In Vitro Activities of Daptomycin, Linezolid, and Quinupristin-Dalfopristin against a Challenge Panel of Staphylococci and Enterococci, Including Vancomycin-Intermediate *Staphylococcus aureus* and Vancomycin-Resistant *Enterococcus faecium*

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ABSTRACT

We assessed the in vitro activities of daptomycin, linezolid, and quinupristin-dalfopristin (QD) against a contemporary challenge panel of 88 staphylococcal and 90 enterococcal isolates. The staphylococci selected included vancomycin-intermediate *Staphylococcus aureus* (VISA), methicillin-resistant *S. aureus*, and coagulase-negative staphylococci. Enterococcal isolates included vancomycin-resistant *Enterococcus faecium* (VREF) containing either vanA, vanB1, or vanD. The MICs of daptomycin, linezolid, and QD were determined using commercial broth microdilution panels. All three VISA isolates were susceptible to daptomycin, linezolid, and QD. QD was the most active agent against staphylococcal isolates (MIC$_{50}$ ≤ 0.5 µg/ml and MIC$_{90}$ = 1 µg/ml), including those with decreased susceptibility to vancomycin. QD was also the most active agent against VREF (MIC$_{90}$ ≤ 0.5 µg/ml). No differences were seen for susceptibility of vanA, vanB1, and vanD VREF strains for daptomycin, linezolid, or QD. Daptomycin was the most effective against *E. faecalis*. On the basis of manufacturer-suggested interpretive criteria, 92% of isolates were susceptible (MIC$_{90}$ ≤ 4 µg/ml). All isolates tested were susceptible to at least one antimicrobial agent for which interpretive criteria have been defined. Population analysis of three *S. aureus* isolates for which the daptomycin MICs were 8 µg/ml showed a pattern of homogeneous resistance.

INTRODUCTION

DAPTOMYCIN, LINEZOLID, AND QUINUPRISTIN-DALFOPRISTIN (QD) are newer antimicrobial agents that are active against gram-positive cocci. Daptomycin, a lipopeptide, has a spectrum of activity very similar to vancomycin and is active against aerobic and anaerobic Gram-positive organisms, including methicillin-susceptible and -resistant staphylococci and enterococci. Linezolid, an oxazolidinone, has excellent activity against most staphylococci, including methicillin-resistant strains, and multidrug-resistant enterococci, although resistance has begun to emerge. QD, a streptogramin, has bactericidal activity against methicillin-susceptible *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), and coagulase-negative staphylococci (CoNS). QD inhibits both vancomycin-susceptible and vancomycin-resistant strains of *Enterococcus faecium*.

We performed susceptibility testing of a challenge panel of contemporary staphylococci and enterococci against daptomycin, linezolid, and QD to assess and compare the in vitro activity of these agents.

MATERIALS AND METHODS

**Bacterial isolates**

A total of 178 staphylococcal and enterococcal isolates were obtained from the Centers for Disease Control and Prevention.
(CDC) and Project ICARE (Intensive Care Antimicrobial Resistance Epidemiology) culture collections. These isolates were collected from 72 hospitals between 1996 and 2001 from 24 U.S. states located in diverse geographical regions and five other countries. Upon receipt, all isolates were subcultured twice onto blood agar plates (Remel, Inc., Lenexa, KS) and then frozen at −80°C in sterile defibrinated sheep blood (Remel, Inc.) until needed. To minimize the inclusion of duplicate staphylococcal isolates, patterns of susceptibility to previously tested antimicrobials were examined for all isolates before including them in the challenge panel. Pulsed-field gel electrophoresis (PFGE) was performed on all enterococci used in the study, and duplicate isolates were not included in the challenge panel (data not shown).

The staphylococcal challenge panel (n = 88) included the following: three vancomycin-intermediate *Staphylococcus aureus* (VISA), *E. faecalis*, and seven MSSA isolates. The CoNS consisted of one vancomycin-intermediate *S. epidermidis*, 13 methicillin-resistant *S. epidermidis* (MRSE), 13 methicillin-resistant *S. haemolyticus*, one methicillin-susceptible *S. warneri*, one methicillin-resistant *S. hominis*, and two *Staphylococcus* spp. isolates.

The enterococcal challenge panel (n = 90) included the following: 63 VREF, one vancomycin-intermediate *E. faecalis*, 13 *E. faecalis* (11 vancomycin-resistant, one vancomycin-intermediate, and one vancomycin-susceptible), 10 *E. gallinarum*, and three *E. casseliflavus* isolates. The 63 VREF isolates included those with either vanA, vanB1, or vanD resistance mechanisms.

**Broth microdilution testing**

Testing was performed using custom broth microdilution MIC plates prepared by Trek Diagnostics (Westlake, OH) according to the manufacturer’s instructions. MIC plates included daptomycin (with calcium supplementation to 50 mg/L), linezolid, oxacillin, QD, vancomycin, a positive-growth control well, and a sterility well. Plates were stored at 35°C for 18–24 hr. Four 10-μl droplets of undiluted culture and four 10-μl droplets of serial 10-fold dilutions were inoculated onto one daptomycin-containing MHA plate at each concentration. The plates were inverted and incubated at 35°C for 24–48 hr. This procedure was performed four times with reproducible results. The average number of colony-forming units (CFU)/ml for each dilution was determined. CFUs were plotted using a logarithmic scale.

**RESULTS**

**Broth microdilution**

Daptomycin was more active against vancomycin-susceptible *S. aureus* (defined as isolates for which vancomycin MICs were ≤2 μg/ml) than against those isolates with decreased susceptibility to vancomycin (defined as isolates for which the vancomycin MIC = 4 μg/ml) (Table 1). The daptomycin MIC50 and MIC90 for vancomycin-susceptible *S. aureus* were 0.5 μg/ml and 1 μg/ml, respectively, whereas for isolates with decreased susceptibility to vancomycin the MIC50 was 2 μg/ml and the MIC90 was 8 μg/ml. The daptomycin MICs for the three VISA isolates were 1 or 2 μg/ml. For CoNS isolates, daptomycin was again more active against vancomycin-susceptible isolates (MIC50 and MIC90 values of 0.5 μg/ml and 1 μg/ml) than against isolates with decreased susceptibility to vancomycin (MIC50 and MIC90, values 1 μg/ml and 4 μg/ml). The three *S. aureus* and two CoNS isolates for which the daptomycin MICs were 8 μg/ml and 4 μg/ml, respectively, remained susceptible to linezolid and QD. The daptomycin MIC50 and MIC90 values for all 90 enterococci were 2–4 μg/ml and 8 μg/ml, respectively; 16 (25%) VREF isolates showed daptomycin MICs of 8 μg/ml.

For one *S. aureus* isolate, the linezolid MC was 8 μg/ml (which is undefined according to NCCLS interpretive criteria), but the isolate remained susceptible to vancomycin and daptomycin (Table 1). All VISA isolates were susceptible to linezolid (MIC = 2 μg/ml). The MIC50 and MIC90 values of linezolid for all staphylococci included in the challenge panel were, respectively, 2 μg/ml and 4 μg/ml for *S. aureus* and 2 μg/ml and 2 μg/ml for CoNS. Overall, 87 (99%) staphylococcal isolates were susceptible to linezolid. For VREF, only 32 (51%) isolates were performed on a third plate according to NCCLS guidelines using *S. aureus* ATCC 25923 and a daptomycin disk (acceptable range 18–23 mm). A fourth plate was inoculated with *Pseudomonas aeruginosa* ATCC 27853 and incubated with a gentamicin disk. Quality control results for the MHA lot tested were in range for both antimicrobial agents; therefore, no additional calcium supplementation was undertaken. These plates prepared in house were supplemented with daptomycin (Cubist Pharmaceuticals, Lexington, MA) in doubling concentrations ranging from 0.5 to 16 μg/ml. The daptomycin stock solution was prepared according to NCCLS guidelines. Each test isolate and one control isolate (*S. aureus* ATCC 29213) were suspended in Mueller–Hinton broth (MHB) (BBL, Cockeysville, MD) to an optical density equivalent to a 0.5 McFarland standard. The inoculum was serially diluted in sterile saline, and 100 μl of a 10−3, 10−3, 10−6 dilution was plated in duplicate onto plain MHA plates to determine the starting population. The plates were inverted and incubated at 35°C for 18–24 hr. Four 10-μl droplets of undiluted culture and four 10-μl droplets of serial 10-fold dilutions were inoculated onto one daptomycin-containing MHA plate at each concentration. The plates were inverted and incubated at 35°C for 24–48 hr. This procedure was performed four times with reproducible results. The average number of colony-forming units (CFU)/ml for each dilution was determined. CFUs were plotted using a logarithmic scale.
isolates were susceptible to linezolid (MIC \( \leq 2 \) mg/ml). Three (23%) \( E. \) faecalis isolates yielded MICs in the intermediate range for linezolid (MIC \( \leq 5 \) mg/ml); all were vanB-containing isolates, two of which were obtained from the same hospital, although during different time periods and with different strain typing profiles. Overall, QD showed the best activity against all staphylococcal isolates included in the challenge panel with MIC \( \leq 0.5 \) mg/ml and MIC \( \leq 0.5–2 \) mg/ml (Table 1). One MRSA isolate gave intermediate results for QD (MIC \( \leq 2 \) mg/ml). This same isolate was nonsusceptible to linezolid (MIC \( \leq 8 \) mg/ml), but was susceptible to both daptomycin and vancomycin. Eighty-seven (99%) staphylococcal isolates were susceptible to QD. QD showed the best activity against VREF; all isolates were susceptible to this agent. QD was not active against \( E. \) faecalis (MIC \( \leq 16 \) mg/ml).

**Population analysis**

The three \( S. \) aureus isolates for which the daptomycin MICs were \( 8 \) mg/ml by broth microdilution were isolated in 1997, 1999, and 2000 in three geographically distinct states. Two isolates (803 and 473) grew readily on MHA plates containing \( 8 \) mg/ml of daptomycin; the third (377) grew at \( 4 \) mg/ml. At a concentration of \( 8 \) mg/ml, plates inoculated with isolates 803 and 473 averaged \( 1 \times 10^7 \) CFUs each, while the average for isolate 377 on plates with \( 4 \) mg/ml was \( 2 \times 10^7 \) CFUs. None of the isolates grew at a daptomycin concentration of \( 16 \) mg/ml. For all three isolates, the subpopulations resistant to daptomycin show a pattern of homogeneous resistance (Fig. 1).

**DISCUSSION**

Daptomycin, linezolid, and QD were active against this challenge panel of Gram-positive organisms, including staphylococci with decreased susceptibility to vancomycin and VREF with a variety of vancomycin resistance mechanisms. All staphylococcal and enterococcal isolates tested in the challenge panel were susceptible to at least one drug for which categorical breakpoints have been defined. The antibacterial activity of daptomycin is highly dependent on the calcium concentration of the testing medium. Calcium supplementation of MHB from the normal 25 mg/L to 50 mg/L enhances the activity of daptomycin two- to four-fold for many bacterial species.\(^ {2,25} \) The levels of daptomycin used for in vitro testing are similar to physiological conditions in humans (47–52 mg/L free calcium).\(^ {19} \) In the study by Barry et al.,\(^ {2} \) the enhanced activity with calcium supplementation did not have a dramatic

### Table 1. Activity of Daptomycin, Linezolid, Quinupristin-Dalfopristin (QD), and Vancomycin against Gram-Positive Bacteria

<table>
<thead>
<tr>
<th>Bacteria (number tested)</th>
<th>Antimicrobial agent</th>
<th>MIC (%): 50%</th>
<th>90%</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DSV</strong> (^ {a} ) Staphylococcus aureus (19)(^ {b} )</td>
<td>Daptomycin</td>
<td>2</td>
<td>8</td>
<td>1–8</td>
</tr>
<tr>
<td></td>
<td>Linezolid</td>
<td>2</td>
<td>4</td>
<td>1–8</td>
</tr>
<tr>
<td></td>
<td>QD</td>
<td>( \leq 0.5 )</td>
<td>( \leq 0.5 )</td>
<td>( \leq 0.5–2 )</td>
</tr>
<tr>
<td></td>
<td>Vancomycin</td>
<td>4</td>
<td>8</td>
<td>4–8</td>
</tr>
<tr>
<td><strong>VS</strong> (^ {c} ) Staphylococcus aureus (38)</td>
<td>Daptomycin</td>
<td>0.5</td>
<td>1</td>
<td>0.12–4</td>
</tr>
<tr>
<td></td>
<td>Linezolid</td>
<td>2</td>
<td>4</td>
<td>1–4</td>
</tr>
<tr>
<td></td>
<td>QD</td>
<td>( \leq 0.5 )</td>
<td>( \leq 0.5 )</td>
<td>( \leq 0.5–1 )</td>
</tr>
<tr>
<td></td>
<td>Vancomycin</td>
<td>1</td>
<td>2</td>
<td>( \leq 0.5–2 )</td>
</tr>
<tr>
<td><strong>DSV</strong> Coagulase-negative staphylococci (17)(^ {d} )</td>
<td>Daptomycin</td>
<td>1</td>
<td>4</td>
<td>0.25–4</td>
</tr>
<tr>
<td></td>
<td>Linezolid</td>
<td>2</td>
<td>2</td>
<td>1–2</td>
</tr>
<tr>
<td></td>
<td>QD</td>
<td>( \leq 0.5 )</td>
<td>( \leq 0.5 )</td>
<td>( \leq 0.5–1 )</td>
</tr>
<tr>
<td></td>
<td>Vancomycin</td>
<td>4</td>
<td>4</td>
<td>4–8</td>
</tr>
<tr>
<td><strong>VS</strong> Coagulase-negative staphylococci (14)</td>
<td>Daptomycin</td>
<td>0.5</td>
<td>1</td>
<td>0.25–2</td>
</tr>
<tr>
<td></td>
<td>Linezolid</td>
<td>2</td>
<td>2</td>
<td>1–4</td>
</tr>
<tr>
<td></td>
<td>QD</td>
<td>( \leq 0.5 )</td>
<td>1</td>
<td>( \leq 0.5–1 )</td>
</tr>
<tr>
<td></td>
<td>Vancomycin</td>
<td>2</td>
<td>2</td>
<td>1–2</td>
</tr>
<tr>
<td><strong>VR</strong> (^ {e} ) Enterococcus faecium (63)</td>
<td>Daptomycin</td>
<td>4</td>
<td>8</td>
<td>2–8</td>
</tr>
<tr>
<td></td>
<td>Linezolid</td>
<td>2</td>
<td>4</td>
<td>2–4</td>
</tr>
<tr>
<td></td>
<td>QD</td>
<td>( \leq 0.5 )</td>
<td>( \leq 0.5 )</td>
<td>( \leq 0.5–1 )</td>
</tr>
<tr>
<td></td>
<td>Vancomycin</td>
<td>( \geq 128 )</td>
<td>( \geq 128 )</td>
<td>( \geq 128 )</td>
</tr>
<tr>
<td>Enterococci, other(^ {f} ) (27)</td>
<td>Daptomycin</td>
<td>2</td>
<td>8</td>
<td>0.5–8</td>
</tr>
<tr>
<td></td>
<td>Linezolid</td>
<td>4</td>
<td>4</td>
<td>2–4</td>
</tr>
<tr>
<td></td>
<td>QD</td>
<td>2</td>
<td>16</td>
<td>( \leq 0.5–32 )</td>
</tr>
<tr>
<td></td>
<td>Vancomycin</td>
<td>8</td>
<td>( \geq 128 )</td>
<td>1–( \geq 128 )</td>
</tr>
</tbody>
</table>

\(^ {a} \)DSV, Decreased susceptibility to vancomycin.
\(^ {b} \)Includes three \( S. \) aureus with vancomycin MIC = \( 8 \) mg/ml.
\(^ {c} \)VS, Vancomycin-susceptible.
\(^ {d} \)Includes one CoNS with vancomycin MIC = \( 8 \) mg/ml.
\(^ {e} \)Includes \( E. \) faecalis, \( E. \) casseliflavus, \( E. \) gallinarum, and vancomycin-intermediate \( E. \) faecium.
\(^ {f} \)VR, Vancomycin-resistant.
effect on staphylococci, i.e., the MIC$_{50}$ and MIC$_{90}$ values remained below 2 µg/ml, which several investigators have proposed as the susceptible breakpoint for daptomycin. However, with calcium supplementation the daptomycin MIC$_{50}$ and MIC$_{90}$ values for *E. faecalis* in the Barry study fell from 4 to 1 and 8 to 2 µg/ml, respectively, and MIC$_{90}$ values for *E. faecium* were 4 µg/ml. Thus, if breakpoints of ≤2 µg/ml (susceptible), 4 µg/ml (intermediate), and ≥8 µg/ml (resistant) proposed by Fuchs et al. are accepted by NCCLS, laboratories testing organisms in standard MHB as opposed to calcium-supplemented MHB would likely report falsely resistant results (i.e., major errors) routinely for enterococci. Our results for vancomycin-susceptible *S. aureus* and CoNS using calcium-supplemented broth were consistent with the MIC results of Barry et al. and Snydman et al. However, among isolates of staphylococci with decreased susceptibility to vancomycin, we noted three isolates of *S. aureus* for which the daptomycin MICs were 8 µg/ml. Our results differ from those of Rybak et al., who reported lower daptomycin MIC$_{50}$ and MIC$_{90}$ values for vancomycin-susceptible *S. aureus* strains.

Our studies suggest a possible link between the mechanisms of reduced susceptibility to vancomycin and daptomycin. However, unlike the population analysis results presented by Silverman et al., our population studies showed homogeneous rather than heterogeneous daptomycin resistance. Our results may well represent a novel mechanism of decreased susceptibility to daptomycin when compared to results for strains tested by Silverman and colleagues because we verified that the calcium concentration in the MHA was acceptable prior to testing, and since low calcium content appears to have little effect on testing staphylococci. Since our disk diffusion results were within the range defined for quality control, we assume that the agar media contained sufficient calcium for testing. However, the discrepancy between the broth microdilution MIC and the agar dilution MIC for isolate 377 may reflect the varying concentrations of calcium ions between the two media. This problem associated with the varying concentrations of calcium in MHA and broth may plague further studies with daptomycin.

For linezolid and QD, no difference in susceptibility was noted among the *S. aureus* isolates with varying susceptibilities to vancomycin. Our MIC results for enterococci and linezolid were consistent with other studies. Linezolid was active against *S. aureus*, CoNS, and a variety of enterococci. Eliopoulos et al. who tested 875 isolates of *E. faecium* collected from around the United States, reported QD MICs up to 32 µg/ml and noted little difference in the QD MIC results between *vanA*- and *vanB*-containing strains. Strains for which the QD MICs were ≥4 µg/ml were all *aac6'-li* positive in their study. These latter isolates were obtained from patients who were treated with QD.

The importance of testing drugs, such as daptomycin, linezolid, and QD, that may be used to treat severe infections due to *S. aureus* is emphasized by the recent appearance of *S. aureus* strains resistant to vancomycin (MIC ≥ 32 µg/ml). These newer antimicrobial agents are likely to become the mainstay for therapy of serious infections when vancomycin resistance becomes prevalent. However, testing daptomycin in the clinical laboratory, because of its need for calcium supplementation, may pose a challenge.

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