In Vitro Pharmacodynamics of Vancomycin and Cefazolin Alone and in Combination against Methicillin-Resistant Staphylococcus aureus

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Previous studies employing time-kill methods have observed synergistic effects when a β-lactam is combined with vancomycin against methicillin resistant *Staphylococcus aureus* (MRSA). However, these time-kill studies have neglected the importance of human-simulated exposures. We evaluated the effect of human-simulated exposures of vancomycin 1g q8h in combination with cefazolin 1g q8h against various MRSA. Four clinical isolates [2 MRSA (vancomycin MIC: 0.5 and 2.0 µg/ml), heterogenous vancomycin intermediate *S. aureus* (hVISA) (MIC: 2.0 µg/ml), and VISA (MIC: 8.0 µg/ml)] were evaluated in an *in vitro* pharmacodynamic model with a starting inoculum of 10^6 and 10^8 colony forming units (cfu)/ml. Bacterial density was measured over 48-72h. Time-kill curves were constructed and the area under the bacterial killing and regrowth curve (AUBC) was calculated. During 10^6 cfu/ml studies, combination therapy achieved greater log_{10} cfu/ml changes at 12h compared with vancomycin alone (-4.31±0.58 vs. -2.80±0.59, p<0.001), but not at 48h. Combination therapy significantly reduced the AUBC from 0 to 48h (122±14) compared with vancomycin alone (148±22, p=0.017). Similar results were observed during 10^8 studies, where combination therapy achieved greater log_{10} cfu/ml changes at 12h compared with vancomycin alone (-4.00±0.20 vs. -1.10±0.04, p<0.001) and significantly reduced the AUBC (275±30 vs. 429±37, p<0.001) after 72h incubation. In this study, the combination of vancomycin and cefazolin at human simulated exposures improved the rate of kill against these MRSA isolates and resulted in greater overall antibacterial effect, but no differences in bacterial density were observed by the end of the experiments.
KEY WORDS: combination therapy, methicillin-resistant *Staphylococcus aureus*, vancomycin, cefazolin
BACKGROUND

*Staphylococcus aureus* causes serious infections in both the hospital and community settings, and the growing prevalence of methicillin-resistant isolates (i.e., MRSA) as a cause of these infections has increased the use of glycopeptide antibiotics such as vancomycin. Although vancomycin is almost universally accepted as the drug of choice for the treatment of most MRSA infection, it is less rapidly bactericidal compared with β-lactams against *S. aureus*, especially at higher inoculums [34]. Additionally, the recent emergence of decreased vancomycin susceptibility in *S. aureus*, including isolates with vancomycin minimum inhibitory concentrations (MICs) of 2 µg/ml, heterogeneous vancomycin-intermediate *S. aureus* (hVISA), and vancomycin-intermediate *S. aureus* (VISA), presents a significant clinical problem [26, 37, 39]. Sakoulas and colleagues reported significant correlation between vancomycin susceptibilities of *S. aureus* isolates from blood and patient outcomes [29], and some studies revealed that MRSA having vancomycin MICs of 1-2 µg/ml are less likely to be successfully treated with vancomycin compared with patients infected with isolates demonstrating lower MICs [14, 18, 21, 24, 36]. Similarly, significantly higher rates of morbidity were observed in patients infected with hVISA and VISA during vancomycin treatment [3, 15, 16].

With few antibiotics historically available to treat MRSA infections, combination therapy with a β-lactam and vancomycin has previously been explored [4, 7, 11, 27]. These studies have been conducted using *in vitro* time-kill methodology, checkerboard synergy studies, or *in vivo* animal studies. Rochon-Edouard and colleagues observed that cefazolin and imipenem were strongly bactericidal against MRSA when combined with vancomycin using a disk diffusion screening method [27]. Similarly, previous *in vitro*
experiments employing time-kill methods have demonstrated synergistic effects when a β-lactam is combined with vancomycin against MRSA with various degree of susceptibility to vancomycin [4]. In contrast, not all animal studies have observed similar synergistic effects of the combination therapy [7]. One shortcoming of previous in vitro studies is that only single drug concentrations of the combination were explored for the duration of the experiments. Hence, these time-kill studies have neglected the importance of human-simulated exposures. In the current study, we evaluated the effect of human-simulated exposures of vancomycin in combination with cefazolin against various MRSA phenotypes.

**MATERIALS AND METHODS**

**Bacterial strains.** Four clinical *S. aureus* isolates with variable degrees of susceptibility to vancomycin were selected for use in the study. The isolates included two MRSA (Hosp 2-23 and STA 336), one hVISA isolate (STA 449), and one VISA isolate (STA 454). The vancomycin MIC for all organisms was determined in triplicate by the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) recommendations [5]. The vancomycin MIC for MRSA (Hosp 2-23), MRSA (STA 336), hVISA (STA 449), and VISA (STA 454) was 0.5, 2, 2, and 8 µg/ml, respectively. All isolates were presumed to have a cefazolin MIC ≥32 µg/ml due to their MRSA phenotype.

**Antibiotics.** Vancomycin analytical powder was acquired from Sigma-Aldrich, Inc. (St. Louis, MO). Cefazolin (Apotex Corp., Weston, FL; lot 101A015; expiration date, June 2012) was obtained from the Department of Pharmacy at Hartford Hospital.
Simulated drug exposures. Vancomycin and cefazolin were administered as a bolus into the model every 8 hours (q8h) to simulate peak and trough free drug concentrations achieved in human serum after intravenous doses of 1 gram (g) q8h of vancomycin infused over 1 hour and 1g q8h of cefazolin infused over 2-3 minutes in healthy volunteers [12, 13]. Protein binding was assumed to be 50% for vancomycin and 90% for cefazolin [1, 35]. The target free antibiotic concentration for the vancomycin 1g q8h regimen was a peak of approximately 17.5 µg/ml (total concentration 35.0 µg/ml) with a half-life of 6.5h, thereby resulting in free trough concentrations of approximately 7.5 µg/ml (total concentration of 15.0 µg/ml). The target free antibiotic concentrations for cefazolin 1g q8h was a peak of 18.8 µg/ml (total concentration 188.0 µg/ml) with a half-life of 2.0h, thereby resulting in free trough concentration of approximately 1.9 µg/ml.

In vitro pharmacodynamic model. A one-compartment in vitro model was used for all experiments. Each experiment consisted of three independent models (two experimental treatment models and one growth control model), which ran simultaneously for each isolate. The models were placed in a 37°C circulating water bath for optimal temperature control. Experiments were performed at 10^6 cfu/ml for 48h against all strains and 10^8 cfu/ml for 72h against 2 of the isolates [MRSA (STA 336) and hVISA (STA 449)]. We explored the higher inoculum and longer study duration in an effort to observe reduced antibacterial effects for the vancomycin monotherapy regimen. As such, we only selected isolates with MICs of 2 µg/ml for these experiments since this made sense clinically (i.e., other therapies would be employed for VISA and vancomycin appears to be effective for isolates with very low MICs). For the lower inoculum studies, three independent starting inoculums of 10^6 cfu/ml were set up from an overnight culture
of the test isolate for all model experiments. Cation adjusted Muller-Hinton broth (CAMHB) (Becton, Dickinson and Company, Sparks, MD) was used as the bacterial growth medium for experiments with MRSA. Brain Heart Infusion (BHI) broth (Becton, Dickinson and Company, Sparks, MD) was used as the bacterial growth medium for hVISA and VISA isolates [note: internal experiments found no difference in growth characteristics between mediums for any of the isolates (data not shown)]. Antibiotic was administered 0.5h after inoculation of bacteria into models and then every 8h. Fresh broth was supplied via a peristaltic pump (Masterflex L/S Model 7524-40; Cole-Palmer Instrument Company) set to achieve the human simulated half-life. For combination regimen experiments, we employed methods originally described by Blaser [2]. Each experiment was conducted over 48h and performed in duplicate to ensure reproducibility.

For the higher inoculum studies, three independent starting inoculums of 10⁸ cfu/ml were set up. Briefly, 10 ml of a 15*10⁸ cfu/ml (5.0 McFarland) bacterial suspension was centrifuged at 2500 revolutions per minute for 20 minutes at 25°C. The cell pellet was then separated and reconstituted with 0.75 ml of 0.9% sodium chloride to achieve a suspension of 10¹⁰ cfu/ml. Three milliliters was drawn up and injected in each 300 ml model to achieve the starting inoculum of 10⁸ cfu/ml. BHI broth was used as the bacterial growth medium for all experiments. Each infection model experiment was conducted over 72 h and performed in duplicate to ensure reproducibility. All other methods were similar to those described for the 10⁶ cfu/ml studies.

To assess bacterial density over time, samples were obtained from each model and serially diluted in normal saline. Aliquots of diluted sample were plated for quantitative culture. Trypticase soy agar plates (100-mm diameter) with 5% sheep blood or BHI were
used for quantitative determinations. Colony counts were read after 18-24 h of incubation at 37ºC. The lower limit of detection for bacterial density was 1.7 log$_{10}$ cfu/ml.

To evaluate the antibiotic activity of each regimen, bacterial density was measured by the change from 0 h in log$_{10}$ cfu/ml compared between treatments at 12, 48, and 72 h. Time-kill curves were constructed and the area under the bacterial killing and regrowth curve (AUBC) was calculated to compare antibiotic efficacy [10].

**Antibiotic concentration and exposure determinations.** Samples of CAMHB and BHI broth taken from each of the treatment models were assayed for vancomycin at 0, 8, 16, 24, 32, 40, 48, 56, 64 and 72h and cefazolin at 0, 4, 8, 12, 16, 20, 24, 28, 32, 40, 48, 56, 64 and 72h. All samples were immediately stored at -80 ºC until analysis. The concentration of vancomycin was assessed using a fluorescence polarization immunoassay (Abbott Diagnosis TDx) at the chemistry lab of Manchester Hospital (Manchester, CT). For the assay, the lower and upper detection limits were 3.5 and 40 µg/ml. The inter- and intra-day coefficient of variation (CV) for low, middle, and high check samples were less than 6%. Cefazolin was analyzed by a validated high-performance liquid chromatography (HPLC) method at the Center for Anti-Infective Research and Development, as described previously [8]. The cefazolin assay was linear over a concentration range of 0.5 to 30 µg/ml. The intraday CV for low and high quality control samples were 2.1% and 3.1%, respectively. The interday quality control samples were 5.9% and 2.6%, respectively. Based on achieved concentrations in each model, the peak and trough concentrations for each drug were reported as that observed, the half-life was calculated by 0.693/$k$, were $k$ was the estimated elimination rate constant calculated by the natural log of peak/trough divided by the dosing interval, and the free area under
the curve over each 24h period ($f_{AUC_{0-24}}$) (mg*h/l) was calculated using the trapezoidal rule.

**Statistical analyses.** The mean log$_{10}$ cfu/ml reduction of vancomycin monotherapy, cefazolin monotherapy and combination therapy at 12, 48, and 72h from the starting inoculum (0h), and AUBC for each therapy were compared with each other using one way analysis of variance (ANOVA). A p-value less than 0.05 was considered significant.

**RESULTS**

**Pharmacokinetic analysis.** Pharmacokinetic parameters observed in the models are shown in Table 1. Observed peak and trough concentrations of vancomycin were within -31% to 5% and -34% to 22% of target values across all experiments. Observed peak and trough concentrations of cefazolin were within -36% to 24% and -23% to 224% of target values across all experiments. These resulted in a mean vancomycin free AUC$_{0-24}$/MIC during of 518 for MRSA-Hosp2-23 (MIC = 0.5 µg/ml), 123 for MRSA-STA 449 (MIC = 2.0 µg/ml), 119 for hVISA-STA449 (MIC = 2.0 µg/ml), and 30 for VISA-STA 454 (MIC = 8.0 µg/ml). Since no cefazolin concentrations were greater than 23.4 µg/ml during experiments, we presume that the time that free drug concentrations were above the MIC ($f/T>MIC$) was 0% for all isolates.

**Antibacterial activity at $10^6$ cfu/ml inoculum.** The average bacterial density of the starting inoculum was 6.29±0.20 log$_{10}$ cfu/ml. Control isolates grew to 8.87±0.22 log$_{10}$ cfu/ml over 48h in the models. Fig. 1 summarizes the time-kill curves for vancomycin alone and in combination with cefazolin for all isolates. The mean changes in bacterial density at 12 and 48h against each of the 4 isolates are provided in Table 2. For the three
MRSA isolates with vancomycin MIC ≥ 2 µg/ml, colony counts began to regrow after 28h incubation, but none approached the initial inoculum. The AUBC for vancomycin alone over 48h was 148±22 and ranged from 129 to 199. A reduction in log_{10} cfu/ml was not observed for cefazolin alone at 48h for any of the isolates. The AUBC for cefazolin alone over 48h was 357±16 and ranged from 345 to 379.

Combination therapy significantly reduced bacterial density compared with cefazolin alone at 12 and 48h (p<0.001 and p<0.05). Compared with vancomycin alone, the reduction in bacterial density was significant at 12h (-4.31±0.58 vs. -2.80±0.59, p<0.001), but not at 48h (-4.00±0.82 vs. -3.07±1.15, p=0.081). Combination therapy significantly reduced the AUBC from 0 to 48h (122±14) compared with vancomycin alone (148±22, p=0.017) and cefazolin alone (357±16, p<0.001).

**Antibacterial activity at 10^8 cfu/ml inoculum.** The average bacterial density of the starting inoculum was 8.52±0.08 log_{10} cfu/ml. Control isolates grew to 8.87±0.16 log_{10} cfu/ml over 72h in the models. Fig. 2 summarizes the time-kill curves for MRSA (STA 336) and hVISA after exposures of human-simulated blood concentration of vancomycin alone and vancomycin plus cefazolin. Cefazolin alone was not studied in these experiments. The mean changes in bacterial density at 12, 48 and 72h against each isolate are provided in Table 3. Vancomycin alone demonstrated bactericidal activity up to 56h with signs of regrowth afterward. The AUBC for vancomycin alone over 72h was 429±37 and ranged from 390 to 474.

For the combination of vancomycin and cefazolin, MRSA (STA 336) did not experience bacterial regrowth over the 72h experiment. In contrast, hVISA demonstrated regrowth after 32h, but this did not reach starting bacterial densities.
Compared with vancomycin alone, the reduction in bacterial density was significant at 12h (-4.00±0.20 vs. -1.10±0.04, p<0.001) and at 48h (-6.24±0.55 vs. -5.35±0.30, p=0.03), but not at 72h (-6.12±0.75 vs. -5.37±0.47, p=0.15). The combination significantly reduced the AUBC compared with vancomycin alone (275±30 vs. 429±37, p<0.001) over the 72h experiment.

Comparison of bacterial density reduction between 10^6 cfu/ml and 10^8 cfu/ml inoculum studies. The starting inoculum affected vancomycin’s killing profile, but only when administered alone. The change in bacterial density for MRSA (STA 336) and hVISA (STA 449) for vancomycin alone was significantly greater for the 10^6 cfu/ml inoculum study versus the 10^8 cfu/ml inoculum study (-2.45 vs. -1.11, p<0.05) at 12h, but not at 48h. For the combination of vancomycin and cefazolin, there were no changes in bacterial density at any tested time point between 10^6 and 10^8 cfu/ml inoculums (p=1.0).

DISCUSSION

Vancomycin has been the mainstay of MRSA therapy for several decades despite of the recognition of its important shortcomings. These shortcomings include poor tissue and intracellular penetration [17, 19, 31], lack of activity against organisms growing in biofilm [28], slow bactericidal effects especially for high bacterial densities [34], and the recent emergence of decreased vancomycin susceptibility in some *S. aureus* [26, 37, 39]. In the absence of many antibacterial options to treat MRSA, the combination of vancomycin and various β-lactams was explored and reported to be a promising alternative to vancomycin monotherapy [4, 7, 27]. As a result, vancomycin is sometimes clinically administered together with an anti-staphylococcus β-lactam during the initial
empiric phase of treatment. In contrast, not all animal infection models were found to replicate these findings [7]. Domenech and colleagues evaluated the antibiotic activities of glycopeptides and β-lactams against 4 strains of *S. aureus* with vancomycin MICs from 1 to 8 µg/ml *in vitro* and *in vivo* [7]. Against the VISA strain, they observed no significant increases in efficacy for combination therapy during *in vivo* studies, but synergistic effects for the combination during *in vitro* studies. One limitation to the *in vitro* studies was the absence of simulating human exposures, which might explain the *in vitro/in vivo* discordance above.

Herein, we evaluated the effect of human-simulated exposures of vancomycin 1g q8h in combination with cefazolin 1g q8h against various MRSA phenotypes. In this study, 4 clinical isolates (2 MRSA, 1 hVISA and 1 VISA) with various vancomycin MICs were tested. We observed that regardless of the starting bacterial inoculum, the combination of vancomycin and cefazolin improved bacterial activity over vancomycin alone when the AUBC was used to evaluate overall antibacterial effects. This difference was primarily due to the quicker reductions (i.e., by 12h) in bacterial density with the combination regimen. By the end of the 48h and 72h experiments, bacterial reductions were similar for the combination and vancomycin alone regimens, eluding to the importance of human-simulated exposures in synergy-type studies. Cefazolin alone had little effect against these isolates, as expected.

To our knowledge, this is the first *in vitro* pharmacodynamic assessment of combination therapy with human-simulated exposures of vancomycin and cefazolin against MRSA, hVISA and VISA. Studies simulating vancomycin alone, however, have been conducted, therefore allowing comparisons with some of our observations. In the
current study, simulating free vancomycin exposures after a 1g q8h dose, we anticipated poor bactericidal effects for isolates with vancomycin MICs ≥2 µg/ml because some studies have shown that a total AUC/MIC of ≥400 is needed for successful outcomes [6, 18, 23]. The mean free AUC$_{0-24}$/MIC of vancomycin observed in our experiments were 518 for Hosp 2-23 with a MIC = 0.5 µg/ml (presumed total AUC/MIC = 1032), 123 for STA 336 with a MIC = 2.0 µg/ml (presumed total AUC/MIC = 246), 119 for STA 449 with a MIC = 2.0 µg/ml (presumed total AUC/MIC = 238), and 30 for STA 454 with a MIC = 8.0 µg/ml (presumed total AUC/MIC = 60). However, we observed substantial bactericidal effects for vancomycin alone against all isolates over the 48h experiments. MacGowan and colleagues simulated free vancomycin concentrations of a 1g q12h regimen against MRSA (MIC: 2 µg/ml) and VISA (MIC: 8 µg/ml) at a starting inoculum of 10$^6$ cfu/ml and noted similar observations to our vancomycin alone results. In that study, the 3.0 log$_{10}$ cfu/ml reduction observed at 48h was similar to the reduction for all isolates with vancomycin monotherapy in the current study (range, -2.31 to -3.57) [22]. LaPlante and colleagues also evaluated the effects of vancomycin monotherapy simulated as a 1g q12h regimen against MRSA (MIC: 2 µg/ml in the presence of albumin) with an in vitro pharmacodynamic model [20]. Against this isolate, vancomycin alone also achieved substantial reductions in bacterial density over the 72h experiment. Turner and colleagues showed that after exposure to human simulated vancomycin concentrations, the vancomycin-resistant subpopulation of VISA was more rapidly eradicated from an in vitro pharmacodynamic model than the susceptible population [38]. Similarly, lower vancomycin AUC/MIC ratios were required for VISA compared with vancomycin-susceptible strains to produce a 50% maximum kill or a maximum kill in an in vivo...
animal study [9]. Hence, we speculate that the need to attain a substantially higher pharmacodynamic target in patient studies has to do with the complexity of the patient (e.g., complicated bacteremia, endocarditis, pneumonia), variability in vancomycin tissue penetration, and that clinical treatment is significantly longer than the 48h to 72h duration of our in vitro experiments.

Nevertheless, we observed that the addition of cefazolin to vancomycin increased the killing early during the experiments (i.e., 12 hours). Reductions in methicillin MICs among mutant MRSA isolates have been described previously, but the mechanisms for regaining susceptibility are not clear [25]. Presumably, these mutant strains have experienced some profound disturbance of cell wall metabolism that results in abnormal peptidoglycan composition and conditions that allow acylation of the penicillin binding protein (in this case, PBP2A) at lower antibiotic concentrations. This disturbance can be caused by exposure to other cell wall inhibitors such as vancomycin [33], and additionally has been observed in highly vancomycin resistant S. aureus strains [32]. In line with these observations, Fox and colleagues found that a combination of vancomycin and a β-lactam was more effective in an in vivo animal endocarditis model caused by vancomycin-resistant Staphylococcus aureus (VRSA) [11]. In VRSA strains caused by the vanA gene complex, the production of cell walls lacking terminal D-Ala-D-Ala residues result in the inability for cells to be cross-linked by PBP2a, thereby explaining how β-lactam susceptibility can be regained [30]. The mechanism in MRSA strains not carrying the vanA gene complex, as was the case with strains in the current experiment, is still unknown. The additive or synergistic activity observed between vancomycin and a
β-lactam may simply be due to the fact that they act at different stages of cell wall synthesis and potentiate each other's effects [4].

While every attempt was made to adequately mimic the pharmacokinetic profiles of both agents, the concentrations observed within some of the models were slightly lower than the targeted concentrations, particularly for vancomycin. Despite achieving lower concentrations than anticipated within the model, vancomycin alone still demonstrated efficacy against all isolates in this study, but one might hypothesize that higher vancomycin exposures than were observed in our studies might further minimize the benefits seen with combination therapy. Additionally, we did not attempt to measure the emergence of vancomycin resistance because there was little regrowth at 48h and 72h in any of the experiments. A final limitation to our in vitro study is that we explored only a single β-lactam, cefazolin, and there are other more potent (i.e., lower MIC) β-lactams available such as the semi-synthetic penicillins and carbapenems. During previous in vitro combination studies, the highest synergist effect was observed with vancomycin when combined with imipenem [27]. However, cefazolin is often used to treat serious S. aureus infections at a dose of 1g q8h, and its thrice daily dosing regimen made for simpler application in the Blaser model.

In conclusion, the combination of vancomycin and cefazolin at human simulated exposures improved the rate of kill against these MRSA isolates and resulted in greater overall antibacterial effect as measured by AUBC regardless of starting bacterial inoculum and experiment duration. Our observations support consideration of human exposures during in vitro combination/synergy studies.
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REFERENCES


that incorporates muramyl dipeptides into the cell wall peptidoglycan. J. Bio. Chem. 269:27246-27250.


Figure legends

**Fig. 1.** Mean bacterial densities over 48h for A) MRSA (Hosp2-23), B) MRSA (STA 336), C) hVISA (STA 449), and D) VISA (STA 454). Growth control, closed circles; vancomycin monotherapy for MRSA (Hosp2-23), closed triangles (up); MRSA (STA 336), closed triangles (down); hVISA (STA 449), closed squares; VISA (STA 454), closed diamond; combination therapy for MRSA (Hosp2-23), open triangles (up); MRSA (STA 336), open triangles (down); hVISA (STA 449), open squares; VISA (STA 454), open diamond. Data are plotted as the means of the two models for the treatments and the mean of all corresponding growth control isolates. The lower limit of detection (dashed line) was 1.7 log₁₀ cfu/ml.

**Fig. 2.** Mean bacterial densities over 72h for MRSA (336), hVISA (STA 449). Growth control, closed circles; vancomycin monotherapy for MRSA (STA 336), closed triangles (down); vancomycin monotherapy for hVISA (STA 449), closed squares; combination therapy for MRSA (STA 336), open triangles (down); combination therapy for hVISA (STA 449), open squares. Data are plotted as the means of the two models for the treatments and the mean of all corresponding growth control isolates. The lower limit of detection (dashed line) was set to 1.7 log₁₀ cfu/ml.
Table 1. Observed concentrations and calculated pharmacokinetic parameters achieved in the in vitro models

<table>
<thead>
<tr>
<th>Antibiotic Regimen</th>
<th>Peak (µg/ml)</th>
<th>Trough (µg/ml)</th>
<th>T½ (h)</th>
<th>AUC₀-2₄ (µg*h/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin 1g q8h</td>
<td>14.8 ± 0.6</td>
<td>6.6 ± 0.5</td>
<td>7.0 ± 0.6</td>
<td>244 ± 25</td>
</tr>
<tr>
<td>Cefazolin 1g q8h</td>
<td>15.3 ± 1.0</td>
<td>1.7 ± 0.2</td>
<td>2.5 ± 0.1</td>
<td>204 ± 12</td>
</tr>
</tbody>
</table>

All data presented as mean ± standard deviation.
Table 2. Mean change in bacterial density at 12 and 48 for vancomycin monotherapy, cefazolin monotherapy, and the combination against all 4 MRSA isolates with varying phenotypes at the $10^6$ cfu/ml inoculum.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Vancomycin MIC (µg/ml)</th>
<th>Change in bacterial density (log$_{10}$ cfu/ml) from 0h$^1$</th>
<th>Vancomycin</th>
<th>Cefazolin</th>
<th>Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12h</td>
<td>48h</td>
<td>12h</td>
<td>48h</td>
<td>12h</td>
</tr>
<tr>
<td>MRSA (Hosp2-23)</td>
<td>0.5</td>
<td>-3.39</td>
<td>-3.57</td>
<td>+1.57</td>
<td>+2.05</td>
</tr>
<tr>
<td>MRSA (STA336)</td>
<td>2</td>
<td>-1.94</td>
<td>-3.40</td>
<td>+1.03</td>
<td>+1.88</td>
</tr>
<tr>
<td>hVISA (STA449)</td>
<td>2</td>
<td>-2.88</td>
<td>-2.31</td>
<td>+1.00</td>
<td>+2.25</td>
</tr>
<tr>
<td>VISA (STA454)</td>
<td>8</td>
<td>-2.98</td>
<td>-3.02</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Mean: -2.80 -3.07 +1.20 +2.06 -4.31 -4.00
Standard deviation: 0.59 1.15 0.30 0.18 0.58 0.82

Note. Positive values indicate growth from 0h bacterial density, while negative numbers indicate reductions in colony forming units from 0h. ND, not done.

$^1$ Bacterial density for each isolate above is the mean of 2-4 independent experiments.
Table 3. Mean change in bacterial density at 12, 48 and 72h for vancomycin monotherapy and the vancomycin plus cefazolin combination against MRSA (STA336) and hVISA (STA449) at the 10^8 cfu/ml inoculum

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Vancomycin MIC (µg/ml)</th>
<th>Change in bacterial density (log_{10} cfu/ml) from 0h (^1)</th>
<th>Vancomycin Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12h</td>
<td>48h</td>
<td>72h</td>
</tr>
<tr>
<td>MRSA (STA336)</td>
<td>2</td>
<td>-1.11</td>
<td>-5.60</td>
</tr>
<tr>
<td>hVISA (STA449)</td>
<td>2</td>
<td>-1.10</td>
<td>-5.11</td>
</tr>
</tbody>
</table>

Mean: -1.10 -5.35 -5.37 -4.00 -6.24 -6.11
Standard deviation: 0.04 0.30 0.47 0.20 0.55 0.75

Note. Positive values indicate growth from 0h bacterial density, while negative numbers indicate reductions in colony forming units from 0h.

\(^1\) Bacterial density for each isolate above is the mean of 2-4 independent experiments.