be communicated to all site investigators prior to publication or presentation. The trial results will be presented at national and international scientific conferences. The trial results will also be submitted for publication to a peer reviewed scientific journal, irrespective of the results. A plain-language summary of the trial results will be made available to individual participants upon request.

The first draft of the manuscript will be written by CIs Davis and Tong, and subsequent drafts will have input from the rest of the study steering committee (meaning all named Chief Investigators and members of the trial management committee) and the CAMERA study group (which is comprised of all CIs and site PIs). The Australian Society for Infectious Diseases Clinical Research Network (ASID CRN) steering committee will be asked to provide feedback on the manuscript prior to submission for publication. The decision where to publish will be made by the study steering committee. The authorship of the paper will include all of the Steering Committee who meet ICJME criteria for authorship. Contributions of other study participants will be recognized by the following language at the end of the named authors' list: " . . .and the CAMERA2 study group for the ASID Clinical Research Network". The CAMERA2 study group will consist of all named site investigators, and will be listed in the collaborators section of the paper.

3.1 Plans for biological specimens

3.1.1 Blood sample collection

Blood cultures will be collected on days 2 and 5 (and every 48 hours thereafter whilst they remain positive) into standard blood culture bottles using the recommended volume of blood. In addition, an EDTA tube (4-6ml) and a Lithium Heparin tube (4-6ml) will be collected for FBC (EDTA tube) and LFTs, EUC, CRP (LiHep tube) on the same days. The clinical team will be asked to arrange this as part of usual care, but if necessary, the research team will make sure this blood is collected and sent.

3.1.2. Microbiology laboratory procedures

All blood cultures which flag positive will be processed as per the local laboratory's usual procedures. The study does not mandate the use of rapid molecular tests, or of any particular identification method.

3.1.3 Storage and testing of biological specimens

All bacterial isolates will be frozen and stored as per standard laboratory practice at each site. Most laboratories store all sterile site isolates routinely, but to ensure availability of relevant isolates for study procedures, the microbiology laboratory of each site will be asked to freeze and store the

isolate from the index blood culture and any subsequent cultures growing MRSA during the first 90 days after randomisation for all CAMERA2 participants. These will later be transported for archiving in -80 degrees centigrade freezers at the Menzies School of Health Research laboratory. Isolates will be transported to Menzies in batches at four specified time points in the study – 90 days after the recruitment of participant # 100, 200, 300 and 440. Isolates will be identified by their CAMERA study number and local laboratory specimen ID number. They will be transported either as colonies subcultured onto agar slopes, or as cotton swabs which have picked up an individual colony of a pure subculture of the organism.

Once recruitment is complete, the complete set of isolates will undergo testing that may include:

- Vancomycin MIC by ETest
- Oxacillin MIC by ETest
- Daptomycin MIC by Etest
- Susceptibility to other antimicrobials using standard methods
- Staphylococcal strain typing and analysis of resistance determinants using a combination of molecular methods
- *In-vitro* synergy testing comparing several methods (see separate synergy sub-study protocol)

3.2 Trial Management Committee

The trial management committee will include:

Joshua Davis, Steven Tong, Jane Nelson, David Chien Lye, Sophia Archuleta, Dafna Yahav, Alan Cass (representing the Menzies School of Health Research), David Paterson (representing the ASID Clinical Research Network) and Matthew Roberts (representing the Australian Kidney Trials Network).

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Appendices 1: Summary of protocol changes

Version 2.0, 11/06/2015 to version 2.1

Section	Original text in version 2.0 (underlined represents deleted text)	Revised Text in version 2.1 (underlined represents added text)	Reason for change
Cover page *Date and version*	V 2.0 11/06/2015	V2.1 11/02/2016	To reflect revised version of protocol
Cover page *& page 43 TMC* *Inclusion of an Investigator & the affiliated institution*		Dr Dafna Yahav Rabin Medical Center, Beilinson Hospital	To update investigators with the inclusion as Israel sites
Cover page *Inclusion of a funding source*		National Medical Research Council, Singapore Project Grant # CTGIITL14Nov001	Additional funder
Inclusion Criteria *(page 6, page 18)*	Likely to remain as inpatient for 7 days following randomization	Likely to remain as inpatient for 7 days following randomization or an outpatient receiving haemodialysis and is accessible for follow up by the site PI).	To be more inclusive of participants that are receiving dialysis.
Abbreviations *(page 10)*		NMRC National Medical Research Council, Singapore	
Background and Rationale *(page 10 & 11)*	**1.2.1 Overview** *S. aureus* causes a broad range of infections, ranging from superficial skin infections, deep skin and tissue abscesses and bone infections, to invasive bloodstream infections. Widespread resistance to the potent β–lactam class of antibiotics (e.g., flucloxacillin) makes treatment of such infections considerably more difficult, as	**1.2.1 Overview** *Staphylococcus aureus* (*S. aureus)* is one of the most important bacterial pathogen of humans, and causes a broad range of infections, ranging from superficial skin infections, deep skin and tissue abscesses and bone infections, to invasive bloodstream infections. Methicillin-resistant *S.*	To make text clearer and more precise.

	therapies for MRSA are either less effective (e.g., vancomycin) or much more expensive (e.g., daptomycin) than β–lactams. Alternative therapies, including novel combinations of existing agents, are therefore urgently required to treat MRSA-B, each episode of which results in a mortality of ~25% [1].	*aureus* (MRSA) is resistant to the mainstay of *S. aureus* therapy, the anti-staphylococcal penicillins (such as flucloxacillin) and is hence more difficult to treat. Therapies for MRSA are either less effective (e.g., vancomycin) or much more expensive (e.g., daptomycin) than the anti-staphylococcal penicillins. Alternative therapies, including novel combinations of existing agents, are therefore urgently required, particularly to treat invasive MRSA infections, each episode of which results in a mortality of ~25% [1].	
Background and Rationale *(page 11)*	This high mortality, both in Australia and in resource limited settings where SAB is common and infection control practices are suboptimal, is a key reason for our proposed RCT.	This high mortality, not only in Australia, Singapore, New Zealand and Israel, but also in resource limited settings where SAB is common and infection control practices are suboptimal, is a key reason for the current described randomised controlled trial (RCT).	Update protocol with status as the trial is current.
Background and Rationale *(page 11)*	**MSSA-B**	methicillin-susceptible *S. aureus* bacteremia (MSSA-B)	Expand acronym
Background and Rationale *(page 11 – 12)*	In addition, strains of MRSA with decreased susceptibility to vancomycin (hVISA) are beginning to emerge worldwide [11]. In recent years, several alternative agents to vancomycin have become available for the treatment of MRSA bacteraemia, including linezolid, daptomycin, tigecycline and ceftaroline	In addition, strains of MRSA with decreased susceptibility to vancomycin (heterogenous vancomycin intermediate resistance *S. aureus* [hVISA]) are beginning to emerge worldwide [11]. In recent years, several alternative agents to vancomycin have become available for the treatment of MRSA bacteraemia, including linezolid, daptomycin and ceftaroline	Expand acronym

Background and Rationale *(page 12)*		Ceftaroline resistance is an additional concern with a recent Australian study finding overall resistance rates of 17% amongst MRSA and 41% in sequence type 239 MRSA .	Updating background with recent publication results
Background and Rationale *(page 12)*	None of linezolid, daptomycin and tigecycline demonstrate synergy with vancomycin against MRSA [18]	Neither linezolid nor daptomycin demonstrate synergy with vancomycin against MRSA [18].	Updating information
Background and Rationale *Page 13*	The one study that did not demonstrate synergy did not actually include any MRSA isolates. In this study, Joukhadar and colleagues tested 10 clinical isolates of MSSA and found evidence of neither synergy nor antagonism in any strain, both using fixed drug concentrations, and in a dynamic model simulating clinical dosing [29]		Protocol updated to reflect finding from recent literature review
Background and Rationale *Page 13*	Synergy has been reported with all ß-lactams tested (including cefazolin), but the largest effect has been observed with oxacillin and naficillin, which are unavailable in Australia but nearly identical chemically to flucloxacillin (part of the same antibiotic class "antistaphylococcal semi-synthetic penicillins")	Synergy has been reported with all ß-lactams tested (including cefazolin), but the largest effect has been observed with oxacillin and naficillin. Flucloxacillin is also considered in the same antibiotic class "antistaphylococcal semi-synthetic penicillins".	To make text clearer and more precise.
Background and Rationale *Page 14*	There are currently no published prospective controlled trials of vancomycin/β-lactam combination therapy in participants with MRSA bacteremia, but one observational study has recently been published [34].	There are few published data on β-lactam based combination therapy for MRSA in humans.	Protocol updated to reflect finding from recent literature review
Background and Rationale *Page 14*		In the only prospective clinical trial to date (CAMERA1), Davis et al. [37] randomised 60 patients with MRSA bacteraemia to	Protocol updated to reflect finding from recent literature review

		standard therapy with vancomycin alone, or to combination therapy with vancomycin and flucloxacillin. The study was conducted in seven centres in Australia and was open-labelled in design. Patients receiving combination therapy cleared bacteraemia at a mean of two days compared to three days with standard therapy (P=0.06).	
Objectives and hypothesis *Page 16*	We hypothesise that the addition of β-lactams to standard therapy will lead to synergistic bacterial killing and hence faster clearance of bacteria from the blood stream and other infected foci, and thereby reduce the risk of disseminated infection and death.	We hypothesise that the addition of β-lactams to standard therapy in adults with MRSA bacteraemia will lead to synergistic bacterial killing and hence faster clearance of bacteria from the bloodstream and other infected foci, thereby reducing the risk of disseminated infection and death.	To make text clearer and more precise.
Trial design *Page 16*	CAMERA is an investigator-initiated, multi-centre, parallel group, open-label, randomised controlled trial powered for superiority.	CAMERA is an investigator-initiated, multi-centre, parallel group, open-label, randomised controlled trial powered for superiority, which compares combination antibiotic therapy with standard antibiotic therapy in adults with MRSA bacteraemia.	To make text clearer and more precise.
Study setting *Page 18*	We are aiming to recruit from 23 Australian, 3 Singaporean and one New Zealand site	We are planning to recruit from 24 Australian, 1 New Zealand, 3 Singaporean and 2 Israeli acute care hospitals. Other sites may be added during the course of the study.	To update current status of trial.
Standard care arm *Page 20*		The choice of vancomycin or daptomycin will be at the clinician's discretion.	To make text clearer.
Combination therapy arm *Page 20*	This β-lactam will be flucloxacillin 2g q6h IVI in Australia, and cloxacillin 2g q6h IVI in Singapore (where flucloxacillin is not generally available).	This β-lactam will be flucloxacillin 2g q6h IVI in Australia and New Zealand, and cloxacillin 2g q6h IVI in Singapore and Israel (where flucloxacillin is not generally available).	Updating to include overseas sites

Combination therapy arm *Page 20*		If flu/cloxacillin is temporarily unavailable at a study site, then cefazolin can be used in its place, even in the absence of β-lactam allergy or of haemodialysis. However, this is not the preferred option, so should only occur if there are genuine supply issues.	For clarification
Adjusting for renal function *Page 21*	Following the initial vancomycin dose (25mg/kg), at subsequent haemodialysis sessions blood is to be taken at the commencement of dialysis and sent for an urgent vancomycin level	For those on haemodialysis, blood is to be taken at the commencement of each dialysis session and sent for an urgent vancomycin level	To make text clearer.
β-lactam use after randomisation Page 23 & 24		The β-lactam should not be switched to a broader spectrum agent (such as piperacillin/tazobactam or meropenem) during the first 7 days of randomisation. If a patient allocated to the combination therapy group receives a β-lactam other than flu/cloxacillin or cephazolin within the first 7 days post randomisation, this will be recorded as a protocol violation, but the patient will remain in the study.	
Training of site PIs *Page 23 & 24*	All site PIs will complete a computer-based training course in Good Clinical Practice conducted through ARCS Australia called 'Applied GCP Training for Investigational Sites and Sponsors'. The project manager or delegate will have regular phone contact with all enrolling site investigators, including after the enrolment of participants number 1, 2 and 5 at each site, and every 5 participants thereafter.	All site PIs will complete a computer-based training course in Good Clinical Practice. The project manager or delegate will have regular phone contact with all enrolling site investigators.	To be consistent for all site requirements
Primary outcome measure	Whilst the key outcome of interest is all-cause mortality, a study powered to detect a	Whilst the key outcome of interest is all-cause mortality, a study powered	To add clarity

Page 25	clinically meaningful 5% absolute mortality reduction would require over 2000 participants, which is beyond the capacity of this study, and would be used as the primary outcome of a possible future trial depending on the results of the current study.	to detect a clinically meaningful 5% absolute mortality reduction would require over 2000 participants, which is beyond the capacity of this study.	
Informed Consent *Page 28*		In the case where the person responsible is not physically present within the recruitment time frame, the consent discussion can take place over the telephone, and the person responsible can give verbal consent via the telephone. The person conducting the consent discussion should then document this in the medical record. The person responsible will then need to sign the consent form as soon as possible afterwards.	To include the ability for phone consent by persons responsible when they are not physically in the hospital
Informed Consent *Page 29*		Where the participant is illiterate, they can sign the consent form with their mark rather than their signature, as long as a witness is able to sign also.	To be inclusive of participants that have low literacy and numeracy
End point assessment *Page 30*	This committee will consist of three infectious diseases physicians (IDPs), to be appointed by the trial management committee. Members of the adjudication committee will not be investigators on the study (CIs or PIs).	This committee will consist of three infectious diseases physicians (IDPs), to be appointed by the trial management committee.	Removing unnecessary statements
Sample size *Page 32*	Hence if we arbitrarily take the midpoint of 12.5%, then the sample size required is 434 (including 10% inflation for drop out). A trial of 394 participants with complete data for the primary outcome will have 80% power to detect a statistically significant difference at the two-sided 5% level	Hence we have arbitrarily taken the midpoint of 12.5%, resulting in a sample size required of 438 (including 11.1% inflation for 10% drop out). A trial of 394 participants with complete data for the primary outcome will have 83% power to detect a statistically significant difference at the two-sided	Updating information after further review.

		5% level.	
Allocation *Page 32*		(Spiral Software, Wellington, New Zealand).	Added details
Data recording and record keeping *Page 34*	Data for this study will be recorded via a secure, Electronic Data Capture (EDC) web-based system using the eCRFs. It will be transcribed by the site PI or their delegate from the paper CRFs onto the eCRF (in no case is the paper CRF or eCRF to be considered as source data for this trial). Data will be stored in a re-identifiable manner in the database, using a unique screening number for each patient.	Data for this study will be recorded via a secure, Electronic Data Capture (EDC) web-based system. It will be transcribed by the site PI or their delegate from the paper CRFs onto the EDC. Data will be stored in a re-identifiable manner in the database, using a unique screening number for each patient.	Removing unnecessary information and add clarity
Statistical analysis plan *Page 35*	No assumptions will be made about those with missing data.		Updating post review
Statistical analysis plan *Page 35, i)*		Similarly, the synergistic effect of a β-lactam may differ depending on the backbone drug.	Updating post review
Statistical analysis plan *Page 36, vi)*	**Participants recruited in Australia/New Zealand vs Singapore**	**Participants recruited in Australia/New Zealand vs Singapore vs Israel.** We expect that ~50% of patients will be recruited from Singapore.	To include overseas sites
Study monitoring *Page 37*	The responsible monitor will contact and visit each site PI at periodic intervals and will be allowed, on request, to inspect the various records (source documents, paper CRFs, eCRFs and other pertinent data) provided that subject confidentiality is maintained in accord with local requirements.	The responsible monitor will visit each study at least once per year and will be allowed, on request, to inspect the various records (source documents, paper CRFs, eCRFs and other pertinent data) provided that subject confidentiality is maintained in accord with local requirements.	To update current information
Safety *Page 38*	All trial medications are licensed for use in Australia, Singapore and New Zealand with established safety profiles.	All trial medications are licensed for use in Australia, Singapore, New Zealand and Israel with established safety profiles.	To include overseas sites

SUSARs *Page 39*		All overseas sites must adhere to their country's regulatory reporting guidelines, any reports or actions they receive from their country's regulatory agency in response to a report involving a participant on the CAMERA2 trial should be copied and forward to the sponsor within 72 hours of receipt.	To be consistent with local and overseas regulatory compliance
Ethical Considerations *Page 40*	All antimicrobials in this study are registered for use in Australia and Singapore. The intervention (the addition of β-lactam to standard therapy) is unlikely to cause harm, and has proven safe both in published human studies and in our own pilot RCT.	All antimicrobials in this study are registered for use in Australia, Singapore, NZ and Israel. The intervention (the addition of β-lactam to standard therapy) is unlikely to cause harm, and has proven safe both in published human studies and in our own pilot RCT.	Include overseas sites
Ethical Considerations *Page 40*	Each HREC reviewing the protocol must be properly constituted according to NHMRC requirements and have the capacity to review the study	Each HREC reviewing the protocol must be properly constituted as per the country's regulatory requirements (according to NHMRC requirements for Australian sites) and have the capacity to review the study	Including requirements of overseas sites
Dissemination policy *Page 41*	The trial results will be communicated to all site investigators by teleconference prior to publication or presentation. The trial results will be presented at at least one national (e.g. the Australian Society for Infectious Diseases) and at least one international (e.g. the Interscience Conference on Antimicrobial Agents and Chemotherapy) scientific conference.	The trial results will be communicated to all site investigators prior to publication or presentation. The trial results will be presented at national and international scientific conferences.	To make text clearer and more precise
Trial Management Committee **Page 43**	Shirin Kalimuddin	Sophia Archuleta, Dafna Yahav	Update membership to reflect overseas participation.