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 against methicillin-resistant Staphylococcus aureus (MRSA) when a β-lactam is combined with vancomycin. However, these time-kill studies have neglected the importance of human-simulated exposures. We evaluated the effect of human simulated exposures of vancomycin at 1 g every 8 h (q8h) in combination with cefazolin at 1 g q8h against various MRSA isolates. Four clinical isolates (two MRSA isolates [vancomycin MICs, 0.5 and 2.0 μg/ml], a heterogeneous vancomycin-intermediate S. aureus [hVISA] isolate [MIC, 2.0 μg/ml], and a vancomycin-intermediate S. aureus [VISA] isolate [MIC, 8.0 μg/ml]) were evaluated in an in vitro pharmacodynamic model with a starting inoculum of 10⁶ or 10⁸ CFU/ml. Bacterial density was measured over 48 to 72 h. Time-kill curves were constructed, and the area under the bacterial killing and regrowth curve (AUBC) was calculated. During 10⁶ CFU/ml studies, combination therapy achieved greater log₁₀ CFU/ml changes than vancomycin alone at 12 h (−4.31 ± 0.58 versus −2.80 ± 0.39, P < 0.001), but not at 48 h. Combination therapy significantly reduced the AUBC from 0 to 48 h (122 ± 14) compared with vancomycin alone (148 ± 22, P = 0.017). Similar results were observed during 10⁸ CFU/ml studies, where combination therapy achieved greater log₁₀ CFU/ml changes at 12 h than vancomycin alone (−4.00 ± 0.20 versus −1.10 ± 0.04, P < 0.001) and significantly reduced the AUBC (275 ± 30 versus 429 ± 37, P < 0.001) after 72 h of 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.

Staphylococcus aureus causes serious infections in both the hospital and community settings, and the growing prevalence of methicillin-resistant S. aureus (MRSA) isolates 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 infections, it is less rapidly bactericidal than β-lactams against S. aureus, especially at higher inocula (34). Additionally, the recent emergence of decreased vancomycin susceptibility in S. aureus, including isolates with vancomycin MICs of 2 μg/ml, heterogenous vancomycin-intermediate S. aureus (hVISA), and vancomycin-intermediate S. aureus (VISA), presents a significant clinical problem (26, 37, 39). Sakoulas and colleagues reported a significant correlation between vancomycin susceptibilities of S. aureus isolates from blood and patient outcomes (29), and some studies revealed that patients infected with MRSA isolates having vancomycin MICs of 1 to 2 μg/ml are less likely to be successfully treated with vancomycin than patients infected with isolates demonstrating lower MICs (14, 18, 21, 23, 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 the in vitro time-kill methodology, checkerboard synergy studies, or in vivo animal studies. Onouch-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 against MRSA isolates with various degrees of susceptibility to vancomycin when a β-lactam is combined with 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 isolates (Hosp2-23 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 MICs for MRSA Hosp2-23, MRSA STA 336, hVISA STA 449, and VISA STA 454 were 0.5, 2, 2, and 8 μg/ml, respectively. All isolates were presumed to have a cefazolin MIC of ≥32 μg/ml due to their MRSA phenotype.

Antibiotics. Vancomycin analytical powder was acquired from Sigma-Aldrich, Inc. (St. Louis, MO). Cefazolin (lot 101A015; expiration date, June 2012; Apotex Corp., Weston, FL) 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 h (q8h) to simulate the peak and trough free drug concentrations achieved in the sera of healthy volunteers after intravenous doses of 1 g q8h of vancomycin infused over 1 h and 1 g q8h of cefazolin infused over 2 to 3 min (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 1 g q8h regimen was a peak of approximately 17.5 μg/ml (total concentration, 35.0 μg/ml) with a half-life of 6.5 h, thereby resulting in free trough concentrations of approximately 7.5 μg/ml (total concentration, 15.0 μg/ml). The target free antibiotic concentration for cefazolin 1 g q8h was a peak of 18.8 μg/ml (total concentration, 188.0 μg/ml) with a half-life of 2.0 h, thereby resulting in a 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 10⁸ CFU/ml for 48 h against all strains and 10⁹ CFU/ml for 72 h 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 selected only 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 inocula of 10⁶ CFU/ml were set up from an overnight culture of the test isolate for all experiments. Cation-adjusted Mueller-Hinton broth (CAMHB; Becton Dickinson and Company, Sparks, MD) was used as the bacterial growth medium for all experiments. Each infection model experiment was conducted over 72 h after inoculation of bacteria into models and then every 8 h. Fresh broth was drawn up and injected in each 300-ml model to achieve the starting inoculum of 10⁷ CFU/ml. Brain heart infusion (BHI) broth (Becton Dickinson and Company, Sparks, MD) was used as the bacterial growth medium for hVISA and VISA isolates (note that internal experiments found no difference in growth characteristics between media for any of the isolates [data not shown]). Antibiotic was administered 0.5 h after inoculation of bacteria into models and then every 8 h. 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 48 h and performed in duplicate to ensure reproducibility. For the higher inoculum studies, three independent starting inocula of 10⁸ CFU/ml were set up. Briefly, 10 ml of a 15 × 10⁸ CFU/ml (5.0 McFarland standard) bacterial suspension was centrifuged at 2,500 rpm for 20 min 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 10⁸ CFU/ml. Three milliliters was drawn up and injected in each 300-ml model to achieve the starting inoculum of 10⁷ 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 10⁷ 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 to 24 h of incubation at 37°C. The lower limit of detection for bacterial density was 1.7 log₁₀ CFU/ml.

To evaluate the antibiotic activity of each regimen, bacterial density was measured by the change in log₁₀ CFU/ml from that at 0 h 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 72 h and cefazolin at 0, 4, 8, 12, 16, 20, 24, 28, 32, 40, 48, 56, 64, and 72 h. All samples were immediately stored at −80°C 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, respectively. The inter- and intraday coefficients of variation (CVs) 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 CVs for low and high quality control samples were 2.1% and 3.1%, respectively. Those for the interday quality control samples were 5.9% and 2.6%, respectively. On the basis of the concentrations achieved in each model, the peak and trough concentrations for each drug were reported as those observed, the half-life was calculated by 0.693/k, where k was the estimated elimination rate constant calculated by the natural log of peak/trough concentration divided by the dosing interval, and the free area under the curve (AUC) over each 24-h period (AUC₀₋₂₄ μg · h/ml) was calculated using the trapezoidal rule.

#### Statistical analyses

The mean log₁₀ CFU/ml reductions of vancomycin monotherapy, cefazolin monotherapy, and combination therapy at 12, 48, and 72 h from the starting inoculum (0 h) and AUBC for each therapy were compared with each other using one-way analysis of variance (ANOVA). A P value of 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, respectively, across all experiments. Observed peak and trough concentrations of cefazolin were within −36% to 24% and −23% to 224% of target values, respectively, across all experiments. These resulted in mean vancomycin free AUC₀₋₂₄/MIC values of 518 for MRSA Hosp2-23 (MIC = 0.5 μg/ml), 123 for MRSA STA 449 (MIC = 2.0 μg/ml), 119 for hVISA STA 449 (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 assume that the cumulative percentage of a 24 h period that the free drug concentration exceeds the MIC under steady-state pharmacokinetic conditions (fT_MIC) was 0% for all isolates.

#### Antibacterial activity at 10⁶ CFU/ml inoculum

The average bacterial density of the starting inoculum was 6.29 ± 0.20 log₁₀ CFU/ml. Control isolates grew to 8.87 ± 0.22 log₁₀ CFU/ml over 48 h in the models. Figure 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 48 h against each of the 4 isolates are provided in Table 2. For the three MRSA

<table>
<thead>
<tr>
<th>Antibiotic regimen</th>
<th>Peak (μg/ml)</th>
<th>Trough (μg/ml)</th>
<th>AUC₀₋₂₄ (μg · h/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin, 1 g q8h</td>
<td>14.8 ± 0.6</td>
<td>6.6 ± 0.5</td>
<td>7.0 ± 0.6</td>
</tr>
<tr>
<td>Cefazolin, 1 g q8h</td>
<td>15.3 ± 1.0</td>
<td>1.7 ± 0.2</td>
<td>2.5 ± 0.1</td>
</tr>
</tbody>
</table>

* All data are presented as mean ± standard deviation. t½, half-life.
isolates with vancomycin MICs of $\geq 2 \mu g/ml$, colonies began to regrow after 28 h of incubation, but the counts for none of the colonies approached the initial inoculum. The AUBC for vancomycin alone over 48 h was $148 \pm 22$ and ranged from 129 to 199. A reduction in the number of $\log_{10}$ CFU/ml was not observed for cefazolin alone at 48 h for any of the isolates. The AUBC for cefazolin alone over 48 h was $357 \pm 16$ and ranged from 345 to 379.

Combination therapy significantly reduced bacterial density compared with cefazolin alone at 12 and 48 h ($P < 0.001$ and $P < 0.05$). Compared with vancomycin alone, the reduction in bacterial density was $2.80 \pm 0.59$ and ranged from 1.15 to 3.07.

### TABLE 2
Mean change in bacterial density at 12 and 48 h for vancomycin monotherapy, cefazolin monotherapy, and the combination against all 4 MRSA isolates with various phenotypes at the $10^6$ CFU/ml inoculum

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Vancomycin MIC (µg/ml)</th>
<th>Vancomycin Change</th>
<th>Cefazolin Change</th>
<th>Combination Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12 h</td>
<td>48 h</td>
<td>12 h</td>
</tr>
<tr>
<td>MRSA Hosp2-23</td>
<td>0.5</td>
<td>-3.39</td>
<td>-3.57</td>
<td>+1.57</td>
</tr>
<tr>
<td>MRSA STA 336</td>
<td>2</td>
<td>-1.94</td>
<td>-3.40</td>
<td>+1.03</td>
</tr>
<tr>
<td>hVISA STA 449</td>
<td>2</td>
<td>-2.88</td>
<td>-2.31</td>
<td>+1.00</td>
</tr>
<tr>
<td>VISA STA 454</td>
<td>8</td>
<td>-2.98</td>
<td>-3.02</td>
<td>ND</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>-2.80</td>
<td>-3.07</td>
<td>+1.20</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>0.59</td>
<td>1.15</td>
<td>0.30</td>
</tr>
</tbody>
</table>

*Positive values indicate growth from 0 h bacterial density, while negative numbers indicate reductions in numbers of CFU/ml from those at 0 h. ND, not done. Bacterial density for each isolate is the mean of 2 to 4 independent experiments.*

![FIG 1](image-url)
and hVISA STA 449 for vancomycin alone was significantly greater for the 10⁶ CFU/ml-inoculum study than the 10⁸ CFU/ml-inoculum study (−2.45 versus −1.11, P < 0.05) at 12 h, but not at 48 h. For the combination of vancomycin and cefazolin, there were no changes in bacterial density at any tested time point between the 10⁶ and 10⁸ CFU/ml inocula (P = 1.0).

**DISCUSSION**

Vancomycin has been the mainstay of MRSA therapy for several decades, despite the recognition of its important shortcomings. These shortcomings include poor tissue and intracellular penetration (17, 19, 35), 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* isolates (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 antistaphylococcus β-lactam during the initial empirical 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 described above.

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

**TABLE 3** Mean change in bacterial density at 12, 48, and 72 h for vancomycin monotherapy and the vancomycin plus cefazolin combination against MRSA STA 336 and hVISA STA 449 at the 10⁶ CFU/ml inoculum

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Vancomycin MIC (µg/ml)</th>
<th>Change in bacterial density (log₁₀ CFU/ml) from 0 h*</th>
<th>Vancomycin</th>
<th>Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12 h</td>
<td>48 h</td>
<td>72 h</td>
</tr>
<tr>
<td>MRSA STA 336 2</td>
<td>2</td>
<td>−1.11</td>
<td>−5.60</td>
<td>−5.59</td>
</tr>
<tr>
<td>hVISA STA 449 2</td>
<td>2</td>
<td>−1.10</td>
<td>−5.51</td>
<td>−5.16</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>−1.10</td>
<td>−5.35</td>
<td>−5.37</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>0.04</td>
<td>0.30</td>
<td>0.47</td>
</tr>
</tbody>
</table>

* Positive values indicate growth from 0 h bacterial density, while negative numbers indicate reductions in numbers of CFU/ml from those at 0 h. Bacterial density for each isolate is the mean of 2 to 4 independent experiments.
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, VISA, 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 1 g q8h dose, we anticipated poor bactericidal effects for isolates with vancomycin MICs of ≥2 μg/ml because some studies have shown that a total AUC/MIC of ≥400 is needed for successful outcomes (6, 18, 24). The mean fAUC0-24/MIC values of vancomycin observed in our experiments were 518 for Hosp2-23 with a MIC of 0.5 μg/ml (presumed total AUC/MIC = 1,032), 123 for STA 336 with a MIC of 2.0 μg/ml (presumed total AUC/MIC = 246), 119 for STA 449 with a MIC of 2.0 μg/ml (presumed total AUC/MIC = 238), and 30 for STA 454 with a MIC of 8.0 μg/ml (presumed total AUC/MIC = 60). However, we observed substantial bactericidal effects for vancomycin alone against all isolates over the 48 h experiments. MacGowan and colleagues simulated free vancomycin concentrations of a 1-g-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 observations similar to our results for vancomycin alone. In that study, the 3.0 log_{10} CFU/ml reduction observed at 48 h was similar to the reduction for all isolates with vancomycin monotherapy in the current study (range, −2.31 to −3.57 log_{10} CFU/ml) (22). LaPlante and Rybak also evaluated the effects of vancomycin monotherapy simulated as a 1-g-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 72 h 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 strains than for vancomycin-susceptible strains to produce a 50% maximum kill or a maximum kill in an in vivo animal model 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, and pneumonia), variability in vancomycin tissue penetration, and the fact that clinical treatment is significantly longer than the 48 h to 72 h 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 h). 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, PBP 2a) at lower antibiotic concentrations. This disturbance can be caused by exposure to other cell wall inhibitors such as vancomycin (32) and has additionally been observed in highly vancomycin-resistant S. aureus strains (31). 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 model of endocarditis caused by vancomycin-resistant Staphylococcus aureus (VRSA) (11). In VRSA strains with resistance caused by the vanA gene complex, the production of cell walls lacking terminal D-Ala-D-Ala residues results in the inability for cells to be cross-linked by PBP 2a, 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 concentrations lower than anticipated within the model, vancomycin alone still demonstrated efficacy against all isolates in this study, but one might hypothesize that vancomycin exposures higher than those 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 48 h and 72 h 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 semisynthetic penicillins and carbapenems. During previous in vitro combination studies, the highest synergistic effect was observed with vancomycin when combined with imipenem (27). However, cefazolin is often used at a dose of 1 g q8h to treat serious S. aureus infections, and its thrice-daily dosing regimen made for simpler application in the Blaser model (2).

In conclusion, the combination of vancomycin and cefazolin at human simulated exposures improved the rate of kill against these MRSA isolates and resulted in a 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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