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ORIGINAL RESEARCH

Evidence from an In Vitro Study: Is Oxacillin Plus Vancomycin a Better Choice for Heteroresistant Vancomycin-Intermediate *Staphylococcus aureus*?

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ABSTRACT

Introduction: Heteroresistant vancomycin-intermediate *Staphylococcus aureus* (hVISA) bacteremia may result in clinical failure of vancomycin therapy, together with prolonged infection and hospitalization. This clinical problem has resulted in a search for more effective treatment options. The current study was designed to further investigate the synergistic effect of oxacillin plus vancomycin against methicillin-resistant *S. aureus* (MRSA) and hVISA using checkerboard and time-kill assays.

Methods: Non-duplicate *S. aureus* isolates including hVISA (n = 29), MRSA (n = 10) and methicillin susceptible *S. aureus* (MSSA, n = 11) were used for combinational testing using checkerboard and time-kill assays.

Results: Twenty-one isolates, 15 hVISA and 6 MRSA, showed synergy between oxacillin and vancomycin by checkerboard assay with fractional inhibitory concentration indices of $\leq$ 0.5. The addition of oxacillin to vancomycin resulted in a reduction in baseline vancomycin MIC from 1–2 to 0.06–0.5 μg/ml against MRSA and hVISA isolates. In the time-kill assay, the combination of oxacillin and vancomycin resulted in synergistic activity against hVISA (n = 23) and MRSA (n = 7) isolates. Regrowth was observed in six hVISA isolates exposed to combination in the time-kill assay, but none of them reached the original inoculum density at 24 h. All re-growth isolates showed a onefold increase in vancomycin MIC (from 1 to 2 μg/ml) and were re-confirmed as hVISA using the population-analysis profile experiment. Overall, for hVISA and MRSA, the combination of oxacillin plus vancomycin had greater antibacterial effect than each individual drug alone.

Conclusion: The present study showed the potential activity of vancomycin plus oxacillin combination against hVISA and MRSA isolates. Further, continued evaluation of this combination is warranted and may have therapeutic benefits in treating complicated MRSA infections.

Keywords: hVISA; MRSA; MSSA; Oxacillin; Vancomycin

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INTRODUCTION

Staphylococcus aureus bacteremia (SAB) is a common cause of mortality and morbidity and a major burden on healthcare around the world. In patients with positive blood cultures (BC) suggestive of gram-positive cocci in clusters or with a high clinical suspicion of staphylococcal bacteremia, empirical therapy with anti-staphylococcal β-lactams such as nafcillin or cefazolin is indicated. Empirical therapy with nafcillin or cefazolin had lower 30-day mortality than second- or third-generation cephalosporins [1–3]. In settings with a methicillin-resistant *S. aureus* (MRSA) prevalence of > 20%, vancomycin is the usual empirical antimicrobial of choice [4]. Alternative agents include linezolid or daptomycin, but neither are superior to vancomycin in treating MRSA bacteremia [5].

A prospective study of 1994 SAB episodes found that 30-day mortality was significantly higher in patients with MRSA (30%) than MSSA (17.7%) infection [6]. Although this difference may be due to host factors, vancomycin shows slow bactericidal activity and poor tissue penetration, resulting in higher relapse rates [7–10]. In recent years, *S. aureus* with reduced susceptibility to vancomycin has become a significant clinical problem. Heteroresistant vancomycin-intermediate *S. aureus* (hVISA) is a strain of *S. aureus* containing a subpopulation of cells (1 in 10^6), that can grow within the vancomycin-intermediate susceptibility range of ≥ 4 μg/ml. hVISA bacteremia may result in clinical failure to vancomycin therapy, together with prolonged infection and hospitalization [11, 12].

This clinical problem has resulted in a search for more effective treatment options. Several in vitro and a few in vivo studies have explored the synergy between glycopeptides and various beta-lactam antibiotics against MRSA isolates with varying susceptibility to vancomycin [13–16]. These studies have described synergistic killing in most but not all tested strains. This potential synergy relies on the "seesaw effect" which demonstrates improved beta-lactam susceptibility, concomitant to reduced glycopeptide susceptibility [17]. In addition, the combination of vancomycin plus beta-lactam prevents the development of reduced vancomycin susceptibility in MRSA [18]. No published studies have documented synergism between vancomycin and daptomycin/linezolid.

Studies have also reported potential in vitro synergy between anti-staphylococcal penicillin (oxacillin or nafcillin) and vancomycin against MRSA and hVISA [19–23]. The current study was designed to further investigate the synergistic effect of oxacillin plus vancomycin against MRSA and hVISA using checkerboard and time-kill assays. These findings were compared with MSSA to establish incremental benefit.

METHODS

Bacterial Strains

A total of 50 non-duplicate *S. aureus* isolated from the blood culture between 2016 and 2017 at a 2600-bed tertiary care hospital, Christian Medical College, Vellore, India, were included in this study. Isolates were identified and characterized using standard culture and biochemical methods [24]. Of 50 isolates, 11 were MSSA, 10 were MRSA and 29 were confirmed as hVISA, using population analysis profile-area under curve (PAP-AUC). In this study, *S. aureus* strains that were resistant to cefoxitin and completely susceptible to vancomycin without any heteroresistant subpopulation were specified as MRSA, while MRSA strains expressing vancomycin heteroresistance were called hVISA. The hVISA strains were subcultured and maintained in brain Heart infusion agar (BHIA) were supplemented with 1 μg/ml of vancomycin and stored at − 70 °C. The study was approved by the institutional review board (IRB. Min. No. 10643 dated April, 2017).

Susceptibility Testing

Methicillin resistance was detected by disk diffusion testing using a 30-μg cefoxitin disk as recommended by the CLSI guidelines [25]. The MICs of oxacillin and vancomycin were

determined by the CLSI-recommended broth microdilution method [26]. Oxacillin and vancomycin powders were obtained from commercial sources (Sigma, St Louis, MO, USA). Cation-adjusted Mueller–Hinton broth (CA-MHB) containing 2% NaCl was used for oxacillin MICs determination. Oxacillin and vancomycin were tested at the concentration of 0.03–512 μg/ml and 0.03–16 μg/ml, respectively. The MICs of oxacillin and vancomycin were read after incubation at 35 °C for 24 h. *S. aureus* ATCC 29213 and *S. aureus* ATCC 43300 strains were used as a quality control strains.

PCR for *mec* A Gene

Bacterial DNA was extracted from colonies grown overnight on blood agar using the QIAamp DNA Mini Kit and the QIAcube instrument (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. PCR was performed for the detection of *mec* A gene in MRSA isolates, as previously described [27]; this is considered as the gold standard method for detection of methicillin resistance in *S. aureus*.

Screening and Confirmation of hVISA

MRSA isolates were screened for hVISA using the glycopeptide resistance detection (GRD) *E* test (bioMérieux, France) and confirmed with the PAP/AUC method. The GRD *E* test was performed using 0.5 McFarland-adjusted inoculum swabbed onto Mueller–Hinton agar (MHA) containing 5% blood. The zone of inhibition was read at 24 and 48 h after incubation at 35 ± 2 °C [28]. The test isolate was considered positive for hVISA if the MIC of vancomycin or teicoplanin was 8 μg/ml. PAP-AUC analysis was performed as previously described [29]. The bacterial suspension was plated onto freshly prepared BHIA plates containing 0.5–8 μg/ml of vancomycin. Colonies were counted after 48 h of growth at 35 ± 2 °C. For calculation of AUC, viable counts were plotted against increasing concentration of vancomycin using the GraphPad Prism™ (v.7.0) software package. All PAP-AUC experiments were performed in duplicate. For vancomycin PAP analysis, the AUC ratio was calculated by dividing the AUC of the test strain by the AUC of the MU3 (hVISA) strain. The PAP-AUC ratio was interpreted as follows, < 0.9 as vancomycin-susceptible *S. aureus* (VSSA), ≥ 0.9 as hVISA phenotype, and > 1.3 as vancomycin-intermediate *S. aureus* (VISA). For each batch of the PAP-AUC experiment, the hVISA (MU3, ATCC 700698), VISA (MU50, ATCC 700699) and *S. aureus* ATCC 29213 (VSSA) were used as the reference, comparator and negative control strains, respectively.

Broth Microdilution Checkerboard Assay

Checkerboard synergy testing was performed by the microbroth dilution method, as previously described [30]. Vancomycin was tested at a concentration of 0.03–8 μg/ml. MHB containing 2% NaCl was used to perform in vitro synergy testing. In the checkerboard assay, vancomycin was combined with oxacillin in the concentration of 1–128 μg/ml for MRSA and hVISA. For MSSA, oxacillin was used at a concentration of 0.25–8 μg/ml. Microtiter plates were incubated at 37 ± 2 °C for 24 h. Growth control and sterility control were included in each test panel. The first non-turbid well in each row and column was used to calculate the fractional inhibitory concentration (FIC) index. An FIC index of ≤ 0.5 was defined as synergy, an FIC index of > 0.5 to 4.0 was defined as indifferent, and an FIC index of > 4.0 was defined as antagonistic.

Time-Kill Assay (TKA)

The time-kill assay was performed in duplicate on all PAP-confirmed hVISA, MRSA, and MSSA isolates. Time-kill assays were performed according to previously published techniques [31]. An initial bacterial inoculum of approximately 5×10^6 for each isolate was inoculated into CA-MHB containing 2% NaCl. Time-kill experiments were performed at half-MIC of oxacillin and vancomycin for the respective isolates. Each antibiotic alone, together with oxacillin in combination with vancomycin,

were tested. The inoculum was diluted 1 in 100 using sterile saline. A volume of 100 µl was plated on the MHA plates at times of 0, 3, 6 and 24 h post-incubation in a shaker-incubator at 35 ± 2 °C. After 24 h of incubation (48 h for hVISA) isolates at 35 ± 2 °C, colonies were counted and results were expressed as $\log_{10}$CFU/ml. Each batch of testing included sterility control and growth control (without any antibiotic). Synergy was defined as ≥ 2 $\log_{10}$ decrease in CFU/ml for combination antibiotics in comparison to a single agent. Antagonism was defined as ≥ 2 $\log_{10}$ increase in CFU/ml for the combination antibiotic in comparison to the most active single agent. Bactericidal activity was defined as a ≥ 3 $\log_{10}$ CFU/ml reduction from baseline. Regrowth was defined as a ≥ 3 $\log_{10}$ decrease in CFU/ml followed by a ≥ 2 $\log_{10}$ increase in CFU/ml at 24 h.

Statistical Analysis

For the time-kill studies, one-way analysis of variance with Tukey's post hoc test was used to compare changes in CFU/ml. A p value of < 0.05 was considered statistically significant. Statistical analyses were performed using Statistical Package for the Social Sciences v.16.0 (SPSS, Chicago, IL, USA).

Compliance with Ethics Guidelines

This article does not contain any studies with human participants or animals performed by any of the authors. The study was approved by an institutional review board (IRB. Min. No. 10643 dated April 2017).

RESULTS

Of the tested *S. aureus*, MICs of oxacillin ranged between 0.5 and 1 µg/ml for MSSA and 4–128 µg/ml for hVISA and MRSA. All isolates were susceptible to vancomycin with the MIC of 1–2 µg/ml. All MRSA isolates carried the *mec* A gene. Twenty-nine isolates were confirmed as hVISA using the PAP-AUC method, with an AUC ratio between 0.92 and 1.33 (Fig. 1).

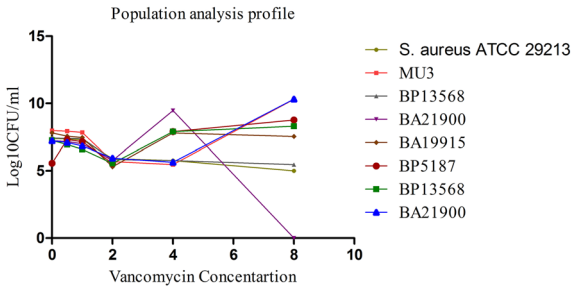

Fig. 1 Population analysis profile (PAP) of vancomycin on five representative methicillin resistant *Staphylococcus aureus* (MRSA) isolate from bacteremia cases. PAP of vancomycin was performed to confirm the presence heterogeneous vancomycin-intermediate strains (hVISA) phenotype. MU3, hVISA as comparator control; MU50, VISA as positive control; ATCC 29213, VSSA as negative control

Twenty-one isolates, 15 hVISA and 6 MRSA, showed synergy between oxacillin and vancomycin by checkerboard assay with FIC indices of ≤ 0.5. This was not seen in any MSSA isolates (FIC 0.75–1; Table 1). The addition of oxacillin to vancomycin resulted in a reduction in baseline vancomycin MIC from 1–2 to 0.06–0.5 µg/ml against MRSA and hVISA isolates.

In time-kill assays, the combination of oxacillin and vancomycin resulted in synergistic activity against hVISA (n = 23) and MRSA (n = 7) isolates (Table 1). Time-kill curves of the oxacillin–vancomycin combination, oxacillin, and vancomycin alone against representative hVISA, MRSA and MSSA are shown in Fig. 2. Although a higher rate of synergy was observed against hVISA, marked heterogeneity in bactericidal activity was observed.

The combination of oxacillin plus vancomycin demonstrated bactericidal activity in 14 of 29 hVISA isolates and 6 of 10 MRSA isolates at 24 h. Regrowth was observed in six hVISA isolates exposed to combination in time-kill assays, but none of them reached the original inoculum density at 24 h. All re-growth isolates showed a onefold increase in vancomycin MIC (from 1 to 2 µg/ml) and were reconfirmed as hVISA using the PAP-AUC method. Overall, for hVISA and MRSA, the combination of oxacillin plus vancomycin had

Table 1 Minimum inhibitory concentration of oxacillin, vancomycin and combination activity of oxacillin plus vancomycin in time kill assay against hVISA, MRSA and MSSA isolates from bloodstream infection

Strains	Oxacillin MIC µg/ml	Vancomycin MIC µg/ml	PAP-AUC ratio	Checker board assay		Time-kill assay ½ MIC of oxacillin + ½ MIC of vancomycin (µg/ml) at 24 h	
				ΣFIC	Activity	Combinational activity	Bactericidal activity
hVISA_1	4	1	0.97	0.5	Synergy	Synergy	Positive
hVISA_2	8	1	1.02	0.37	Synergy	Synergy	Positive
hVISA_3	4	2	0.9	0.5	Synergy	Synergy	Positive
hVISA_4	4	1	0.98	0.5	Synergy	Synergy	Positive
hVISA_5	64	1	1.16	0.5	Synergy	Synergy	Positive
hVISA_6	4	1	0.95	0.37	Synergy	Synergy	Positive
hVISA_7	32	2	0.9	0.62	Indifferent	Synergy	Positive
hVISA_8	8	1	1.03	0.75	Indifferent	Synergy	Positive
hVISA_9	8	1	1.28	0.62	Indifferent	Synergy	Positive
hVISA_10	8	1	0.97	0.75	Indifferent	Synergy	Positive
hVISA_11	4	1	1.12	1.00	Indifferent	Synergy	Positive
hVISA_12	8	2	0.9	1.00	Indifferent	Synergy	Positive
hVISA_13	8	1	1.02	0.62	Indifferent	Synergy	Positive
hVISA_14	16	1	1.03	0.75	Indifferent	Synergy	Positive
hVISA_15	4	1	0.98	0.5	Synergy	Synergy	Negative
hVISA_16	8	1	1.04	0.5	Synergy	Synergy	Negative
hVISA_17	4	1	1.00	0.5	Synergy	Synergy	Negative
hVISA_18	64	2	0.93	0.5	Synergy	Synergy	Negative
hVISA_19	4	1	0.92	0.06	Synergy	Synergy	Negative
hVISA_20	64	1	1.1	0.5	Synergy	Synergy	Negative
hVISA_21	4	1	0.92	0.07	Synergy	Synergy	Negative
hVISA_22	4	2	1.00	0.5	Synergy	Synergy	Negative
hVISA_23	4	1	1.01	0.37	Synergy	Indifferent	Negative
hVISA_24	8	1	1.52	0.75	Indifferent	Synergy	Regrowth
hVISA_25	4	1	0.91	0.5	Indifferent	Indifferent	Regrowth
hVISA_26	8	1	1.33	0.56	Indifferent	Indifferent	Regrowth
hVISA_27	64	1	1.01	1.00	Indifferent	Indifferent	Regrowth
hVISA_28	4	1	1.14	0.75	Indifferent	Indifferent	Regrowth
hVISA_29	8	1	1.23	0.62	Indifferent	Indifferent	Regrowth

Table 1 continued

Strains	Oxacillin MIC μg/ml	Vancomycin MIC μg/ml	PAP-AUC ratio	Checker board assay		Time-kill assay ½ MIC of oxacillin + ½ MIC of vancomycin (μg/ml) at 24 h	
				ΣFIC	Activity	Combinational activity	Bactericidal activity
MRSA_1	128	1	0.28	0.5	Synergy	Synergy	Positive
MRSA_2	64	1	0.39	0.5	Synergy	Synergy	Positive
MRSA_3	4	1	0.37	0.75	Indifferent	Synergy	Positive
MRSA_4	8	1	0.48	0.62	Indifferent	Synergy	Positive
MRSA_5	8	1	0.55	0.62	Indifferent	Synergy	Positive
MRSA_6	64	1	0.37	0.5	Synergy	Indifferent	Positive
MRSA_7	4	1	0.47	0.5	Synergy	Synergy	Negative
MRSA_8	8	1	0.32	0.5	Synergy	Synergy	Negative
MRSA_9	8	1	0.35	0.5	Synergy	Indifferent	Negative
MRSA_10	4	1	0.72	0.62	Indifferent	Indifferent	Negative
MSSA_1	0.5	1	ND	0.75	Indifferent	Indifferent	Positive
MSSA_2	1	1	ND	0.75	Indifferent	Indifferent	Positive
MSSA_3	1	0.5	ND	1.00	Indifferent	Indifferent	Positive
MSSA_4	0.5	1	ND	0.70	Indifferent	Indifferent	Positive
MSSA_5	0.5	1	ND	0.74	Indifferent	Indifferent	Positive
MSSA_6	1	0.5	ND	1.007	Indifferent	Indifferent	Negative
MSSA_7	0.5	1	ND	1.007	Indifferent	Indifferent	Positive
MSSA_8	1	0.25	ND	0.75	Indifferent	Indifferent	Positive
MSSA_9	0.5	0.12	ND	0.75	Indifferent	Indifferent	Positive
MSSA_10	1	0.5	ND	1.007	Indifferent	Indifferent	Negative
MSSA_11	1	0.5	ND	1.007	Indifferent	Indifferent	Positive

ND not done

greater antibacterial effect than each individual drug alone.

Table 2 documents the mean change in $\log_{10}$ CFU/ml of bacterial inoculum treated with oxacillin, vancomycin, and the oxacillin–vancomycin combination against hVISA, MRSA, and MSSA at 24 h. The combination of oxacillin plus vancomycin was superior at inhibiting hVISA and MRSA than vancomycin alone ($p < 0.01$). The combinations resulted in a 1.4-fold reduction in hVISA and a 0.7-fold reduction in MRSA than with vancomycin alone.

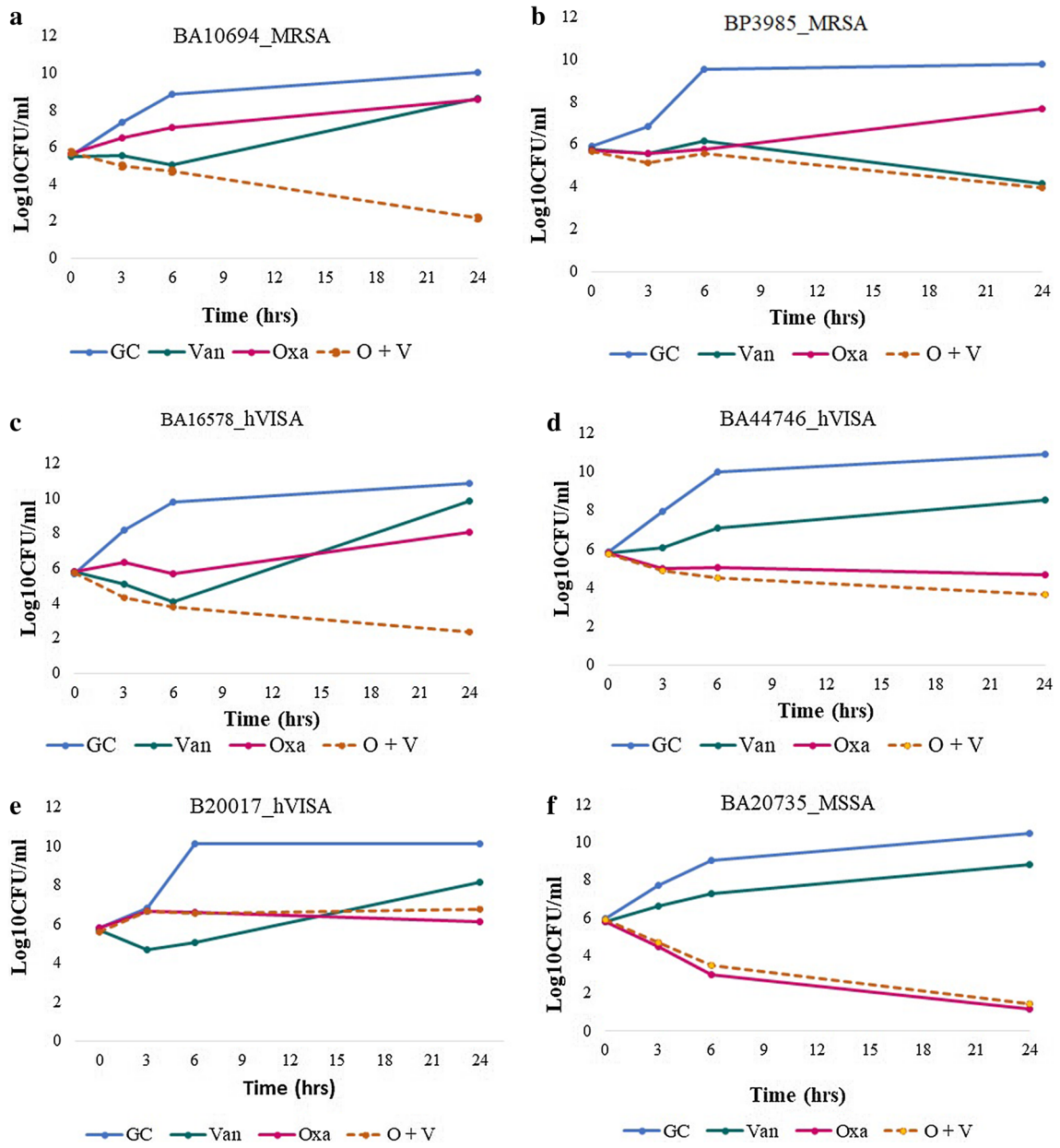

Fig. 2 Time-kill curves of oxacillin (1/2 MIC) in combination with vancomycin (1/2 MIC), vancomycin, oxacillin alone and vancomycin alone. *MRSA* showing **a** synergy and **b** indifferent with oxacillin with vancomycin combination; *hVISA* displaying **c** synergy, **d** indifferent, **e** regrowth with this combination, **d** *MSSA* exhibiting indifferent effect with this combination in time-kill assays

In time-kill studies, the combination of oxacillin plus vancomycin showed indifferent activity against all MSSA isolates. Oxacillin was more effective than combination therapy at inhibiting MSSA ($p < 0.01$). No antagonism was seen in any of the tested isolates with either checkerboard or time-kill assays.

Table 2 Mean $\log_{10}$ colony-forming unit (CFU)/ml of hetero-resistant vancomycin intermediate *S. aureus* (*hVISA*), methicillin resistant *S. aureus* (*MRSA*) and methicillin susceptible *S. aureus* (MSSA) isolates treated with oxacillin alone, vancomycin alone and oxacillin plus vancomycin combination in time-kill assays

Organism	Growth control $\log_{10}$ CFU/ml ± SD	Time kill assay [a]Mean $\log_{10}$ CFU/ml ± SD		
		Oxacillin	Vancomycin	[b]Oxacillin at ½ X MIC + vancomycin ½ X MIC
hVISA (n = 29)	8.3 ± 0.3	6.5 ± 0.7	6.4 ± 0.5	5.0 ± 0.7
MRSA (n = 10)	8.1 ± 0.1	5.1 ± 0.3	4.6 ± 0.3	3.9 ± 0.4
MSSA (n = 5)	8.4 ± 0.3	3.7 ± 0.3	6.9 ± 0.2	3.6 ± 1.0

[a] All data are presented as mean ± standard deviation (SD)
[b] 1/2 MICs of oxacillin and vancomycin are derived from hVISAMIC of individual isolates as listed in Table 1

DISCUSSION

Clinical guidelines recommend at least 14 days of antibiotic therapy to treat for treating uncomplicated *S. aureus* bacteremia [32]. Empiric therapy for *S. aureus* bacteremia often includes beta-lactam with additional vancomycin until susceptibility of the isolate are known [33].

MRSA is inherently resistant to all β-lactams except ceftaroline fosamil and ceftobiprole. Several studies have established that beta-lactams and vancomycin show synergy against MRSA with varying vancomycin susceptibility (MIC, ≤ 2 to ≥ 4 μg/ml). [13–16, 19–23, 34]. Recently, Tran et al. have reported that vancomycin in combination with various beta-lactams including nafcillin, cefazolin, cefepime and ceftaroline resulted in a 4- to 16-fold reduction in baseline vancomycin MIC values [15]. Further, a marked "seesaw effect" was demonstrated in MRSA isolates with increased susceptibility to ceftaroline associated with decreased glycopeptide and daptomycin susceptibility [17]. An in vitro study has shown that sub-MIC concentrations of oxacillin plus vancomycin prevents the selection of vancomycin heteroresistance [19]. However, strong evidence to support this hypothesis has not been established through in vivo studies.

Sieradzki et al. have reported that increased glycopeptide MICs were associated with reduced β-lactam resistance in MRSA isolates [35, 36]. Notably, the addition of oxacillin resulted in a fourfold reduction (32 to 2 μg/ml) of vancomycin MIC in VRSA isolates [37]. An in vitro pharmacokinetic/pharmacodynamic study demonstrated that nafcillin in combination with vancomycin resulted in more rapid killing of MRSA (6.3 h) than vancomycin alone (72 h) [23].

Although, studies have attempted to assess the activity of nafcillin/oxacillin and vancomycin, the mechanism behind this synergistic effect is unclear. Vancomycin and beta-lactam have different targets. Vancomycin binds with the D-ala-D-ala peptide and beta-lactam suicide-inhibits transpeptidase [21, 36]. Specifically, beta-lactam antibiotics bind to PBPs other than PBP2′ in hVISA and vancomycin-intermediate *S. aureus* (VISA).

Regardless of the in vitro methodology, our study agrees with previous studies in demonstrating synergistic activity against MRSA and hVISA isolates [14–18]. The present study clearly demonstrates that the inhibitory activity of vancomycin is enhanced by the addition of oxacillin. Consistent with previous studies [13–16, 19–23, 34, 38], we found that vancomycin had the least bactericidal activity against MRSA than all other antibiotics. Cell wall thickening in hVISA/VISA leads to clogging of vancomycin and prevents binding at the target site [12]. Alteration in the cell wall structure is induced by binding of beta-lactam to PBPs other than PBP2′ which promotes the

Table 3 In vitro and in vivo evidence for increased antibacterial activity of anti-staphylococcal penicillin/beta-lactam with vancomycin

Study	hVISA/VISA	MRSA	MSSA
In vitro	In nearly all studies, consistent synergistic bacterial killing was reported in most but not all strains tested. Synergy was proportional to vancomycin MIC, and increasing degree of synergy was seen with increasing vancomycin MIC	Consistently reported synergistic bacterial killing in most but not all strains tested	Least effect against MSSA. Neither synergy nor antagonism were evident in any strain, using both fixed dose concentration and dynamic model stimulating clinical dosing
In vivo	No data	Higher microbiological eradication, improved outcome and shortens the duration of MRSA bacteremia [33, 43, 44]	β-lactam is superior to vancomycin in treating MSSA infection [40–42]

binding of vancomycin to the D-ala-D-ala subunit [18]. In addition, an inverse relationship between vancomycin and oxacillin MIC was reported in an in vitro study [36]. This finding suggests that the "seesaw effect" may contribute to the enhanced synergistic activity of oxacillin plus vancomycin against hVISA, in comparison to single agent vancomycin alone. Neither antagonism nor synergism was observed with the vancomycin–oxacillin combination against MSSA isolates. This was similar to the findings of Joukhadar et al. [39]. It is well established that beta-lactam is superior to vancomycin in treating MSSA infections [40–42].

Dilworth et al. have studied the microbiological impact of adding an anti-staphylococcal β-lactams to vancomycin and reported increased microbiological eradication with this combination [43]. Similarly, McConeghy et al. have reported that the combination of vancomycin with beta-lactam could improve the outcome of *S. aureus* infection [33]. Furthermore, a multicenter randomized controlled trial (ACTRN12610000940077) found that the duration of MRSA bacteremia was shortened from 3 days in patients receiving vancomycin alone to 1.94 days in patients receiving the combination of vancomycin and flucloxacillin. However, no significant difference in 90-day mortality was noted [44]. Collectively, this suggests that combination therapy has a role in the treatment of MRSA bacteremia (Table 3).

There are certain limitations to the present study as the data from presented here represent in vitro synergistic activity. However, additional in vivo studies are required to support these findings. Further, this may not be translated into clinical benefit for patients. Thus, additional clinical studies are warranted to establish the superiority of this combination to vancomycin alone in treating severe MRSA infections.

CONCLUSION

This present study confirms previous findings that vancomycin and oxacillin are synergistic against MRSA including hVISA strains. However, oxacillin alone was more potent against MSSA than combination therapy. A randomized controlled trial is warranted to establish whether combination therapy should be recommended as standard therapy to reduce morbidity and mortality among recurrent or difficult-to-treat hVISA/MRSA infections.

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Compliance with Ethics Guidelines. This article does not contain any studies with human participants or animals performed by any of the authors The study was approved by an institutional review board (IRB. Min. No. 10643 dated April 2017).

Data Availability. All data generated or analyzed during this study are included in this published article.

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