Combination of Vancomycin and -Lactam Therapy for Methicillin-Resistant Staphylococcus aureus Bacteremia

A Pilot Multicenter Randomized Controlled Trial

Davis, Joshua S.; Sud, Archana; O’Sullivan, Matthew V N; Robinson, James O.; Ferguson, Patricia E.; Foo, Hong; Van Hal, Sebastiaan J.; Ralph, Anna P.; Howden, Benjamin P.; Binks, Paula M.; Kirby, Adrienne; Tong, Steven Y C; Tong, Steven; Binks, Paula; Majumdar, Suman; Ralph, Anna; Baird, Rob; Gordon, Claire; Jeremiah, Cameron; Leung, Grace; Brischetto, Anna; Crowe, Amy; Dakh, Farshid; Whykes, Kelly; Kirkwood, Maria; Menon, Mahesh; Somerville, Lucy; Subedi, Shrada; Owen, Shirley; O’Sullivan, Matthew; Liu, Eunice; Zhou, Fei; Robinson, Owen; Coombs, Geoffrey; Ferguson, Patrician; Pollet, Simon; Van Hal, Sebastian; Davis, Rebecca; CAMERA Study Group; CAMERA Study Group; CAMERA Study Group; CAMERA Study Group; CAMERA Study Group; CAMERA Study Group; CAMERA Study Group; CAMERA Study Group; CAMERA Study Group; CAMERA Study Group

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Combination of vancomycin and β-lactam therapy for methicillin-resistant Staphylococcus aureus bacteremia: A pilot multicenter randomized controlled trial

Joshua S. Davis1,2,*, Archana Sud3,4, Matthew V. N. O’Sullivan6,8, James O. Robinson6,7, Patricia E. Ferguson4,8, Hong Foo9, Sebastiaan J. van Hal10, Anna P. Ralph1,11, Benjamin P. Howden12,13,14, Paula M. Binks1, Adrienne Kirby15 and Steven Y. C. Tong1,11,*, for the Combination Antibiotics for MEthicillin Resistant Staphylococcus Aureus (CAMERA) study group8 and the Australasian Society for Infectious Diseases Clinical Research Network

1Global and Tropical Health Division, Menzies School of Health Research, Darwin, Northern Territory, Australia
2Department of Infectious Diseases, John Hunter Hospital, Newcastle, New South Wales, Australia
3Department of Infectious Diseases, Nepean Hospital and University of Sydney, Sydney, New South Wales, Australia
4Marie Bashir Institute for infectious Diseases and Biosecurity, University of Sydney, Sydney, New South Wales, Australia
5Centre for Infectious Diseases and Microbiology, Westmead Hospital, Sydney, New South Wales, Australia
6Department of Microbiology and Infectious Diseases, PathWest Laboratory Medicine-WA, Royal Perth Hospital & Fiona Stanley Hospital, Perth, Western Australia, Australia
7Australian Collaborating Centre for Enterococcus and Staphylococcus Species (ACCESS) Typing and Research, School of Veterinary and Life Sciences, Murdoch University and School of Biomedical Sciences, Curtin University, Perth, Western Australia, Australia
8Department of Infectious Diseases, Blacktown Hospital, Sydney, New South Wales, Australia
9Department of Microbiology and Infectious Diseases, Liverpool Hospital, Sydney, New South Wales, Australia

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We randomized 60 patients with methicillin-resistant *Staphylococcus aureus* bacteremia to treatment with vancomycin or vancomycin and flucloxacillin. The mean bacteremia duration was 3.0 and 1.9 days in the standard and combination therapy groups respectively (P=0.06). Further trials are warranted.

*JSD and SYCT contributed equally to this manuscript*

*Study group members listed in Acknowledgement section*
ABSTRACT

Background

In vitro laboratory and animal studies demonstrate a synergistic role for the combination of vancomycin and anti-staphylococcal β-lactams for methicillin-resistant Staphylococcus aureus (MRSA) bacteremia. Prospective clinical data are lacking.

Methods

In this open-label, multicenter, clinical trial, adults with MRSA bacteremia received vancomycin 1.5g intravenously (IV) twice daily and were randomly assigned (1:1) to flucloxacillin 2g IV 6 hourly for seven days (combination group) or no additional therapy (standard therapy group). Participants were stratified by hospital and randomized in permuted blocks of variable size. Randomization codes were kept in sealed, sequentially numbered, opaque envelopes. The primary outcome was the duration of MRSA bacteremia in days.

Results

We randomly assigned 60 patients to receive vancomycin (n=29), or vancomycin plus flucloxacillin (n=31). The mean duration of bacteremia was 3.00 days in the standard therapy group and 1.94 days in the combination group. According to a negative binomial model, the mean time to resolution of bacteremia in the combination group was 65% (95% confidence interval [CI] 41%, 102%; P=0.06) of that in the standard therapy group. There was no difference in the secondary endpoints of 28 and 90 day mortality, metastatic infection, nephrotoxicity, or hepatotoxicity.

Conclusions

Combining an anti-staphylococcal β-lactam with vancomycin may shorten the duration of MRSA bacteremia. Further trials with a larger sample size and objective clinically relevant endpoints are warranted.

Australian New Zealand Clinical Trials Registry; www.anzctr.org.au; ACTRN12610000940077.
BACKGROUND

Invasive methicillin-resistant *Staphylococcus aureus* (MRSA) infection imposes a substantial burden on healthcare systems throughout the world [1]. A recent national Australian study conducted over one year found that 450 of 1,994 episodes (24%) of *S. aureus* bacteremia were caused by MRSA [2]. More importantly, this study found that the all-cause 30-day mortality was 30% for MRSA compared with 17.7% for methicillin-susceptible *S. aureus* (MSSA; *p*<0.001). Studies from elsewhere have also reported infection with MRSA to have a higher mortality than MSSA [3]. The reasons for this difference in outcome are unclear, but may relate to differences in host factors [4] or to the limitations of vancomycin, the most commonly used antibiotic for invasive MRSA infections [5].

Compared with the anti-staphylococcal β-lactam oxacillin and its derivatives for treatment of MSSA infections, vancomycin demonstrates slower bacterial killing [6], poorer tissue penetration [7], slower clearance of bacteremia [8], and is associated with higher mortality [9]. In recent years, several alternative agents to vancomycin have become available for the treatment of MRSA bacteremia, including linezolid, daptomycin, and ceftaroline. Each of these has been found to be non-inferior to vancomycin for selected MRSA infections, but none have been shown to be superior for MRSA bacteremia [10] and are all associated with a high cost and/or a substantial risk of adverse effects.

An alternative strategy to improve outcomes from MRSA bacteremia is to combine vancomycin with a second agent, aiming for synergistic bacterial killing. Unfortunately, neither daptomycin nor linezolid demonstrate *in vitro* synergy with vancomycin. In contrast, at least 17 *in vitro* studies have demonstrated synergy of vancomycin combined with various β-lactams against MRSA and vancomycin-intermediate *S. aureus* [11]. These studies varied in their methodology (checkerboard synergy testing or time-kill curves) and the β-lactams used, but consistently found synergistic bacterial killing in the majority of tested strains. Animal studies have also demonstrated evidence of synergy between vancomycin and β-lactams [11]. The mechanisms for this observed synergy are not clear but may include β-lactam induced potentiation of host defense peptide activity against *S. aureus*. 
aureus [12], and a “see-saw” effect whereby reduced vancomycin susceptibility results in reduced transcription of mecA and increased susceptibility to β-lactams [13].

Thus there is considerable in vitro and limited animal model evidence to suggest that the combination of vancomycin and an anti-staphylococcal β-lactam may be more effective than vancomycin alone for MRSA bacteremia. In a retrospective analysis, Dilworth et al. described a higher rate of clearance of MRSA bacteremia in patients receiving empiric vancomycin plus a β-lactam compared to patients receiving vancomycin alone [14]. However, no prospective human clinical studies addressing this question have been performed. We therefore conducted a pilot randomized controlled trial (RCT), to assess feasibility, proof of concept and safety of this strategy.

Objectives

We hypothesized that the anti-staphylococcal β-lactam flucloxacillin combined with vancomycin would have a greater clinical efficacy compared to vancomycin alone in patients with MRSA bacteremia. The primary objective of this study was to determine and compare the average duration in days of MRSA bacteremia by allocated treatment.

METHODS

Study design and setting

We performed a pilot, multicenter, open-label, parallel group RCT at seven Australian hospitals (see acknowledgements section for details). Participants were recruited between January 2011 and May 2014. Institutional ethics approval was obtained at each site, and written informed consent was obtained from the participant or a surrogate decision maker prior to enrolment in the study. The trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12610000940077). The study protocol can be accessed at http://www.menzies.edu.au/page/Research/Projects/Staphylococcus/CAMERA Protocols/.

Participants

Participants were hospital inpatients, and were eligible if they met all of the following inclusion criteria: (1) A positive blood culture with MRSA; (2) Able to be randomized within 48 h of the first
positive blood culture being drawn; (3) Age ≥18 years; and (4) Judged as likely to remain as a hospital inpatient for at least seven days following randomization. Patients were excluded if they met any of the following criteria: (1) Previous history of significant allergy to β-lactams or glycopeptides (defined as previous type 1 hypersensitivity reaction to any β-lactams or glycopeptides, OR definite history of rash or serious non-type 1 hypersensitivity reaction to flucloxacillin, any penicillin, or vancomycin); (2) Renal failure with an estimated glomerular filtration rate <10 ml/minute; (3) Polymicrobial bacteremia; (4) Previous participation in the trial; (5) Known pregnancy; (6) Treating clinicians unwilling for patient to be enrolled; or (7) Patient currently receiving β-lactam therapy that could not be ceased or substituted for a non-β-lactam antibiotic.

**Randomization and masking**

Randomization was stratified by site, with a 1:1 treatment allocation using permuted blocks of variable size. Randomization codes were computer generated by the trial statistician who had no involvement in the day to day running of the trial. Allocation concealment was achieved using sequentially numbered opaque sealed double envelopes.

**Procedures**

Participants were randomized to receive either standard care (intravenous [IV] vancomycin dosed as per Australian Therapeutic Guidelines: Antibiotic, version 14 [15] with adjustment to maintain trough vancomycin levels of 15+/−3mg/L), or combination therapy (vancomycin plus IV flucloxacillin 2g four times daily for the first seven days post randomization). Both groups also received standard management of MRSA bacteremia as per the Australasian Society for Infectious Diseases 2006 guidelines [16]. The total duration of vancomycin was clinician-determined.

**Outcomes and measurements**

The primary endpoint was the duration of MRSA bacteremia in days. Secondary endpoints were: (1) Combined safety endpoint of nephrotoxicity (a rise in serum creatinine of >50% from baseline) or hepatotoxicity (plasma alanine transaminase or gamma-glutaryl transferase >2.5x the upper limit of
normal) within the first 10 days post randomization; (2) All cause 28-day and 90-day mortality; (3) Relapsed bacteremia during the index hospital admission (defined as a positive blood culture for MRSA ≥48 hours after a preceding negative blood culture); (4) Metastatic complications during the first 10 days; and (5) Requirement for ICU admission OR development of septic shock after randomization. Post-hoc secondary endpoints were duration of bacteremia >3 days and >7 days.

Blood cultures were collected daily for the first seven days of the study in all patients. Those with a positive blood culture on study day seven had ongoing blood cultures collected every 48 h until they became negative. Blood was also collected for routine analyses and vancomycin levels on days 1–7 inclusive and on day 10.

**Laboratory methods**

Oxacillin and vancomycin Etest® (bioMérieux, Marcy l’Etoile, France) minimum inhibitory concentrations (MICs) for each bacterial isolate were determined according to manufacturer’s instructions. We genotyped the isolates by a coagulase gene PCR-Restriction Fragment Length Polymorphisms assay, contour clamped Homogenous Electric Field Electrophoresis [17], binary typing [18], and spa sequence typing on selected isolates. These typing results were used to assign a predicted multi-locus sequence type (ST). The binary typing method was also used to determine the presence of the gene encoding Panton-Valentine leucocidin [18].

**Sample size**

A sample size of 60 was calculated to determine the duration of bacteremia in each group with a 95% confidence interval of +/- 2 days, assuming that the duration of bacteremia has a normal distribution with a standard deviation of 5.1 days (derived from Fowler at al. [19]) and a 10% correction factor for drop-out.

**Statistical Methods**

The primary efficacy analysis compared the duration of bacteremia from randomization by treatment group. We defined the duration of bacteremia as the time in days from randomization until one day following the last positive BC. For example, if the last positive BC was on the third day
following randomization, the duration of bacteremia was four days. If the BC obtained on the day of randomization was negative for bacterial growth, the duration of bacteremia was considered to be one day. As the duration of bacteremia was not normally distributed, but rather had a negative binomial distribution, we analysed these data using a generalized linear model with a negative binomial link. A negative binomial distribution can be thought of as the number of failures until the \( r \)th success. In our case it is the number of days of bacteremia before the first \( (r=1) \) day of persistently negative blood cultures [20]. The estimate of the effect of the combination therapy is the ratio of the mean duration in the two interventions. The duration of bacteremia was also assessed with a time to event analysis, including a Kaplan-Meier plot and a Cox model in which deaths, prior to the resolution of bacteremia, were treated as censored observations. A sensitivity analysis where the deaths were treated as a competing risk was also performed. Binary secondary outcomes were analysed using a chi-square test and the result presented as a relative risk. Secondary analyses were done on a ‘per-protocol’ data set where the following patients were excluded: If on standard therapy the participant received at least one dose of any \( \beta \)-lactam; or if on combination therapy the participant received less than 12 doses of flucloxacillin. The pre-specified subgroups for assessment of the primary outcome were: (1) Complicated vs uncomplicated SAB (complicated SAB defined as SAB with any of the following: the presence of indwelling intravascular devices [cardiac valves, implantable cardiac devices, intravascular grafts] or a prosthetic joint; ongoing fever \( >38.0^\circ \text{C} \) at days 3 and 4; a primary focus of infection of infective endocarditis, osteoarticular, intraabdominal or central nervous system infection; the presence of metastatic complications. Duration of bacteremia was not included in this definition as this was the primary endpoint of the study); (2) \textit{S. aureus} vancomycin MIC \( <1.5 \mu \text{g/mL} \) vs. \( \geq 1.5 \mu \text{g/mL} \); (3) received at least one dose of \( \beta \)-lactam in the 48h prior to randomization vs. did not; (4) health-care associated (HCA) MRSA (based on genotype; specifically ST239 and ST22) vs. community-associated (CA) MRSA (non ST239 or ST22) [21]. P values were 2 sided and no adjustment was made for multiple comparisons. Analyses used SAS, version 9.3 (SAS Institute, Cary, NC) or STATA, version 13.1 (StatCorp LP, College Station, TX). We did not have a
data monitoring committee. The trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12610000940077).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. JSD and SYCT had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

Between January 6 2011 and April 20 2014, 380 patients were screened for eligibility and 60 patients were enrolled (Supplementary Figure 1): 20 at Royal Darwin Hospital, 12 at Nepean Hospital, 8 at Westmead Hospital, 7 at Royal Perth Hospital, 6 at Blacktown Hospital, 5 at Liverpool hospital and 1 at Royal Prince Alfred Hospital. 29 were randomly assigned to vancomycin (standard therapy group), and 31 to vancomycin plus flucloxacillin (combination group). In the 7 days following randomization, 3 patients in the standard therapy group received ≥1 dose of a β-lactam, and 3 patients in the combination group received <12 doses of flucloxacillin. These were considered protocol violations, leaving 26 and 28 patients respectively in the per-protocol population.

The two treatment groups were well balanced in terms of baseline characteristics and focus of infection (Table 1). The mean serum vancomycin levels during the first 10 days were 19.2mg/L (standard deviation [sd] 5.1) and 20.3mg/L (sd 4.2), and the median time to achieve levels ≥15 mg/L were 3 (interquartile range [IQR] 2, 4.5) and 3 (IQR 2, 3) days, for the standard therapy and combination groups respectively. The distribution of MICs for vancomycin and oxacillin and genotypic characterization of isolates were also similar (Table 2, Supplementary figure 2).

In the intention to treat (ITT) population, the mean duration of bacteremia was 3.00 (sd 3.35) days in the standard therapy group and 1.94 (sd 1.79) days in the combination group (Table 3). The distribution of duration of bacteremia in both groups followed a negative binomial distribution (Figure 1a). The rate ratio of means was 0.65 (95% confidence interval [CI] 0.41, 1.02; P=0.062),
indicating that the mean time to resolution of bacteremia in the combination group was 65% of that in the standard therapy group. Results of the per-protocol analysis (Table 3) were similar with mean durations of bacteremia of 2.92 (sd 3.37) in the standard therapy group and 1.82 (sd 1.59) in the combination group, and a rate ratio of means of 0.62 (95% CI 0.38, 1.01; P=0.055). For the ITT population, the number of participants with bacteremia for >3 days was 8/29 (28%) in the standard therapy group and 4/31 (13%) in the combination group (Fisher’s exact, P=0.20); and the number with bacteremia for >7 days was 4/29 (14%) in the standard therapy group and 1/31 (3%) in the combination group (Fisher’s exact, P=0.19). In the combination group 90% of patients cleared bacteremia within 4 days, compared with 9 days in standard therapy group. Kaplan-Meier curves and a proportional hazards regression model also indicated that there was a non-significant earlier time to clearance of bacteremia in the combination group (hazard ratio [HR] 0.74, 95% CI [0.43, 1.26]); (Figure 1b) (logrank test, P=0.14). When the 3 deaths prior to clearance of bacteremia were treated as a competing risk, the hazard ratio was 0.75 (95% CI 0.50, 1.13).

Analysis by pre-specified subgroups did not reveal any statistically significant differences, but suggested a greater benefit in the subgroup infected with S. aureus with vancomycin MIC ≥1.5µg/mL than in those with vancomycin MIC <1.5µg/mL; and also in those with HCA-MRSA genotypes compared to those with CA-MRSA genotypes (Supplementary Table 1).

There was no significant difference between groups in hospital, 28 or 90 day mortality, relapsed bacteremia, the incidence of nephrotoxicity or hepatotoxicity, development of septic shock or need for ICU, or metastatic complications (Table 4).

**CONCLUSIONS**

Our findings support the continued investigation of combining an anti-staphylococcal β-lactam with vancomycin for MRSA bacteremia. We found a non-statistically significant reduction of one day in the duration of bacteremia in the combination therapy group, and that fewer patients had persistent bacteremia at days 3 and 7 following randomization. Although the study was not powered to
determine if these differences were statistically significant, the differences warrant further investigation in larger numbers of patients.

Notably, participants in the two allocated treatment groups were well matched with regards to baseline demographics, comorbidities and clinical syndromes. There were more patients with line-related and native osteoarticular infections in the combination therapy group, and all three pleuropulmonary infections were in the standard therapy group. However, illness severity was similar in both groups. The groups were also balanced in terms of mean serum vancomycin levels, MRSA isolate MICs to vancomycin and oxacillin, and *S. aureus* genotypes.

The trend to reduction in duration of bacteremia with combination therapy was consistent in both the ITT and per-protocol groups. For the pre-specified subgroups, the direction of effect was also towards a shorter duration of bacteremia with combination therapy in all subgroups. For the subgroup where infection was due to MRSA with vancomycin MIC $\geq 1.5 \mu g/mL$, the duration of bacteremia for the four patients receiving standard therapy was 1, 2, 4, and 9 days and for the two patients receiving combination therapy was 1 and 2 days. If there is indeed a greater benefit for combination therapy in the group with vancomycin MIC $\geq 1.5 \mu g/mL$, this would be consistent with *in vitro* studies where synergy is more consistently seen for isolates with higher vancomycin MICs [11].

Prior to this trial, there have been retrospective reports suggesting that the addition of β-lactams for MRSA bacteremia may be beneficial. Patients with persistent MRSA bacteremia during treatment with daptomycin, appear to quickly clear their bacteremia with the addition of nafcillin or oxacillin [22] or ceftaroline [23]. In a single-center retrospective cohort, Dilworth et al. compared 50 patients with MRSA bacteremia who received combination therapy with vancomycin and at least 24 h of β-lactam, and 30 patients who received vancomycin alone. They found a higher rate of microbiological eradication in the combination therapy group (96 vs. 80%, P=0.02) [14]. Thus the results of our trial extend the existing clinical experience in combining β-lactams with standard therapy of either vancomycin or daptomycin for MRSA bacteremia.
An additional therapeutic agent may increase the risk for adverse effects. Acute interstitial nephritis is a known, albeit uncommon, adverse effect of anti-staphylococcal β-lactams [24]. In the setting of peri-operative prophylaxis, Challagundla et al. found that high-dose compared to low-dose flucloxacillin (both with gentamicin) was associated with renal impairment [25]. Although we found no statistically significant increase in renal impairment in the combination therapy group, there were 8 (28%) cases of a rise in serum creatinine of >50% over baseline with combination therapy compared to 3 (11%) cases in the standard therapy group. Close monitoring of renal impairment will be essential for future studies of combination therapy.

Strengths of this study include the randomized, multi-center design, well-matched groups, and inclusion of detailed bacterial genotypic and MIC investigations. As this was designed as a pilot proof of feasibility study, the sample size is too small to make clinical recommendations. Even if we had found a statistically significant reduction in the duration of bacteremia with combination therapy, without clinically relevant endpoints there would be no cause to change practice. The experience with gentamicin is salutary. A trial demonstrating that adding gentamicin to standard therapy reduced the duration of bacteremia by one day but did not affect mortality [26], still led to a widespread practice to use gentamicin in this setting [27]. More recent data has confirmed a lack of mortality benefit [28] and indeed increased nephrotoxicity with the addition of gentamicin [29]. Hence we feel it is important that β-lactam based combination therapy is not adopted as standard clinical practice for MRSA bacteremia until and unless stronger evidence of safety and efficacy emerges from subsequent RCTs.

In conclusion, our study provides an encouraging signal that the combination of vancomycin with an anti-staphylococcal β-lactam may be useful for the treatment of MRSA bacteremia. This pilot RCT adds considerably to the growing literature from in vitro laboratory, in vivo animal, and retrospective clinical studies, and provides the impetus for future clinical trials involving objective clinically relevant endpoints.
ACKNOWLEDGMENTS

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POTENTIAL CONFLICT OF INTERESTS

SvH has received honoraria and is on an advisory board for Pfizer. All other authors declare no relevant conflicts of interests.

CAMERA study group: Royal Darwin Hospital – Steven Tong, Joshua Davis, Paula Binks, Suman Majumdar, Anna Ralph, Rob Baird, Claire Gordon, Cameron Jeremiah, Grace Leung, Anna Brischetto, Amy Crowe, Farshid Dakh, Kelly Whykes, Maria Kirkwood. Nepean Hospital – Archana Sud, Mahesh Menon, Lucy Somerville, Shrada Subedi, Shirley Owen. Westmead Hospital – Matthew O’Sullivan, Eunice Liu, Fei Zhou. Royal Perth Hospital – Owen Robinson, Geoffrey Coombs. Blacktown Hospital – Patrician Ferguson, Anna Ralph, Eunice Liu, Simon Pollet. Liverpool Hospital – Sebastian Van Hal, Hong Foo. Royal Prince Alfred Hospital - Sebastian Van Hal, Rebecca Davis. We would also like to thank Prof David Paterson, of the ASID CRN for advice throughout the course of the study.
REFERENCES


FIGURE LEGEND

Figure 1: Comparison of duration of bacteremia according to allocated treatment group, represented as histograms (A), and cumulative hazard curve (B).
### Table 1: Baseline characteristics of participants

<table>
<thead>
<tr>
<th></th>
<th>Standard therapy</th>
<th>Combination therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N=29)</td>
<td>(N=31)</td>
</tr>
<tr>
<td><strong>Age (years; mean, sd)</strong></td>
<td>65 (21)</td>
<td>64 (19)</td>
</tr>
<tr>
<td><strong>Male gender</strong></td>
<td>17 (59%)</td>
<td>22 (71%)</td>
</tr>
<tr>
<td><strong>Weight (kg; mean, sd)</strong></td>
<td>75.6 (14.3)</td>
<td>78.8 (15.5)</td>
</tr>
<tr>
<td><strong>Comorbidities</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Charlson comorbidity index (median [IQR])</td>
<td>3 (1-5)</td>
<td>2 (1-6)</td>
</tr>
<tr>
<td>Charlson index ≥3</td>
<td>16 (55%)</td>
<td>15 (48%)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>11 (38%)</td>
<td>13 (42%)</td>
</tr>
<tr>
<td>Chronic lung disease</td>
<td>10 (34%)</td>
<td>6 (19%)</td>
</tr>
<tr>
<td>Chronic renal impairment</td>
<td>5 (17%)</td>
<td>7 (23%)</td>
</tr>
<tr>
<td>Chronic liver disease</td>
<td>3 (10%)</td>
<td>2 (6%)</td>
</tr>
<tr>
<td>Hazardous alcohol use</td>
<td>2 (7%)</td>
<td>4 (13%)</td>
</tr>
<tr>
<td><strong>Acquisition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nosocomial</td>
<td>4 (14%)</td>
<td>11 (35%)</td>
</tr>
<tr>
<td>Community onset, health care associated</td>
<td>15 (52%)</td>
<td>9 (29%)</td>
</tr>
<tr>
<td>Community-acquired</td>
<td>10 (34%)</td>
<td>11 (35%)</td>
</tr>
<tr>
<td><strong>Indwelling foreign material</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central venous line</td>
<td>2 (7%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Permanent pacemaker</td>
<td>4 (14%)</td>
<td>4 (13%)</td>
</tr>
<tr>
<td>Prosthetic joint</td>
<td>5 (17%)</td>
<td>3 (10%)</td>
</tr>
<tr>
<td><strong>Antibiotic use in 48 hours prior to randomization</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any antibiotics</td>
<td>26 (90%)</td>
<td>25 (81%)</td>
</tr>
<tr>
<td></td>
<td>Reference 1</td>
<td>19 (45%)</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------</td>
<td>----------</td>
</tr>
<tr>
<td></td>
<td>Reference 2</td>
<td>13 (42%)</td>
</tr>
<tr>
<td>Any vancomycin</td>
<td></td>
<td>13 (45%)</td>
</tr>
<tr>
<td>Any β-lactam</td>
<td></td>
<td>13 (42%)</td>
</tr>
</tbody>
</table>

**Baseline investigations and illness severity**

- **SOFA score**
  - (median, IQR)
  - Reference 1: 1 (1-3)
  - Reference 2: 1 (0-2)

- **APACHE2 score**
  - (mean, sd)
  - Reference 1: 11.0 (6.0)
  - Reference 2: 10.2 (5.9)

- **Septic shock**
  - Reference 1: 7 (24%)
  - Reference 2: 8 (26%)

- **C-reactive protein**
  - (median, IQR)
  - Reference 1: 130 (67-255)
  - Reference 2: 150 (49-290)

- **Total white blood cell count (median, IQR)**
  - Reference 1: 12.4 (9-16.2)
  - Reference 2: 11.8 (8.3-14.8)

**Primary focus of infection**

- **Primary blood stream**
  - Reference 1: 10 (34%)
  - Reference 2: 7 (23%)

- **SSTI**
  - Reference 1: 6 (21%)
  - Reference 2: 7 (23%)

- **Line-related**
  - Reference 1: 2 (7%)
  - Reference 2: 6 (19%)

- **Osteoarticular - native**
  - Reference 1: 0 (0%)
  - Reference 2: 4 (13%)

- **Osteoarticular – device**
  - Reference 1: 1 (3%)
  - Reference 2: 2 (6%)

- **Pleuropulmonary**
  - Reference 1: 3 (10%)
  - Reference 2: 0 (0%)

- **Urinary tract**
  - Reference 1: 2 (7%)
  - Reference 2: 1 (3%)

- **Endocarditis**
  - Reference 1: 0 (0%)
  - Reference 2: 1 (3%)

- **Othera**
  - Reference 1: 4 (14%)
  - Reference 2: 2 (6%)

**Note:** Sequential Organ Failure Assessment – SOFA. Acute Physiology and Chronic Health Evaluation – APACHE. SSTI – skin and soft tissue infection.

- a. Other comprised ‘unknown’ (2), intra-abdominal (1), CNS (1), surgical site infection (2).
### Table 2: Antibiotic susceptibility and genotypic characteristics of bacterial strains

<table>
<thead>
<tr>
<th></th>
<th>Standard therapy (N=28)</th>
<th>Combination therapy (N=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antibiotic susceptibility</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vancomycin MIC (µg/mL), (median, IQR)</td>
<td>0.75 (0.63-1.0)</td>
<td>0.75 (0.50-1.0)</td>
</tr>
<tr>
<td>Vancomycin MIC≥1.5 (µg/mL)</td>
<td>4 (14%)</td>
<td>2 (6%)</td>
</tr>
<tr>
<td>Oxacillin MIC (µg/mL), (median, IQR)</td>
<td>256 (96-256)</td>
<td>256 (128-256)</td>
</tr>
<tr>
<td><strong>Genotypic features</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Panton-Valentine leukocidin positive</td>
<td>8 (28%)</td>
<td>10 (32%)</td>
</tr>
<tr>
<td>Sequence Type 22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5 (18%)</td>
<td>9 (29%)</td>
</tr>
<tr>
<td>Sequence Type 93&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4 (14%)</td>
<td>5 (16%)</td>
</tr>
<tr>
<td>Sequence Type 239&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6 (21%)</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>Other genotype&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13 (46%)</td>
<td>14 (45%)</td>
</tr>
</tbody>
</table>

**Note:** Minimum inhibitory concentration – MIC.

- a. One isolate could not be recovered.
- b. These are considered healthcare-associated MRSA genotypes.
- c. These are considered community-associated MRSA genotypes.
Table 3: Primary outcome measure (duration of bacteremia in days)

<table>
<thead>
<tr>
<th>Population</th>
<th>Standard therapy</th>
<th>Combination therapy</th>
<th>Ratio of means (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intention to treat population</td>
<td>N=29</td>
<td>N=31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (sd)</td>
<td>3.00 (3.35)</td>
<td>1.94 (1.79)</td>
<td>0.65 (0.41, 1.02)</td>
<td>0.062</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>1 (1–2)</td>
<td>1 (1–4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per-protocol population</td>
<td>N=26</td>
<td>N=28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (sd)</td>
<td>2.92 (3.37)</td>
<td>1.82 (1.59)</td>
<td>0.62 (0.38, 1.01)</td>
<td>0.055</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>1 (1–2)</td>
<td>1 (1–4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note:
Duration of bacteremia in days follows a negative binomial distribution.
<table>
<thead>
<tr>
<th>Table 4: Secondary outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Intention to treat population</td>
</tr>
<tr>
<td>Hospital mortality</td>
</tr>
<tr>
<td>28 day mortality</td>
</tr>
<tr>
<td>90 day mortality</td>
</tr>
<tr>
<td>Duration of bacteremia &gt;3 days</td>
</tr>
<tr>
<td>Duration of bacteremia &gt;7 days</td>
</tr>
<tr>
<td>Relapsed bacteremia</td>
</tr>
<tr>
<td>ICU admission OR development of septic shock after randomization</td>
</tr>
<tr>
<td>Grade 2 or above nephrotoxicity or hepatotoxicity</td>
</tr>
<tr>
<td>Metastatic complications during the first 10 days</td>
</tr>
<tr>
<td>Per protocol population</td>
</tr>
<tr>
<td>Hospital mortality</td>
</tr>
<tr>
<td>28 day mortality</td>
</tr>
<tr>
<td>90 day mortality</td>
</tr>
<tr>
<td>Duration of bacteremia &gt;3 days</td>
</tr>
<tr>
<td>Duration of bacteremia &gt;7 days</td>
</tr>
<tr>
<td>Event</td>
</tr>
<tr>
<td>------------------------------------------------------</td>
</tr>
<tr>
<td>Relapsed bacteremia</td>
</tr>
<tr>
<td>ICU admission OR development of septic shock after randomization</td>
</tr>
<tr>
<td>Grade 2 or above nephrotoxicity or hepatotoxicity</td>
</tr>
<tr>
<td>Metastatic complications during the first 10 days</td>
</tr>
</tbody>
</table>

**Note:**

ICU – intensive care unit.