

Efficacy of Vancomycin- β -lactam Combinations Against Heterogeneously Vancomycin-Resistant *Staphylococcus aureus* (hetero-VRSA)

There have been conflicting data about the interactions between vancomycin and β -lactam agents against *Staphylococcus aureus* strains with heterogeneous resistance to vancomycin. We evaluated the efficacy of these combinations against Mu 3 and heterogeneously vancomycin-resistant *S. aureus* (hetero-VRSA) strains which were isolated from Korean patients using a population analysis method. Antagonistic effects were observed when less than 1 $\mu\text{g/mL}$ of β -lactam antibiotics was combined with vancomycin, whereas synergistic effects were noticed with more than 4 $\mu\text{g/mL}$ of β -lactam antibiotics. The antagonistic effects at low concentrations of β -lactams were most prominent at 2 $\mu\text{g/mL}$ of vancomycin, which were the vancomycin MICs of tested hetero-VRSA strains. This study showed the variable effects of vancomycin- β -lactam combinations depending on the concentrations of β -lactam antibiotics and this property could be used to develop screening methods for hetero-VRSA strains.

Key Words : Vancomycin; Vancomycin Resistance; Antibiotics Lactam; Oxacillin; Cefotaxime; *Staphylococcus aureus*

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INTRODUCTION

Heterogeneously vancomycin-resistant *Staphylococcus aureus* (hetero-VRSA) may account for treatment failure with vancomycin in methicillin-resistant *S. aureus* (MRSA) infections and act as a precursor of *S. aureus* that are fully or intermediately resistant to vancomycin (1). Since it was introduced as a different type of vancomycin resistance, there have been many tests to isolate hetero-VRSA from clinical MRSA strains and to evaluate their clinical relevance. The detection rates varied depending on the researchers, which might have resulted from a confusing definition and unreliable screening methods of hetero-VRSA (1-3). Hetero-VRSA was defined by Aritaka et al.: as an *S. aureus* strain whose vancomycin minimum inhibitory concentration (MIC) is less than 8 $\mu\text{g/mL}$ determined by the NCCLS-based broth dilution method, which contains subpopulations of cells that are resistant to higher concentrations of

vancomycin (including 4 $\mu\text{g/mL}$ of vancomycin), and whose mutant strains with increased vancomycin resistance (a MIC of ≥ 8 $\mu\text{g/mL}$) can be obtained by the one-step vancomycin-selection procedure with a frequency of 1 in 10^6 or greater (4).

While investigators continue to search for effective combination therapies against infection caused by *S. aureus* strains with reduced susceptibilities to vancomycin, there have been contradictory results on the interaction between vancomycin and β -lactam antibiotics. Vancomycin and β -lactams showed antagonistic interactions against Mu 3, whereas other reports documented synergistic interactions between these antibiotics against other *S. aureus* strains with reduced susceptibilities to vancomycin (4-8). In order to evaluate the efficacy of vancomycin- β -lactam combinations against hetero-VRSA strains, we performed population analyses of Mu 3 and other hetero-VRSA strains in a wide range of antibiotic concentrations.

MATERIALS AND METHODS

Bacterial strains and antibiotics

Mu 3 and two other hetero-VRSA strains (Sm 1272 and Sm 1299) were used in this study. Sm 1272 and Sm 1299 were isolated from two patients who had been treated with vancomycin for MRSA infections at Samsung Medical Center, Seoul, Korea. Tested strains were cultured overnight on tryptic soy agar (BD, Cockeysville, MD, U.S.A.) with 5% sheep blood at 37 °C with aeration. Oxacillin, cefotaxime, and vancomycin were used (Sigma Chemical Co., St. Louis, MO, U.S.A.).

Susceptibility tests to vancomycin

The MIC of vancomycin was determined by both broth dilution and population analysis methods. Mueller-Hinton broth (Difco Laboratories, Detroit, MI, U.S.A.) and brain-heart infusion (BHI) agar (BD, Cockeysville, MD, U.S.A.) were used for broth dilution and population analyses, respectively. Broth dilution was performed according to the NCCLS guidelines (9). In population analysis methods, vancomycin was increased by 2 µg/mL increments from 2 to 10 µg/mL. Population analyses were done by spreading 50 µL of starting cell suspension (10^8 cfu/mL) cultured overnight and its diluents on BHI agar plates containing various concentrations of vancomycin. These plates were incubated at 37 °C for 48 hr.

Population analyses for the efficacy of vancomycin- β -lactam combinations

In order to evaluate the interaction between vancomycin and β -lactam antibiotics, population analyses with BHI agar were performed. The β -lactam antibiotics used in the study were oxacillin and cefotaxime. Vancomycin was increased by 2 µg/mL increments in the range of 0-8 µg/mL and the concentrations of β -lactam antibiotics combined with vancomycin were 0.06, 0.125, 0.5, 4, or 8 µg/mL. Each bacterial strain was cultured overnight and then plated at several dilutions on BHI agar plates containing various concentrations of vancomycin and β -lactam antibiotics. Conditions for population analysis were the same as those for the determination of MIC. All the tests were repeated over five times.

RESULTS

Vancomycin MICs of Mu 3, Sm 1272, and Sm 1299 were all 2 µg/mL by the broth dilution and population analysis methods. Oxacillin MICs of Sm 1272 and Sm 1299 were more than 128 µg/mL by disc diffusion methods. Population analysis profiles of Sm 1272 and Sm 1299 were similar to those of Mu 3 (Fig. 1). Although vancomycin MICs of

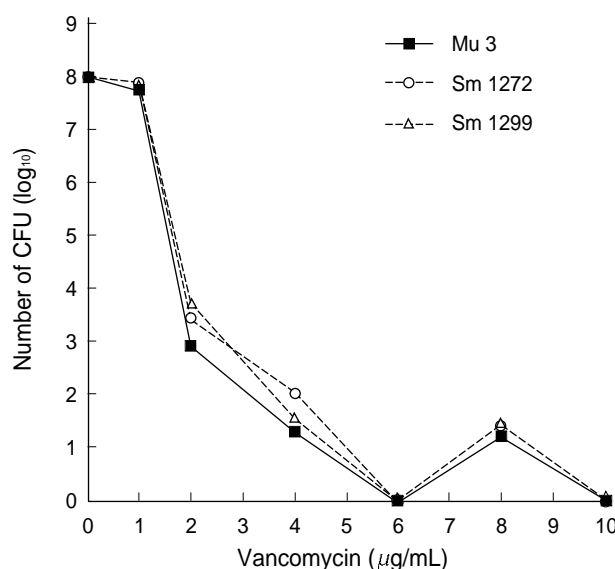


Fig. 1. Susceptibility tests to vancomycin by population analyses. Mu 3, Sm 1272, and Sm 1299 have similar population analysis profiles against vancomycin. The MICs of vancomycin, as determined from the population analysis profiles, are 2 µg/mL for Mu 3, Sm 1272, and Sm 1299. Sm 1272 grows on the agar plates containing 4 µg/mL of vancomycin at a frequency of more than 10^{-6} colonies, and some subpopulations of Sm 1299 and Mu 3 grow on the agar plates containing 8 µg/mL of vancomycin.

the tested strains were 2 µg/mL, some subpopulations of Sm 1272 and Sm 1299 grew on the agar plates containing 4 to 8 µg/mL of vancomycin, and mutant strains with vancomycin MIC of ≥ 8 µg/mL were also obtained from these strains by one-step vancomycin-passage with a frequency of $\geq 10^{-6}$. These two strains could be designated as hetero-VRSA according to definition of Aritaka et al. (4).

The results of population analyses of Mu 3, Sm 1272, and Sm 1299 against combinations of vancomycin and oxacillin are shown in Fig. 2. The number of surviving colonies increased when 0.06, 0.125, or 0.5 µg/mL of oxacillin was combined with vancomycin. When 4 or 8 µg/mL of oxacillin was combined with vancomycin, the number of surviving colonies decreased for all of the three hetero-VRSA strains. Fig. 3 shows the population analysis profiles of these strains against vancomycin and another β -lactam agent, cefotaxime. Similarly, more colonies survived when less than 1 µg/mL of cefotaxime was combined with vancomycin, while fewer colonies survived at 4 or more µg/mL of cefotaxime combined with vancomycin. The antagonistic effects were most prominent when oxacillin or cefotaxime was combined with 2 µg/mL of vancomycin, which were vancomycin MICs of tested hetero-VRSA strains.

DISCUSSION

S. aureus continues to be one of the most common causes

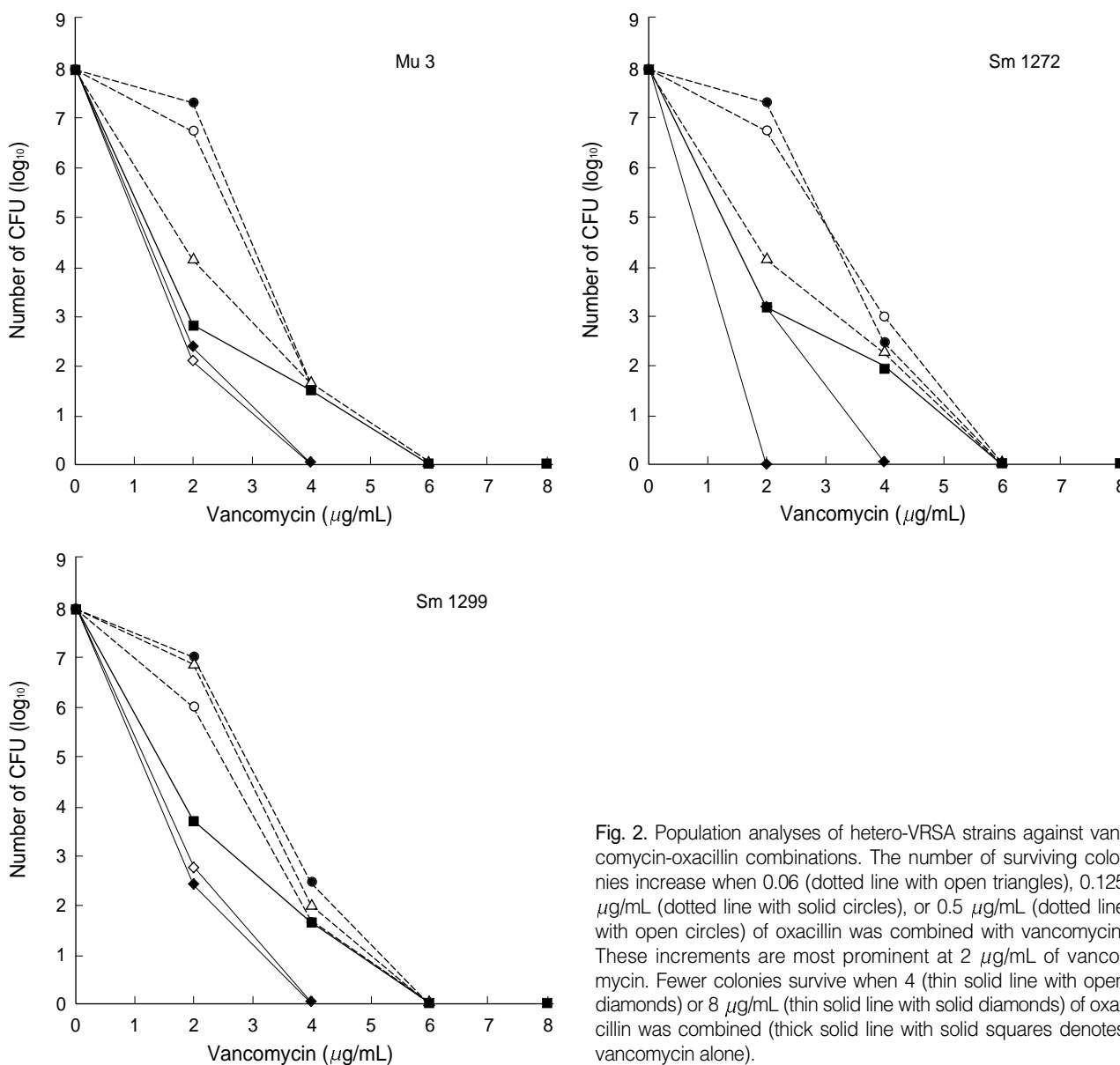


Fig. 2. Population analyses of hetero-VRSA strains against vancomycin-oxacillin combinations. The number of surviving colonies increase when 0.06 (dotted line with open triangles), 0.125 $\mu\text{g/mL}$ (dotted line with solid circles), or 0.5 $\mu\text{g/mL}$ (dotted line with open circles) of oxacillin was combined with vancomycin. These increments are most prominent at 2 $\mu\text{g/mL}$ of vancomycin. Fewer colonies survive when 4 (thin solid line with open diamonds) or 8 $\mu\text{g/mL}$ (thin solid line with solid diamonds) of oxacillin was combined (thick solid line with solid squares denotes vancomycin alone).

of nosocomial and community-acquired infections in the world. The spread of MRSA in hospitals and in the community resulted in widespread vancomycin use in *S. aureus* infections (10, 11). With the widespread use of vancomycin, the first clinical isolate of *S. aureus* with intermediate resistance to vancomycin (VISA) was reported from Japan in 1996 and multiple strains of *S. aureus* with reduced susceptibilities to vancomycin have been reported in other countries (12-17). The emergence of reduced vancomycin susceptibility in *S. aureus* made many clinicians concerned about the possibility that some strains would become fully resistant, and actually vancomycin-resistant *S. aureus* (VRSA) strains have been isolated from Michigan and Pennsylvania in 2002 (18, 19).

The advance of VRSA has added a grave concern to the

therapeutic dilemma caused by the presence of multidrug-resistant organisms in recent years. Therapeutic failures with vancomycin, however, have already been reported for infections caused by MRSA strains (20). It was suggested that the presence of hetero-VRSA strains among MRSA strains might be responsible for vancomycin failure against MRSA infections, and that VRSA strains might have originated from pre-existing MRSA strains after prolonged exposure to vancomycin through hetero-VRSA (4). This possibility indicated a need to survey for the presence of hetero-VRSA strains among MRSA isolates and to evaluate their clinical relevance. The original screening methods suggested by Hiramatsu et al. (1), however, were found to have poor reproducibility and may select vancomycin resistance (21). This may partly explain the variable detection rates (0-47%) of hetero-VRSA strains and

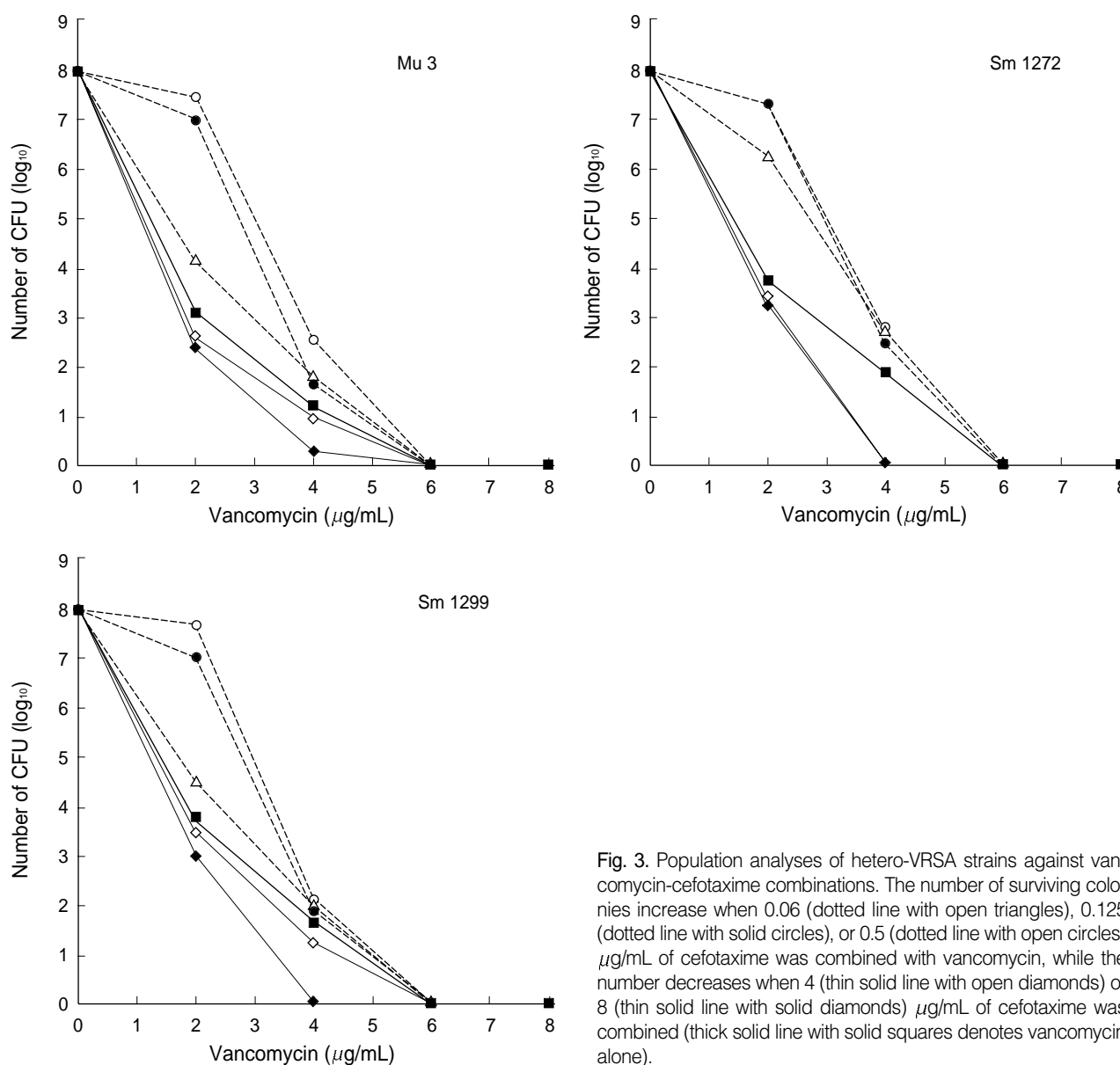


Fig. 3. Population analyses of hetero-VRSA strains against vancomycin-cefotaxime combinations. The number of surviving colonies increase when 0.06 (dotted line with open triangles), 0.125 (dotted line with solid circles), or 0.5 (dotted line with open circles) $\mu\text{g/mL}$ of cefotaxime was combined with vancomycin, while the number decreases when 4 (thin solid line with open diamonds) or 8 (thin solid line with solid diamonds) $\mu\text{g/mL}$ of cefotaxime was combined (thick solid line with solid squares denotes vancomycin alone).

the conflicting data about their clinical significance (1-3, 16). Some researchers reported vancomycin failure for treatment of hetero-VRSA infections, while others presented most of hetero-VRSA infections had been controlled successfully with vancomycin alone (2, 16). The exact clinical significance of hetero-VRSA infection awaits a well-designed prospective case-controlled study.

For the purpose of discovering effective therapeutic regimens for infections caused by *S. aureus* strains with reduced susceptibility to vancomycin, combination methods of current antibiotics have been tested in many laboratories. Hanaki *et al.* documented antagonistic interactions between vancomycin and ceftizoxime against Mu 3, and he developed Mu 3 agar on the basis of these findings for the detection of hetero-VRSA (6). Besides these, many other results have been

reported regarding the efficacy of vancomycin- β -lactam combinations against *S. aureus* strains with reduced susceptibilities to vancomycin-synergistic (or additive) and antagonistic effects (7, 8, 22-26).

This study showed antagonistic effects against hetero-VRSA strains when low concentrations ($<1.0 \mu\text{g/mL}$) of oxacillin or cefotaxime were combined with vancomycin, while synergistic effects were attained when high concentrations ($>4.0 \mu\text{g/mL}$) of β -lactams were combined (oxacillin MICs of hetero-VRSA strains tested in this study were more than 128 $\mu\text{g/mL}$). Haraga *et al.* also performed population analyses with several hetero-VRSA strains to evaluate combination effects (22). Their data demonstrated similar results to ours, that is antagonistic effects at $<1 \mu\text{g/mL}$ but synergistic effects at $>8 \mu\text{g/mL}$ of β -lactam antibiotics (in their data, they did

not evaluate combination effects with 4 $\mu\text{g/mL}$ of β -lactams). Aritaka et al. (4) and Howe et al. (23) each presented that β -lactams showed antagonism at concentrations below their MICs but synergy at concentrations near the MICs, against hetero-VRSA strains, when combined with vancomycin. In data presented by Hiramatsu et al. the concentrations of β -lactams showing antagonism against Mu 3 were 0.125–4 $\mu\text{g/mL}$ for ampicillin and 0.03–4 $\mu\text{g/mL}$ for oxacillin, while those demonstrating synergy were 8–16 $\mu\text{g/mL}$ for ampicillin (MIC, 32 $\mu\text{g/mL}$) and 512 $\mu\text{g/mL}$ for oxacillin (MIC, 1,024 $\mu\text{g/mL}$). Most of data consistently presented antagonism when low concentrations (<1.0 $\mu\text{g/mL}$) of β -lactams were combined with vancomycin, regardless of the testing methods. On the contrary, there was great discrepancy between the concentrations of β -lactams showing synergy of the individual data: from 4 or 8 $\mu\text{g/mL}$ of oxacillin in our data to 512 $\mu\text{g/mL}$ of the same antibiotic. In data presented by Hiramatsu et al. This discrepancy may come from the difference of testing methods. Instead of population analyses used by our and Haraga's study, Hiramatsu et al. adopted the broth dilution methods and Howe used the E-test to evaluate the combination effects (4, 22, 23). There are also other reports regarding the combination effects of vancomycin- β -lactams (7, 8, 24–26). It seems difficult, however, to compare those data with ours directly since the testing methods and tested strains were different from ours. The proper methods for evaluating vancomycin- β -lactam combination effects against hetero-VRSA strains as well as the more reliable screening methods for hetero-VRSA should be developed.

Although the mechanism of antagonism or synergy depending on the concentrations of β -lactams is unknown, it is suggested that the existence of low concentrations of β -lactam antibiotics may induce activation of cell wall synthesis or reduce the cross-linking of peptidoglycan of hetero-VRSA strains. Reduced cross-linking increases the number of D-alanyl-D-alanine residues in the cell wall, which trap more vancomycin molecules within the cell wall layers, thereby preventing the access of vancomycin molecules to their target on the cytoplasmic membrane (4). Inversely, high concentrations of β -lactam antibiotics may cause inhibition of synthesis of proteins essential for the expression of vancomycin resistance, producing synergy (23).

The concentrations of oxacillin showing synergy in this study (4 or 8 $\mu\text{g/mL}$) are clinically achievable, but those in data presented by Hiramatsu et al. (512 $\mu\text{g/mL}$) are not (4). With all data accumulated so far, it is difficult to conclude that vancomycin- β -lactam combinations are synergistic or antagonistic against hetero-VRSA strains at clinically achievable concentrations of β -lactams. The more in vitro and in vivo studies are required to elucidate the real efficacy of vancomycin- β -lactam combinations at clinically achievable concentrations against hetero-VRSA strains. Interestingly, synergistic or antagonistic effects of vancomycin- β -lactam combinations depending on the concentrations of β -lactams seem to

be unique for hetero-VRSA strains, not observed against most of MRSA or VISA strains. This property can be used to develop the more reliable methods to screen hetero-VRSA from MRSA strains.

Despite continuing debates about the efficacy of vancomycin- β -lactam combinations, this study presents evidence reminding that we must be prudent in choosing such a combination for treating infections caused by *S. aureus* with possibly reduced susceptibilities to vancomycin in patients who have a history of prolonged exposure to vancomycin.

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