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Induction of Daptomycin Heterogeneous Susceptibility in *Staphylococcus aureus* by Exposure to Vancomycin

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We studied vancomycin and daptomycin susceptibility in methicillin-resistant *Staphylococcus aureus* from patients exposed to vancomycin, glycopeptide-intermediate *S. aureus*, and *S. aureus* passaged in vancomycin-containing medium. A correlation between vancomycin and daptomycin heteroresistance was noted in some strains, suggesting that exposure of *S. aureus* to vancomycin may affect susceptibility to daptomycin.

Daptomycin demonstrates potent bactericidal activity against methicillin-resistant *Staphylococcus aureus* (MRSA) (1, 5). By population analysis, *S. aureus* exhibits calcium-dependent heterogeneous-type susceptibility to daptomycin (17). Preliminary daptomycin population analysis of MRSA recovered from vegetations from vancomycin-treated rats during a previous study of experimental aortic valve endocarditis demonstrated a subtle but consistent increase in heterogeneity of susceptibility to daptomycin. This led us to further investigate possible cross-heteroresistance between vancomycin and daptomycin.

We examined several previously described glycopeptide-intermediate *S. aureus* (GISA) and hetero-GISA isolates: Mu3 (hetero-GISA, Japan), Mu50 (GISA, Japan), PC-3 (GISA, New York), and HIP5836 (GISA, New Jersey). We also evaluated series of isolates from patients treated with vancomycin for MRSA bloodstream infections in whom subsequent isolates with hetero-GISA or GISA emerged. These include patient 1 (Maryland) strains A8090 (MRSA), A8091 (hetero-GISA), and A8094 (GISA); patient 2 (Massachusetts-1) strains A6224 (MRSA) and A6226 (GISA); patient 3 (Massachusetts-2) strains A6300 (MRSA) and A6298 (hetero-GISA); and patient 4 (Missouri) strains A5937 (MRSA) and A5940 (hetero-GISA). Isolates from patient 1 were kindly provided by James Dick (Johns Hopkins University, Baltimore, MD) (16). Isolates from patients 2 to 4 were characterized previously (11). All isolates within each series were indistinguishable by pulsed-field gel electrophoresis (8). These patients had not been treated with daptomycin.

MRSA32, a strain previously used to evaluate the efficacy of daptomycin in a rat model of aortic valve endocarditis, (14), was passaged in brain heart infusion (BHI) broth containing incrementally higher concentrations of daptomycin, yielding daptomycin-nonsusceptible MRSA32-D. MRSA A8821 (the first of a series of MRSA clinical bloodstream isolates from a patient with recurrent MRSA bacteremia) (9) and the well-studied strain MRSA COL were passaged in BHI broth containing incrementally higher concentrations of vancomycin,

TABLE 1. Susceptibility of *S. aureus* strains to daptomycin and vancomycin

Strain	Daptomycin MIC (μg/ml)			Vancomycin MIC (μg/ml)
	Broth method with Ca^{2+} at:		Etest	
	50 μg/ml	100 μg/ml		
Patient series				
A8090	0.5	1	0.38	1
A8091	1	1	ND[a]	2
A8094	2	2	2.0	8
A6224	0.5	0.5	0.5	2
A6226	2	1	2.0	8
A6300	1	1	0.5	2
A6298	2	1	2.0	4
A5937	0.25–0.5	0.5	0.5	2
A5940	0.5	0.5	0.5	4
Laboratory strains				
MRSA COL	0.5–1	ND	ND	1
MRSA COL (GISA)	1	ND	ND	8
A8821	0.5–1	ND	ND	1
A8821-GISA	1	ND	ND	8
MRSA 32	1	0.5	0.75	1
MRSA 32D	2–4	2	3.0	1
Mu3	1	0.5	ND	2
Mu50	0.5	0.5	ND	4
PC-3	1	1	ND	8
HIP-5836	0.5	0.5	ND	8
Controls				
S. aureus ATCC 33591	0.5	0.5	0.5	1
S. aureus ATCC 29213	0.5	0.5	0.5	ND

[a] ND, not determined.

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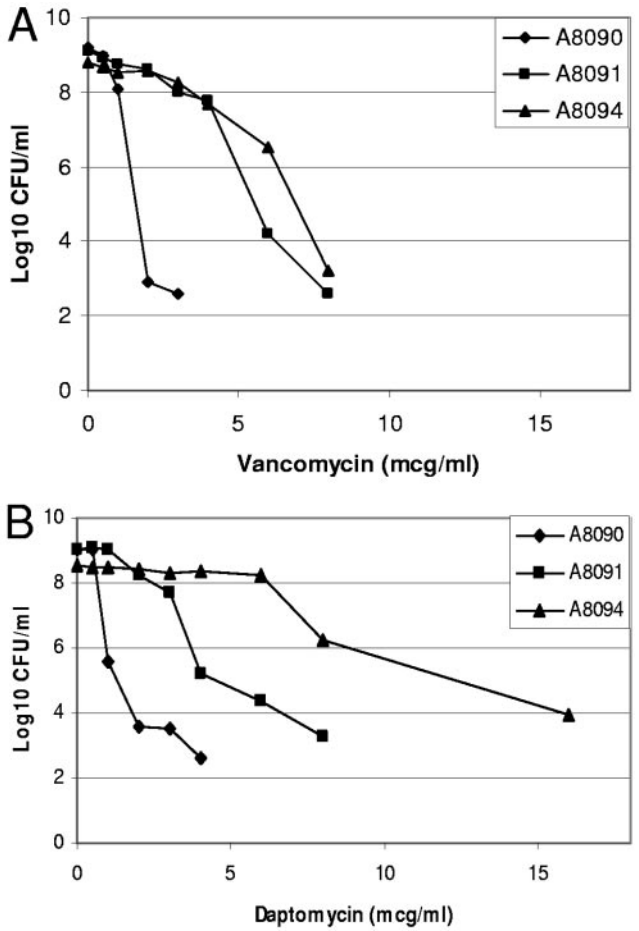

FIG. 1. Population analysis for vancomycin (A) and daptomycin (B) of serial bloodstream isolates from patient 1 (A8091, A8092, and A8095).

yielding A8821-GISA and MRSA-COL (GISA), respectively. *S. aureus* ATCC 33591 (MRSA) and *S. aureus* ATCC 29213 (methicillin-susceptible *Staphylococcus aureus*) were used as controls.

Daptomycin susceptibility testing was performed in duplicate by gradient diffusion using daptomycin Etest strips (AB Biodisk, Solna, Sweden) and by broth macrodilution (inoculum, 5×10^5 CFU/ml) using cation-adjusted Mueller-Hinton broth (Becton Dickinson, Cockeysville, MD) supplemented to contain final calcium concentrations of 50 μg/ml or 100 μg/ml. Susceptibility to vancomycin (American Pharmaceutical Partners, Inc., Los Angeles, CA) was performed by the agar dilution methods of the Clinical and Laboratory Standards Institute (10).

Population analyses with vancomycin and daptomycin were performed in duplicate as previously described (11, 12). For daptomycin analyses, BHI agar was supplemented with a calcium concentration of 30 μg/ml. Isolates with reduced susceptibility to glycopeptides were referred to as GISA if they demonstrated a vancomycin MIC of 8 to 16 μg/ml using CLSI methods and hetero-GISA if the MIC of vancomycin was ≤4 μg/ml, but detectable subpopulations were demonstrated in

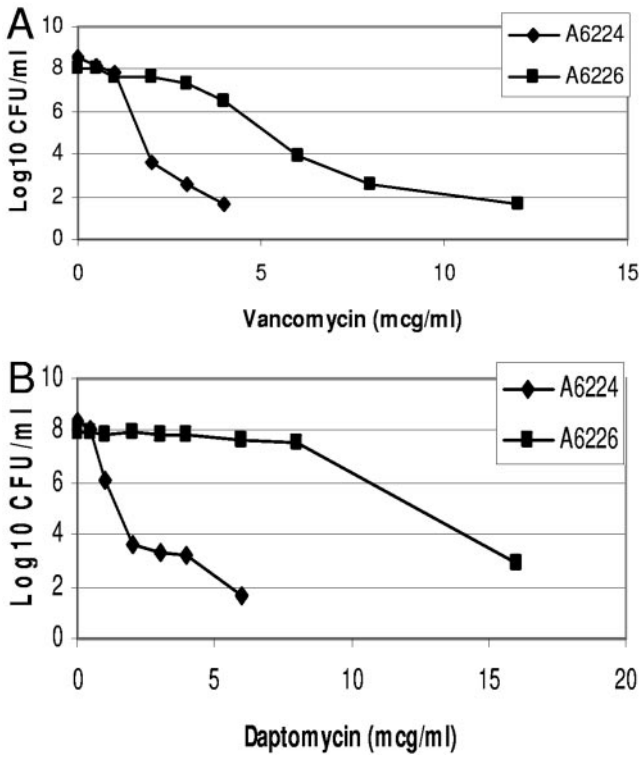

FIG. 2. Population analysis for vancomycin (A) and daptomycin (B) of serial bloodstream isolates from patient 2 (A6224 and A6226).

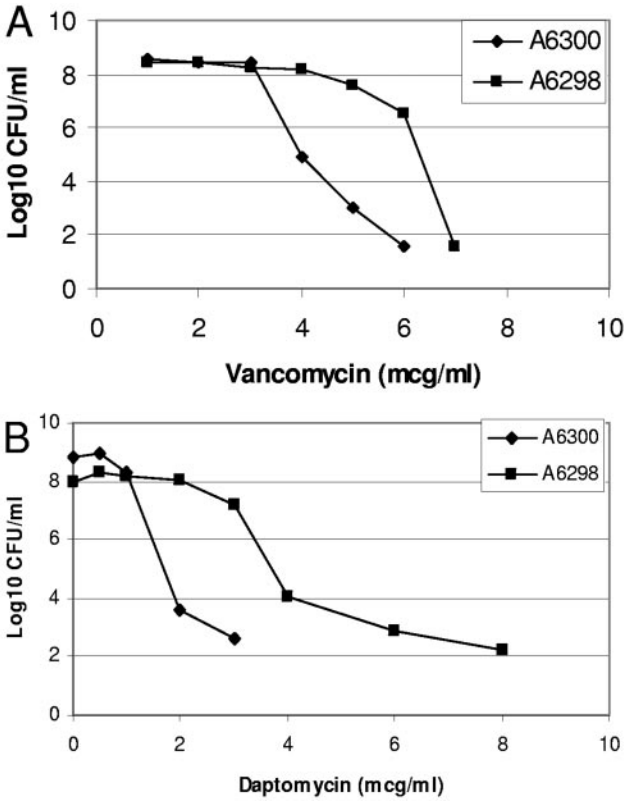

FIG. 3. Population analysis for vancomycin (A) and daptomycin (B) of serial bloodstream isolates from patient 3 (A6300 and A6298).

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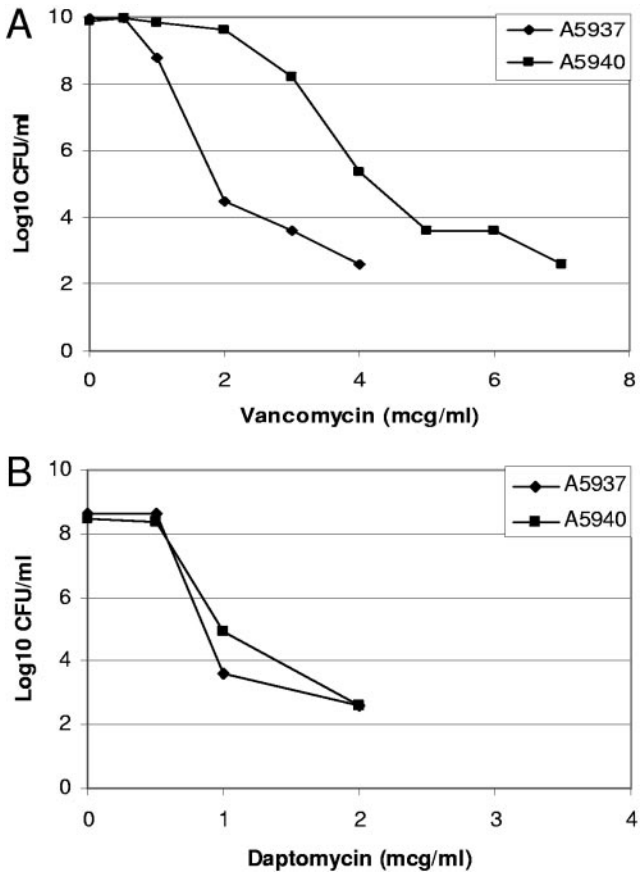

FIG. 4. Population analysis for vancomycin (A) and daptomycin (B) of serial bloodstream isolates from patient 4 (A5937 and A5940).

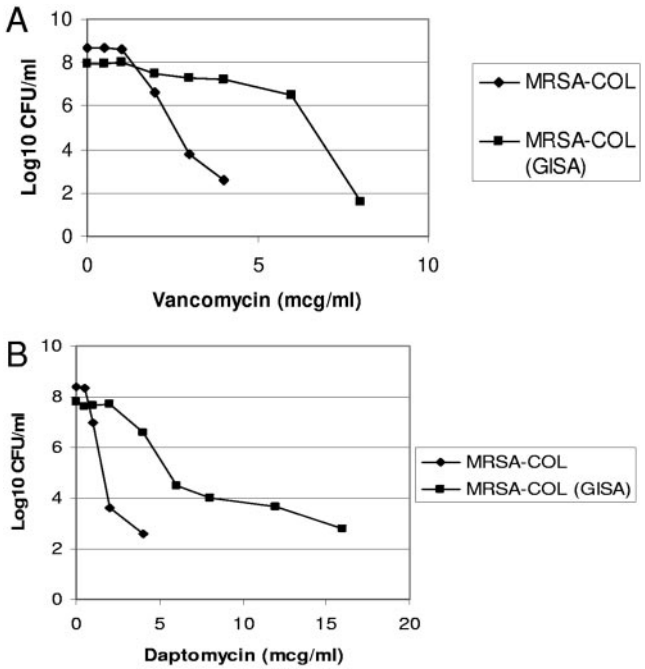

FIG. 5. Population analysis for vancomycin (A) and daptomycin (B) for MRSA COL and MRSA COL (GISA). The latter strain was derived by serial passage of MRSA COL in BHI broth containing incrementally higher concentrations of vancomycin.

solid medium containing >4 μg/ml vancomycin during population analysis (3, 19).

For killing curve assays, 10^7 CFU obtained after 12 to 16 h of growth in Mueller-Hinton broth was inoculated into 20 ml Mueller-Hinton broth containing antibiotics at the specified concentrations. Killing assays utilizing daptomycin (32 μg/ml) were performed in broth supplemented to a final calcium concentration of 50 μg/ml.

Antimicrobial susceptibilities for the bacterial strains are shown in Table 1. For three of the four MRSA strains which developed glycopeptide heteroresistance on vancomycin therapy, there was a small increase in the MIC of daptomycin into the nonsusceptible range (A6298, A6226, and A8094). The fourth patient series yielded an isolate (A5940) for which the daptomycin MIC was not increased from that of the original strain (A5937). The use of medium containing 100 μg/ml attenuated but did not necessarily eliminate the differences in daptomycin heteroresistance that emerged on vancomycin therapy.

The population analysis for daptomycin and vancomycin are shown for the bacteremia cases in Fig. 1 to 4. In three of the four patient series, treatment with vancomycin was associated with the development of vancomycin heterogeneous resistance and accompanied by daptomycin heteroresistance. A minimal if any difference in heteroresistance was seen between A5937 and A5940, the fourth pair of isolates shown in Fig. 4.

The vancomycin and daptomycin population analyses of MRSA-COL (GISA) and A8821-GISA are shown in Fig. 5 and 6, respectively. There was clear and reproducible heteroresistance to daptomycin which appeared coincident with the development of the GISA phenotype. Differences in daptomycin MICs were less evident (Table 1) than in the clinical series and daptomycin susceptibility was preserved.

MRSA 32D retained susceptibility to vancomycin, but demonstrated growth of subpopulations with vancomycin at 2 μg/ml and 3 μg/ml, a feature not present in the parent strain, MRSA 32 (Fig. 7).

Killing assays of two clinical pairs of isolates where heterogeneous resistance to vancomycin developed on therapy are shown in Fig. 8, demonstrating that daptomycin retained bactericidal activity against both the parent and the glycopeptide-heteroresistant progeny but that killing was less pronounced at 4 h.

These results suggest that, in MRSA, physiologic changes may occur with vancomycin exposure that influence daptomycin susceptibility. Daptomycin exerts its bactericidal effects by binding to the bacterial cell membrane, resulting in efflux of potassium ions into the cell and subsequent cell death (2, 17, 18). Vancomycin exerts its activity on the cell wall (6, 7). GISA strains demonstrate thicker cell walls with binding sites that sequester the drug (4, 6, 15). It is conceivable that the cell wall changes observed in GISA strains may interfere with daptomycin activity by decreasing the ability of daptomycin, a relatively large molecule, to access relevant binding regions on the bacterial cell membrane.

These subtle microbiological effects may have consequences on the development of daptomycin resistance. We have previ-

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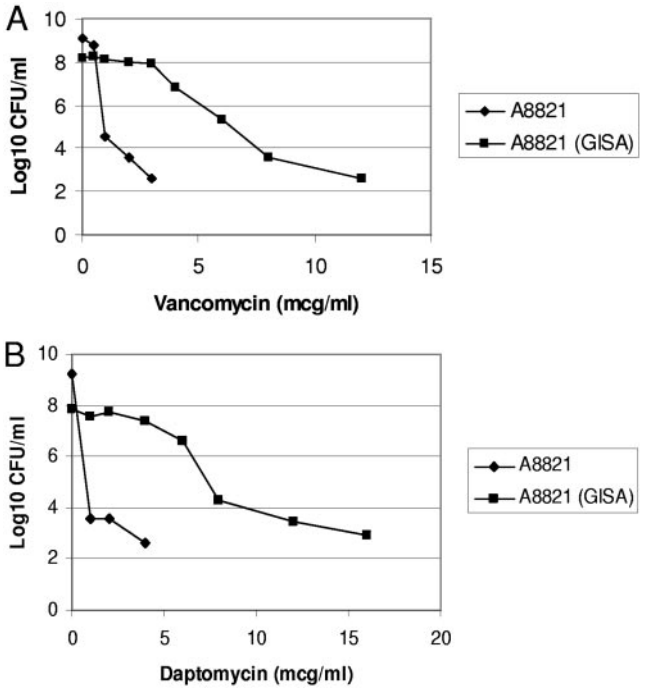

FIG. 6. Population analysis for vancomycin (A) and daptomycin (B) for MRSA bloodstream isolates A8821 and A8821-GISA, the latter derived from the former by in vitro vancomycin exposure methods similar to those described for MRSA COL.

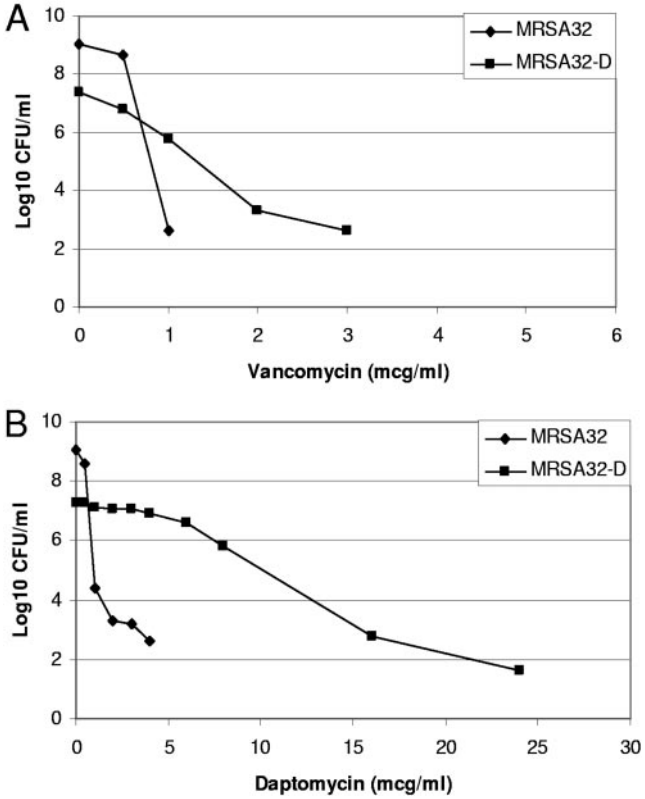

FIG. 7. Population analysis for vancomycin (A) and daptomycin (B) for MRSA 32 and MRSA 32-D. MRSA 32-D was obtained after sequential passage of MRSA 32 in BHI broth containing incrementally higher concentrations of daptomycin.

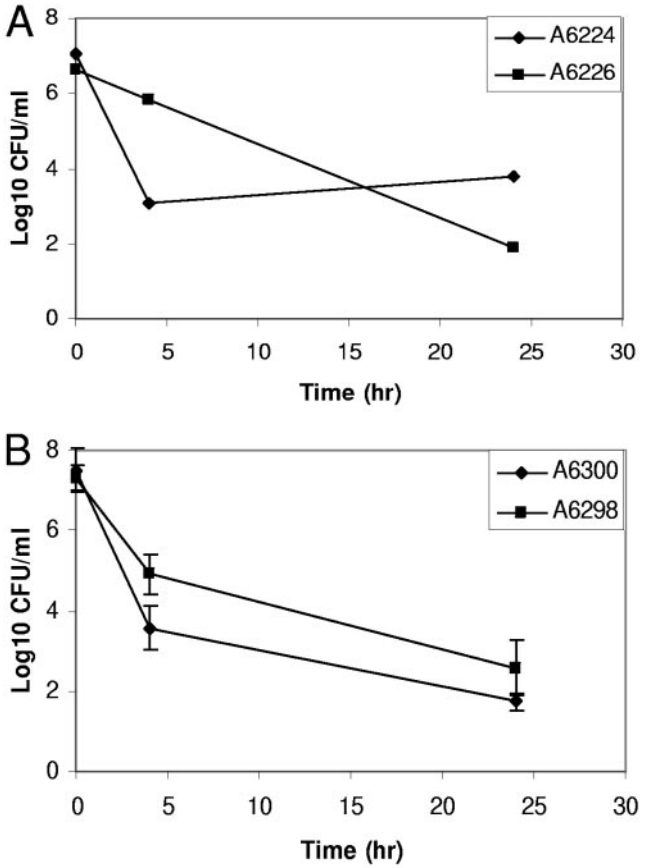

FIG. 8. Killing assays in cation-adjusted Mueller Hinton broth containing 32 μg/ml of daptomycin supplemented with 50 μg/ml calcium chloride. Panel A shows results for MRSA isolates A6224 and A6226 obtained from patient 2 (population analyses shown in Fig. 2). Panel B shows results for isolates A6300 and A6298 obtained from patient 3 (population analyses shown in Fig. 3). Panel A shows the means of one experiment sampled in duplicate. Panel B shows the means of two experiments performed in duplicate, with standard errors.

ously reported that the rate of clinical treatment failures of vancomycin for MRSA bacteremia may increase with minor increases in vancomycin MIC (13). The emergence of subpopulations with heterotypic decreases in susceptibility to daptomycin among organisms sequestered in compartments where daptomycin concentrations do not reach bactericidal concentrations can be envisioned as a first step leading to the emergence of resistance to this antibiotic.

Recent data comparing daptomycin with vancomycin in MRSA bacteremia suggest that the two drugs are comparable (44% versus 32%, respectively, intent-to-treat population) (V. Fowler, S. Cosgrove, E. Abrutyn, H. Boucher, H. Chambers, G. Corey, I. Demeyer, S. Filler, D. Levine, A. Link, M. Rupp, and A. W. Karchmer, Abstr. 45th Intersci. Conf. Antimicrob. Agents Chemother., abstr. K-426a, 2005). Of interest, daptomycin MICs increased to the nonsusceptible range in 6 of 19 patients with persistent or relapsing MRSA infection, all of whom had received vancomycin previously. In the context of these clinical data, our microbiologic findings suggest that the activity of daptomycin against MRSA may be exploited to best effect in patients who are treated with daptomycin early in their

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course. Patients heavily exposed to vancomycin prior to treatment with daptomycin may be at increased risk of a suboptimal response to daptomycin if a nonsusceptible subpopulation emerges.

Further studies are needed to evaluate the clinical significance of these findings and the probability of the development of daptomycin resistance in *S. aureus* with reduced susceptibility to vancomycin.

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