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Activity of Telavancin against Staphylococci and Enterococci Determined by MIC and Resistance Selection Studies[▽]

Klaudia Kosowska-Shick, Catherine Clark, Glenn A. Pankuch, Pamela McGhee, Bonifacio Dewasse, Linda Beachel, and Peter C. Appelbaum*

Department of Pathology, Hershey Medical Center, Hershey, Pennsylvania 17033

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This study used CLSI broth microdilution to test the activity of telavancin and comparator antimicrobial agents against 67 methicillin (meticillin)-resistant *Staphylococcus aureus* (MRSA) isolates. Twenty-six vancomycin-intermediate *S. aureus* (VISA) strains were among the isolates tested; all strains were susceptible to telavancin at ≤1 μg/ml, whereas 12/26 (46%) of these isolates were nonsusceptible to daptomycin at the same concentration. All strains were susceptible to quinupristin-dalfopristin, while resistance was found to all other drugs tested. Telavancin demonstrated potent activity against all vancomycin-susceptible isolates as well as against heterogeneously VISA and VISA resistance phenotypes. In multistep resistance selection studies, telavancin yielded one stable mutant after 43 days in one MRSA strain out of the 10 MRSA strains tested with the MIC rising eightfold from 0.25 μg/ml (parent) to 2 μg/ml. MICs for this clone did not increase further when passages were continued for the maximum 50 days. In contrast, daptomycin selected stable resistant clones (MIC increase of >4×) after 14 to 35 days in 4 of 10 MRSA strains with MICs increasing from 1 to 2 μg/ml (parents) to 4 to 8 μg/ml (resistant clones). Sequencing analysis of daptomycin resistance determinants revealed point mutations in the *mprF* genes of all four stable daptomycin-resistant clones. Teicoplanin gave rise to resistant clones after 14 to 21 days in 2 of 10 MRSA strains with MICs rising from 1 to 2 μg/ml (parents) to 4 to 16 μg/ml (stable resistant clones). Linezolid selected stable resistant clones after 22 to 48 days in 2 of 10 MRSA strains with MICs rising from 2 to 4 μg/ml (parents) to 32 μg/ml (resistant clones). Vancomycin yielded no resistant clones in 10 MRSA strains tested; however, MICs increased two- to fourfold from 1 to 8 μg/ml to 2 to 16 μg/ml after 50 days. No cross-resistance was found with any clone/antimicrobial combination. The two enterococci developed resistance to daptomycin, and one developed resistance to linezolid. Single-step mutation frequencies for telavancin (<4.0×10^{-11} to <2.9×10^{-10} at 2× MIC) were lower than the spontaneous mutation frequencies obtained with the comparators.

Staphylococcus aureus is becoming increasingly resistant to antibiotics. Methicillin (meticillin)-resistant *S. aureus* (MRSA) strains are increasingly encountered all over the world and cannot be treated with existing ß-lactams. Additionally, the majority of hospital-acquired methicillin-resistant (and also some methicillin-susceptible) strains are resistant to all currently available quinolones. The situation has become more complicated by the appearance of heterogeneously vancomycin-intermediate *S. aureus* (hVISA) strains, vancomycin-intermediate *S. aureus* (VISA) strains, and recently nine reported vancomycin-resistant *S. aureus* (VRSA) strains (1). Two recent papers emphasize the recent spread of VISA strains in Turkey (47) and France (15), and a recent alert from the New York City Department of Health (11) has documented six cases of VISA infections in New York City, NY, between February and October 2007, which led to four fatalities. It seems clear that VISA phenotypes occur everywhere but that they are not being routinely detected due to lack of standardized methodology (1, 20). Recently, Rybak et al. (43) have indicated, with Etest macromethod and population analysis testing, that the incidence of hVISA strains has increased over the past 22 years to an overall incidence of 2.2%. Yusof et al. (55) have recently described the utility of the Etest macromethod using a double-sided vancomycin-teicoplanin Etest strip which accurately differentiates between hVISA and VISA strains. Utilization of the latter method will surely increase reports of the incidence of hVISA and VISA strains. As of this time, the pathogenicity of VRSA strains awaits confirmation.

The situation has become further complicated by the appearance and rapid spread, especially in the United States, of community-acquired MRSA strains that are especially virulent, possibly by virtue of production of Panton-Valentine leukocidin (4, 10, 16, 28, 34, 37). Although these strains are currently more susceptible to antimicrobial agents than hospital-acquired strains are, this situation will surely change. Additionally, treatment of the community-acquired MRSA strains with glycopeptides will increase the selective pressure leading to nonsusceptibility to vancomycin and teicoplanin. Recently, we and others have documented clinical development of daptomycin resistance after daptomycin therapy (25), and not all VISA strains are daptomycin susceptible (1, 19, 25, 26). There is an urgent need for new agents to treat MRSA infections.

Telavancin is an investigational lipoglycopeptide active (MICs of ≤1 μg/ml) against gram-positive organisms including MRSA (17, 18, 22, 29, 31, 38, 48). Barcia-Macay and coworkers (3) have reported telavancin MICs of 0.5 μg/ml against two strains of VISA and of 2 to 4 μg/ml against two strains of VRSA. Leuthner and coworkers in a study of 50 glycopeptide-nonsusceptible staphylococci and 3 VRSA strains showed tela-

* Corresponding author. Mailing address: Department of Pathology, Hershey Medical Center, P.O. Box 850, Hershey, PA 17033. Phone: (717) 531-5113. Fax: (717) 531-7953. E-mail: pappelbaum@psu.edu.

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TABLE 1. Microdilution MICs (μg/ml) of 67 MRSA strains

Drug	MICs for VSSA strains (n = 33)		MIC range for hVISA strains (n = 2)	MICs for VISA strains (n = 26)		MIC range for VRSA strains (n = 6)
	Range	MIC_{50}/MIC_{90}		Range	MIC_{50}/MIC_{90}	
Telavancin	0.25–1	0.25/0.25	0.25–0.5	0.25–1	0.5/1	2–4
Vancomycin	0.5–1	1/1	2	2–8	4/8	>32
Teicoplanin	0.25–2	0.5/1	2–8	1–32	4/16	8–>32
Daptomycin	0.25–1	0.5/0.5	0.5–1	0.5–>1	1/>1	0.25–1
Linezolid	2–>4	4/4	2–4	≤0.5–4	2/4	2–4
Telithromycin	0.12–>8	0.12/>8	>8	0.06–>8	>8/>8	>8
Oxacillin	>4	>4/>4	>4	0.5–>4	>4/>4	>4
Quinipristin-dalfopristin	0.25–1	0.5/1	0.5	≤0.12–2	0.5/1	0.5–1
Clindamycin	≤0.5–>4	≤0.5/>4	>4	≤0.5–>4	>4/>4	>4
Ciprofloxacin	0.25–>8	0.5/>8	>8	1–>8	>8/>8	>8
Erythromycin	2–>16	>16/>16	>16	0.25–>16	>16/>16	>16
Gentamicin	0.25–>16	0.5/1	0.25–0.5	0.25–>16	1/>16	0.5–>16
Trimethoprim-sulfamethoxazole[a]	≤0.5–>4	≤0.5/≤0.5	≤0.5	≤0.5–>4	≤0.5/>4	≤0.5–>4

[a] MICs reported based on trimethoprim concentration.

vancin to be potent against all 37 hVISA and VISA strains (13 coagulase-negative strains; resistance phenotypes not differentiated from one another) with MICs of <1 μg/ml, with higher MICs of 2 to 4 μg/ml against the 3 VRSA strains (33). The antibacterial mode of action of telavancin results from inhibition of bacterial cell wall synthesis and interference with the barrier function of the bacterial cell membrane (22). The mode of action of daptomycin also involves targeting the bacterial cell membrane to initiate antimicrobial activity (2, 24, 26). This fact is supported by the involvement of staphylococcal genes regulating cell membrane surface charge (e.g., *mprF*) (39) and fatty acid synthesis (e.g., *yycG*) (36) in the development of daptomycin nonsusceptibility.

In an effort to expand the comparative activity of telavancin against MRSA strains of various resistance phenotypes, we have investigated the activity of telavancin against MRSA strains by determining the activities of telavancin and comparator agents against 67 vancomycin-susceptible and -nonsusceptible MRSA strains using broth microdilution and also by testing the potential of telavancin and the comparator agents vancomycin, teicoplanin, daptomycin, and linezolid to select for resistance in 10 MRSA strains as well as two strains of enterococci by single-step and multistep selection methodology. Additionally, we conducted molecular genetic studies to characterize the mechanism(s) of daptomycin resistance in isolated clones.

(Part of this study was presented at the 48th Interscience Conference on Antimicrobial Agents and Chemotherapy and the 46th Annual Meeting of the Infectious Disease Society of America, a joint meeting held in Washington, DC, in 2008 [12].)

MATERIALS AND METHODS

Bacteria. The antimicrobial activities of telavancin and comparator agents were determined against 67 recently isolated strains of MRSA. These comprised 33 vancomycin-susceptible *S. aureus* (VSSA) strains, 2 hVISA strains, 26 VISA strains, and 6 VRSA strains. The hVISA strains were isolated and identified in our laboratory by the Etest macromethod using vancomycin and teicoplanin Etest strips (8) and confirmed by vancomycin population analysis profiling (53). The detection of VISA and VRSA strains were done by overnight Etest assay. With the exception of VRSA strains and 22 VISA strains obtained from the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA), all strains were recent isolates (2007 to 2008) from blood samples, wounds, or sputum samples from patients at Hershey Medical Center (25). The VISA strain isolated from a patient who developed the VISA and daptomycin nonsusceptibility phenotype from an MRSA strain while on therapy with the two respective agents has been reported in detail (25).

For single-step and multistep resistance selection studies, 10 MRSA strains as well as two enterococci (all recent Hershey isolates) were tested. The MRSA strains comprised two hVISA strains, four VISA strains (including one strain that developed the VISA and daptomycin resistance phenotype after sequential therapy with both compounds) and four vancomycin-susceptible strains. Additionally, one vancomycin-susceptible *Enterococcus faecalis* strain and one vancomycin-susceptible *Enterococcus faecium* strain were tested.

MIC determinations. Both broth microdilution and broth macrodilution methods were used for MIC testing. Broth microdilution was conducted by the Clinical and Laboratory Standards Institute (CLSI) method using commercially prepared trays (TREK, Inc., Cleveland, OH) (13), with added calcium for daptomycin and 24-h incubation for vancomycin. Broth macrodilution was also conducted according to CLSI methodology (13). Telavancin was obtained from Theravance, Inc. (South San Francisco, CA), and the other antimicrobial agents were obtained from their respective manufacturers.

Multistep resistance selection. Multistep resistance selection was performed as previously described using the broth macrodilution method (1, 6, 7, 13). Serial passages were performed daily in Mueller-Hinton broth (MHB; BBL Microbiology Systems, Inc., Cockeysville, MD; added calcium for daptomycin testing) for each strain in subinhibitory concentrations of all antimicrobials. For each subsequent daily passage, an inoculum was taken from the tube 1 to 2 dilutions below the MIC that matched the turbidity of a growth control tube. Daily passages were performed until resistance, defined as a greater than fourfold increase in the macrodilution MIC, was obtained. A minimum of 14 passages was performed in every case. A maximum of 50 daily passages was performed. Quality controls *E. faecalis* ATCC 29212 and *S. aureus* ATCC 29213 were included. Stability of acquired resistance was determined by MIC determinations after 10 daily passages of the clone on blood agar (BBL) without antibiotics. A stable clone was defined as having an MIC after the drug-free passages within 1 dilution of the MIC before the drug-free passages (6, 7). For multistep testing, confirmation of identity of parent and resistant clones was confirmed by DNA fingerprinting: multiple-locus variable number tandem repeats for staphylococci (44) and pulsed-field gel electrophoresis for enterococci (27).

Molecular characterization of daptomycin-resistant clones. All daptomycin-resistant clones (stable and unstable) and their parental strains were subjected to sequence analysis of *mprF*, *yycF*, *yycG*, *rpoB*, and *rpoC* genes. PCR amplicons were generated and sequenced directly (CEQ8000 genetic analysis system; Beckman Coulter, Fullerton, CA) using the conditions and primers described previously (19).

Single-step resistance selection. Bacterial cells were grown overnight, scraped off the plates, washed once with MHB, and resuspended in the same medium to a final concentration of 1×10^{10} to 1×10^{11} CFU/ml. An aliquot (50 μl) of bacterial suspension was spread on drug-containing Mueller-Hinton agar at two, four, and eight times the agar dilution MIC. To ensure that the colonies could be quantitated (0 to 300/plate), dilutions of the latter inoculum were also made. The

TABLE 2. Telavancin multistep resistance selection results

Strain	Time of MIC[a]	MIC (μg/ml)[b]				
		Telavancin	Vancomycin	Teicoplanin	Daptomycin	Linezolid
SA525 (VSSA)	Initial	0.5	2	1	1	4
	Final (passage no.)	1 (50)	8 (50)	8 (21)	8 (20)	16 (50)
	After subculture	–	–	**4**	2	–
SA547 (VSSA)	Initial	0.5	2	2	1	4
	Final (passage no.)	2 (50)	8 (50)	16 (36)	8 (21)	32 (48)
	After subculture	–	–	4	**4**	**32**
SA262 (VSSA)	Initial	0.5	2	1	1	4
	Final (passage no.)	4 (34)	4 (50)	4 (50)	8 (16)	>32 (22)
	After subculture	1	–	–	**4**	**32**
SA248 (VSSA)	Initial	0.25	1	0.5	0.5	4
	Final (passage no.)	2 (43)	2 (50)	4 (32)	4 (41)	16 (50)
	After subculture	**2**	–	1	0.5	–
SA618 (hVISA)	Initial	0.5	2	4	2	4
	Final (passage no.)	2 (50)	8 (50)	16 (50)	16 (14)	16 (50)
	After subculture	–	–	–	**8**	–
SA873 (hVISA)	Initial	0.5	2	2	1	2
	Final (passage no.)	2 (50)	4 (50)	16 (27)	2 (50)	8 (50)
	After subculture	–	–	4	–	–
SA555 (VISA)	Initial	1	8	16	16	2
	Final (passage no.)	2 (50)	8 (50)	32 (50)	32 (50)	4 (50)
	After subculture	–	–	–	–	–
SA1287 (VISA)	Initial	0.5	4	4	4	4
	Final (passage no.)	2 (50)	8 (50)	16 (50)	8 (50)	16 (50)
	After subculture	–	–	–	–	–
SA1948 (VISA)	Initial	0.5	4	2	4	4
	Final (passage no.)	2 (50)	16 (50)	32 (14)	8 (50)	8 (50)
	After subculture	–	–	**16**	–	–
SA770 (VISA)	Initial	0.5	4	4	1	4
	Final (passage no.)	2 (50)	8 (50)	16 (50)	8 (35)	8 (50)
	After subculture	–	–	–	**4**	–
E. faecalis HMC568 (VSE)[c]	Initial	1	2	1	2	2
	Final (passage no.)	1 (50)	2 (50)	2 (50)	16 (21)	32 (35)
	After subculture	–	–	–	**16**	**32**
E. faecium HMC651 (VSE)	Initial	0.25	1	1	4	2
	Final (passage no.)	1 (50)	1 (50)	2 (50)	32 (24)	2 (50)
	After subculture	–	–	–	**16**	–

[a] Time the MIC was measured.
[b] The MICs were determined by broth macrodilution. The MIC values in boldface type indicate that the clones were stable, which was defined as meaning that the MIC after the drug-free passages was within one dilution of the MIC before the drug-free passages. A minus sign means that no MIC increase greater than fourfold (as defined in Materials and Methods) occurred.
[c] VSE, vancomycin-susceptible enterococci.

plates were incubated aerobically at 35°C for 48 h. When bacterial growth was observed on antibiotic-containing medium, the colonies were counted, and up to six randomly selected colonies per drug concentration were retested by agar dilution to determine the proportion of resistant colonies. A resistant colony in single-step studies was defined as a colony with an agar MIC of ≥4× that of the parent. Resistance frequencies at each MIC for each strain/antibiotic pair were calculated as the proportion of resistant colonies per inoculum (6, 7).

RESULTS

MIC determinations. MIC results for telavancin and comparator agents are presented in Table 1. As can be seen, among the 67 MRSA isolates tested, vancomycin-susceptible and -intermediate isolates had telavancin MICs of ≤1 μg/ml, whereas 12 of 26 VISA isolates (46%) were nonsusceptible to daptomycin at the same breakpoint. In contrast, VRSA strains were more susceptible to daptomycin (MICs of 0.25 to 1 μg/ml) than to telavancin (MICs of 2 to 4 μg/ml). All strains were susceptible to quinupristin-dalfopristin.

Multistep resistance selection. Multistep resistance selection results are presented in Table 2. Ten MRSA strains and two enterococcal strains were subjected to daily passages in the presence of subinhibitory concentrations of telavancin, vancomycin, teicoplanin, daptomycin, and linezolid for a maximum

TABLE 3. Molecular characterization of *mprF* genes in daptomycin-resistant clones recovered in multistep selection

Strain	Time of MIC[a]	Daptomycin MIC (μg/ml)[b]	MprF amino acid change[c]
SA525 (VSSA)	Initial	1	E44V
	Final (passage no.)	8 (20)	
	After subculture	2	
SA547 (VSSA)	Initial	1	S295L
	Final (passage no.)	8 (21)	
	After subculture	**4**	
SA262 (VSSA)	Initial	1	I420T
	Final (passage no.)	8 (16)	
	After subculture	**4**	
SA248 (VSSA)	Initial	0.5	–
	Final (passage no.)	4 (41)	
	After subculture	0.5	
SA618 (hVISA)	Initial	2	I506M
	Final (passage no.)	16 (14)	
	After subculture	**8**	
SA770 (VISA)	Initial	1	I420S
	Final (passage no.)	8 (35)	
	After subculture	**4**	

[a] Time the MIC was measured.
[b] The MIC values in boldface type indicate that the clones were stable, which was defined as meaning that the MIC after the drug-free passages was within one dilution of the MIC before the drug-free passages.
[c] A minus sign means that no mutation was detected.

of 50 days. None of the 12 strains tested produced clones with MICs against vancomycin that increased more than fourfold. However, four of the parent strains were VISA isolates with vancomycin MICs of 4 to 8 μg/ml and would therefore not be considered susceptible to vancomycin. Four staphylococcal isolates and both enterococcal isolates produced daptomycin-resistant clones after 14 to 35 days with MICs ranging up to16 μg/ml. Resistant clones were recovered from 2 of 12 teicoplanin-treated isolates, with eightfold increases in MIC observed at 14 or 21 days. Linezolid resistance developed in two staphylococci (VSSA SA547 and VSSA SA262) and *E. faecalis* HMC568. The MICs for these strains were ≥32 μg/ml.

Strain VSSA SA248 produced a stable clone after telavancin passage day 43 with an increase in macrobroth MIC from 0.25 μg/ml to 2 μg/ml. This same strain, when passaged in the presence of either daptomycin or teicoplanin, produced unstable eightfold increases in their respective MICs within a similar time frame. This strain did not produce clones with elevated MICs to either vancomycin or linezolid during 50 serial passages. No other strain produced a stable clone with >4-fold increase in telavancin MIC. Additionally, no cross-resistance was observed between telavancin and any other agent evaluated in this study (data not shown).

Molecular characterization of daptomycin-resistant clones. Sequencing analysis of *mprF*, *rpoC*, *rpoB*, *yycG*, and *yycF* genes in all parental strains but one (SA618) (Table 3) showed the highest homology (99% to 100%) of the obtained nucleotide sequences to genes from *S. aureus* N315 (genome GenBank accession number NC_002745) (32) and hVISA Mu3 (genome GenBank accession number NC_009782) (23). *S. aureus* N315 is a vancomycin-susceptible MRSA and caused major hospital-acquired infections in 1982 with its ability to acquire antibiotic resistance (32). Mu3 is an hVISA MRSA isolate obtained from a sputum sample from a patient with pneumonia after surgery who failed vancomycin therapy (23). The *mprF*, *rpoC*, *rpoB*, *yycG* and *yycF* genes from the parental strain SA618 had the highest homology to strain RF122 (genome GenBank accession number NC_007622) (21), a clonal group which has been isolated from one patient and is also associated with bovine mastitis.

No mutations were detected in the *rpoC*, *rpoB*, *yycG*, and *yycF* genes of the six selected clones, and amino acid changes were present only in MprF in five out of six mutants compared to parental strains (Table 3).

Single-step resistance selection. Single-step resistance selection results obtained by subculturing high inocula of 1×10^{10} to 1×10^{11} CFU/ml on plates with 2×, 4×, and 8× MIC, are presented in Table 4. The same 10 MRSA strains, 2 enterococci, and comparators used in multistep selection were tested for their propensity to produce spontaneous mutations. Attempts to select isolates resistant to telavancin were unsuccessful, regardless of the telavancin concentration used, the bacterial species, or resistance phenotype. No mutants with increased telavancin MICs were detected. The spontaneous mutation frequency to telavancin resistance ranged between $<4.0 \times 10^{-11}$ and $<2.9 \times 10^{-10}$. A low potential for selection of spontaneous mutants with decreased susceptibility was observed for comparator agents such as vancomycin (spontaneous mutation frequency ranged between $<3.3 \times 10^{-11}$ and $>1.0 \times 10^{-8}$), teicoplanin ($<5.0 \times 10^{-11}$ to 1.0×10^{-5}), daptomycin ($<3.3 \times 10^{-11}$ to 1.0×10^{-6}), and linezolid ($<3.7 \times 10^{-11}$ to $<2.5 \times 10^{-9}$). In summary, telavancin appears to have a low potential for selection of spontaneous resistant mutants independently of bacterial species or resistance phenotype.

DISCUSSION

The in vitro activity of telavancin described in this study is consistent with previously reported data (17, 18, 29, 31). In our hands, all hVISA and VISA strains were susceptible to ≤1 μg/ml telavancin, but VRSA strains had higher telavancin MICs (2 to 4 μg/ml) (33). In contrast, 46% of VISA strains were daptomycin nonsusceptible, but all VRSA strains were susceptible to daptomycin at the same breakpoint of ≤1 μg/ml.

In multistep resistance selection studies, telavancin yielded only a single stable clone out of 10 MRSA strains tested with an MIC eight times the parental MIC. In addition, neither of the enterococcal strains yielded clones with significant reduction in susceptibility to telavancin. Additionally, single-step mutation frequencies for telavancin ($<4.0 \times 10^{-11}$ to $<2.9 \times 10^{-10}$ at 2× MIC) were lower than the spontaneous mutation frequencies obtained with the comparators. These findings are consistent with previous resistance studies conducted with telavancin (30, 45) as well as with findings from telavancin clinical studies, where no cases of isolates with reduced susceptibility to telavancin have been recovered from cultures after the baseline culture (51).

The ability of daptomycin to select for resistant clones of MRSA in multistep passage experiments has been described previously by our research group (6) and by others (49). This is

TABLE 4. Single-step resistance selection results

Strain	Selecting drug	Spontaneous mutation frequency to drug resistance at[b]:		
		2 × MIC	4 × MIC	8 × MIC
SA525 (VSSA)	Telavancin	$<4.0 \times 10^{-11}$	$<4.0 \times 10^{-11}$	$<4.0 \times 10^{-11}$
	Vancomycin	$<3.3 \times 10^{-11}$	$<3.3 \times 10^{-11}$	$<3.3 \times 10^{-11}$
	Teicoplanin	1.6×10^{-6}	3.8×10^{-8}	$<6.3 \times 10^{-9}$
	Daptomycin	$<4.0 \times 10^{-11}$	$<4.0 \times 10^{-11}$	$<4.0 \times 10^{-11}$
	Linezolid	$<3.7 \times 10^{-11}$	$<3.7 \times 10^{-11}$	$<3.7 \times 10^{-11}$
SA547 (VSSA)	Telavancin	$<4.3 \times 10^{-11}$	$<4.3 \times 10^{-11}$	$<4.3 \times 10^{-11}$
	Vancomycin	$<1.7 \times 10^{-10}$	$<1.7 \times 10^{-10}$	$<1.7 \times 10^{-10}$
	Teicoplanin	$>4.0 \times 10^{-6}$	6.7×10^{-8}	$<6.7 \times 10^{-9}$
	Daptomycin	$<1.9 \times 10^{-10}$	$<1.9 \times 10^{-10}$	$<1.9 \times 10^{-10}$
	Linezolid	$<3.8 \times 10^{-11}$	$<3.8 \times 10^{-11}$	$<3.8 \times 10^{-11}$
SA262 (VSSA)	Telavancin	$<4.0 \times 10^{-11}$	$<4.0 \times 10^{-11}$	$<4.0 \times 10^{-11}$
	Vancomycin	$<1.0 \times 10^{-10}$	$<1.0 \times 10^{-10}$	$<1.0 \times 10^{-10}$
	Teicoplanin	1.0×10^{-5}	1.7×10^{-8}	3.3×10^{-9}
	Daptomycin	$<9.1 \times 10^{-11}$	$<9.1 \times 10^{-11}$	$<9.1 \times 10^{-11}$
	Linezolid	$<2.5 \times 10^{-9}$	$<2.5 \times 10^{-9}$	$<2.5 \times 10^{-9}$
SA248 (VSSA)	Telavancin	$<4.8 \times 10^{-11}$	$<4.8 \times 10^{-11}$	$<4.8 \times 10^{-11}$
	Vancomycin	$<2.5 \times 10^{-9}$	$<2.5 \times 10^{-9}$	$<2.5 \times 10^{-9}$
	Teicoplanin	8.6×10^{-9}	$<1.4 \times 10^{-9}$	$<1.4 \times 10^{-9}$
	Daptomycin	$<6.7 \times 10^{-11}$	$<6.7 \times 10^{-11}$	$<6.7 \times 10^{-11}$
	Linezolid	$<2.5 \times 10^{-9}$	$<2.5 \times 10^{-9}$	$<2.5 \times 10^{-9}$
SA618 (hVISA)	Telavancin	$<4.3 \times 10^{-11}$	$<4.3 \times 10^{-11}$	$<4.3 \times 10^{-11}$
	Vancomycin	$>1.0 \times 10^{-8}$	1.0×10^{-8}	$<5.0 \times 10^{-10}$
	Teicoplanin	1.6×10^{-6}	1.2×10^{-7}	1.6×10^{-8}
	Daptomycin	5.0×10^{-7}	5.0×10^{-8}	5.0×10^{-9}
	Linezolid	$<5.3 \times 10^{-11}$	$<5.3 \times 10^{-11}$	$<5.3 \times 10^{-11}$
SA873 (hVISA)	Telavancin	$<1.3 \times 10^{-10}$	$<1.3 \times 10^{-10}$	$<1.3 \times 10^{-10}$
	Vancomycin	$<1.3 \times 10^{-10}$	$<1.3 \times 10^{-10}$	$<1.3 \times 10^{-10}$
	Teicoplanin	$>1.4 \times 10^{-7}$	1.4×10^{-7}	$<1.2 \times 10^{-10}$
	Daptomycin	6.7×10^{-7}	$<6.7 \times 10^{-11}$	$<6.7 \times 10^{-11}$
	Linezolid	$<1.9 \times 10^{-10}$	$<1.9 \times 10^{-10}$	$<1.9 \times 10^{-10}$
SA555 (VISA)	Telavancin	$<1.0 \times 10^{-10}$	$<1.0 \times 10^{-10}$	$<1.0 \times 10^{-10}$
	Vancomycin	$<1.6 \times 10^{-10}$	$<1.6 \times 10^{-10}$	$<1.6 \times 10^{-10}$
	Teicoplanin	$<7.1 \times 10^{-11}$	$<7.1 \times 10^{-11}$	$<7.1 \times 10^{-11}$
	Daptomycin	$<2.0 \times 10^{-10}$	$<2.0 \times 10^{-10}$	$<2.0 \times 10^{-10}$
	Linezolid	$<1.8 \times 10^{-10}$	$<1.8 \times 10^{-10}$	$<1.8 \times 10^{-10}$
SA1287 (VISA)	Telavancin	$<5.0 \times 10^{-11}$	$<5.0 \times 10^{-11}$	$<5.0 \times 10^{-11}$
	Vancomycin	$<4.3 \times 10^{-11}$	$<4.3 \times 10^{-11}$	$<4.3 \times 10^{-11}$
	Teicoplanin	$>8.6 \times 10^{-6}$	4.3×10^{-7}	$<1.4 \times 10^{-10}$
	Daptomycin	$<3.3 \times 10^{-11}$	$<3.3 \times 10^{-11}$	$<3.3 \times 10^{-11}$
	Linezolid	$<1.3 \times 10^{-10}$	$<1.3 \times 10^{-10}$	$<1.3 \times 10^{-10}$
SA1984 (VISA)	Telavancin	$<4.5 \times 10^{-11}$	$<4.5 \times 10^{-11}$	$<4.5 \times 10^{-11}$
	Vancomycin	$<4.8 \times 10^{-11}$	$<4.8 \times 10^{-11}$	$<4.8 \times 10^{-11}$
	Teicoplanin	1.3×10^{-6}	5.0×10^{-8}	4.0×10^{-8}
	Daptomycin	$<3.3 \times 10^{-11}$	$<3.3 \times 10^{-11}$	$<3.3 \times 10^{-11}$
	Linezolid	$<6.3 \times 10^{-11}$	$<6.3 \times 10^{-11}$	$<6.3 \times 10^{-11}$
SA770 (VISA)	Telavancin	$<1.1 \times 10^{-10}$	$<1.1 \times 10^{-10}$	$<1.1 \times 10^{-10}$
	Vancomycin	$<5.0 \times 10^{-11}$	$<5.0 \times 10^{-11}$	$<5.0 \times 10^{-11}$
	Teicoplanin	$<5.0 \times 10^{-11}$	$<5.0 \times 10^{-11}$	$<5.0 \times 10^{-11}$
	Daptomycin	1.0×10^{-6}	1.7×10^{-7}	$<3.3 \times 10^{-11}$
	Linezolid	$<7.1 \times 10^{-11}$	$<7.1 \times 10^{-11}$	$<7.1 \times 10^{-11}$
E. faecalis HMC568 (VSE)[a]	Telavancin	$<2.9 \times 10^{-10}$	$<2.9 \times 10^{-10}$	$<2.9 \times 10^{-10}$
	Vancomycin	$<8.3 \times 10^{-11}$	$<8.3 \times 10^{-11}$	$<8.3 \times 10^{-11}$
	Teicoplanin	$<1.4 \times 10^{-10}$	$<1.4 \times 10^{-10}$	$<1.4 \times 10^{-10}$
	Daptomycin	$<6.3 \times 10^{-11}$	$<6.3 \times 10^{-11}$	$<6.3 \times 10^{-11}$
	Linezolid	$<6.7 \times 10^{-11}$	$<6.7 \times 10^{-11}$	$<6.7 \times 10^{-11}$
E. faecium HMC651 (VSE)	Telavancin	$<2.6 \times 10^{-10}$	$<2.6 \times 10^{-10}$	$<2.6 \times 10^{-10}$
	Vancomycin	$<1.4 \times 10^{-10}$	$<1.4 \times 10^{-10}$	$<1.4 \times 10^{-10}$
	Teicoplanin	$<1.7 \times 10^{-10}$	$<1.7 \times 10^{-10}$	$<1.7 \times 10^{-10}$
	Daptomycin	$<6.7 \times 10^{-11}$	$<6.7 \times 10^{-11}$	$<6.7 \times 10^{-11}$
	Linezolid	$<5.6 \times 10^{-11}$	$<5.6 \times 10^{-11}$	$<5.6 \times 10^{-11}$

[a] VSE, vancomycin-susceptible enterococci.
[b] Calculated as the number of resistant colonies per inoculum.

confirmed in the current study in which daptomycin-resistant clones with MICs of >1 μg/ml were present in 4 out of 10 MRSA strains (including one hVISA strain and one VISA strain). Both enterococcal strains also yielded daptomycin-resistant clones. This in vitro property of daptomycin may be related to development of daptomycin resistance while on therapy (25). Ongoing studies with synergy at subinhibitory concentrations (14) and other methods aimed at application of

pharmacokinetic/pharmacodynamic principles to increase the maximum concentration of drug in serum and area under the concentration-time curve of daptomycin (5) may counteract this resistance, but the latter might lead to increased toxicity.

Two types of resistance mechanisms have previously been observed in daptomycin-resistant isolates: (i) unstable, nonsusceptible isolates where antibiotic pressure (daptomycin or vancomycin) may play a role in reduced daptomycin susceptibility (26, 40, 41) and (ii) stable, nonsusceptible isolates with point mutations in certain genes (*mprF*, *yycF*, *yycG*, *rpoB*, and *rpoC*) (19, 40). In our study, stable daptomycin-resistant clones that had amino acid substitutions in MprF had daptomycin MICs of 4 to 8 μg/ml. Two unstable isolates had no changes in any of the genes tested or else change occurred in MprF (E44V) close to the N terminus, which may not affect activity of the protein (C. Ernst, P. Staubitz, G. Hornig, and A. P. D. Kraus, presented at the 13th International Symposium on Staphylococci and Staphylococcal Infections, Cairns, Australia, 2008). Recent studies have shown an important role for MprF in daptomycin resistance (9, 35, 54). Recently, the MprF putative synthase domain has been localized close to the hydrophilic C terminus (Ernst et al., International Symposium on Staphylococci and Staphylococcal Infections) which may explain the fact that mutations at position I420T/S and I506M have resulted in stable resistance to daptomycin. Amino acid substitution at position I420N has been described previously associated with clinical isolates with daptomycin MICs of 4 and 8 μg/ml which developed while on therapy (25). MprF alteration S295L has been described in a laboratory selected mutant while on passage on daptomycin and associated with a MIC of 4 μg/ml (19). To our knowledge, the MprF I506M mutation has not yet been described. The unstable resistance mechanism detected in two clones in our study may be linked to reduced susceptibility to vancomycin, producing thickened cell wall due to excess production of peptidoglycan (9, 41, 46). There is an ongoing debate whether the latter may be seen as a primary or adaptive nonspecific secondary resistance mechanism (35).

In our study we did not observe correlation between the early appearance of mutations in *mprF* followed by alteration in *rpoB* and *rpoC* as reported by Friedman and coworkers (19).

The significance of the high rates of spontaneous mutations obtained with teicoplanin in our single-step experiments is not known. Teicoplanin is widely used in several European countries and may constitute an important selective pressure for glycopeptide resistance in MRSA. The high rates of glycopeptide nonsusceptibility reported by Sancak and coworkers (47) may reflect widespread use of teicoplanin as the glycopeptide of choice in Turkey. This would not be expected from the low mutation rates obtained for telavancin in the current study.

Linezolid-resistant MRSA clones in multistep experiments have been described previously (6). In the current study, linezolid selected resistant clones in 2 of 10 MRSA isolates and 1 enterococcal isolate with MICs of 32 μg/ml.

In clinical trials, emergence of resistance to telavancin has not been observed. Four separate clinical studies of complicated skin and soft tissue infections (50–52) have shown that telavancin given once daily is at least as effective as vancomycin, including in patients infected with MRSA. No persistent isolates that had a >2-fold increase in telavancin MIC compared to the baseline isolate were recovered from the phase 3 ATLAS (assessment of telavancin in complicated skin and skin structure infections) program (51). Telavancin has also been evaluated in two phase 3 (*A*ssessment of *T*elavancin for Hospi*t*al-acqu*i*red P*n*eumonia ATTAIN) studies (42) for the treatment of hospital-acquired pneumonia (HAP), including patients with ventilator-associated pneumonia (VAP). MRSA is an important pathogen causing HAP worldwide. Data from the ATTAIN studies support the once-daily use of telavancin for the treatment of gram-positive HAP, including patients with VAP.

Results of the current study add to clinical results pointing to potential usefulness of telavancin in treatment of MRSA infections such as those causing skin and soft tissue disease and for the treatment of gram-positive HAP, including patients with VAP.

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