False Daptomycin-Nonsusceptible MIC Results by Microscan Panel PC 29 Relative to Etest Results for Staphylococcus aureus and Enterococci

Elizabeth L. Palavecino, Jacqueline M. Burnell
Department of Pathology, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA

This study correlated the daptomycin MIC results obtained by Microscan and by Etest in Staphylococcus aureus and enterococci and found that the Microscan panel GP 29 had a high rate of false nonsusceptible results. Of the isolates interpreted as nonsusceptible by Microscan, 87% of Staphylococcus aureus, 90% of Enterococcus faecalis, and 88% of Enterococcus faecium isolates were interpreted as susceptible by Etest. In turn, Etest also has false nonsusceptible results compared to reference methods.

Daptomycin is used for treatment of severe infections caused by methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococcus (VRE), and although daptomycin remains active against Staphylococcus aureus and Enterococcus isolates, nonsusceptible strains associated with treatment failure have been reported (1–3), highlighting the importance of performing susceptibility testing of daptomycin. Further, a daptomycin MIC in the nonsusceptible range may limit the clinician’s choices for treatment of severe infections caused by MRSA and VRE. It is therefore important for clinical laboratories to accurately detect staphylococci and enterococci with reduced susceptibility to daptomycin to help clinicians decide therapy. The Clinical and Laboratory Standards Institute (CLSI) currently provides only a susceptible category for daptomycin in staphylococci (MIC ≤ 1 µg/ml) and enterococci (MIC ≤ 4 µg/ml) and recommends confirmation of nonsusceptible isolates by a second method (4). At our institution, the Microscan Walkaway System (Siemens Healthcare Diagnostics, West Sacramento, CA) is used to perform routine susceptibility testing of daptomycin for all staphylococci and enterococci isolated from clinical specimens, and the Etest (bioMérieux, Durham, NC) is used as a confirmatory second method. The rate of nonsusceptibility for Staphylococcus aureus and Enterococcus faecalis has remained unchanged at <1% since starting testing of daptomycin in 2008. In contrast, the rate of daptomycin nonsusceptibility in Enterococcus faecium according to Microscan results has increased from <1% in 2008 to 3% in 2009, 7% in 2010, and 12% in 2011. The purpose of this study was to confirm the nonsusceptible category of the isolates by correlating the MIC results obtained by Microscan to those obtained by Etest.

Approximately 3,000 S. aureus, 1,000 E. faecalis, and 350 E. faecium isolates are routinely tested for daptomycin susceptibility every year at our institution using the Microscan Gram-positive panel type 29 (GP 29) with the standard turbidity technique. Daptomycin dilutions in the panel ranged from 0.25 to 4 µg/ml and, according to the manufacturer, included the calcium supplementation recommended by the CLSI (4). Etest strips are overlaid with a constant level of calcium equivalent to 40 µg/ml. Mueller-Hinton (BBL) agar plates were used for the Etest. CLSI breakpoints were used for interpretation of daptomycin MIC results (4). Clinical isolates read as nonsusceptible by Microscan were retested to determine reproducibility of results, and those that repeated as nonsusceptible were saved for further testing. The panels were also read manually and agreed with the instrument’s readings. From these, all S. aureus and E. faecalis isolates and half of the E. faecium isolates saved from 2008 to 2011 were selected for testing with Etest, for a total of 23 Staphylococcus aureus (14 MRSA and 9 methicillin-susceptible S. aureus [MSSA]) isolates and 60 enterococci (10 Enterococcus faecalis and 50 Enterococcus faecium isolates). Only one isolate per patient was included, and because outbreaks of enterococci with decreased susceptibility to daptomycin have been reported (5), we selected E. faecium strains isolated at different time intervals and from inpatient and outpatient locations to decrease the likelihood of selecting epidemiologically related strains. Quality control strains were tested according to standard recommendations. The majority of Staphylococcus aureus isolates classified as nonsusceptible by Microscan were isolated from sterile sites, followed by wounds and respiratory sources. Most enterococci were isolated from urine, followed by blood and other sterile sources. These specimen sources are representative of specimen sources of staphylococci and enterococci isolated at our institution.

The results of our study are as follows. Of the 23 Staphylococcus aureus isolates with a daptomycin MIC of >1 µg/ml by Microscan and interpreted as nonsusceptible, 20 had an MIC of ≤1 µg/ml by Etest and were therefore considered susceptible. Among the enterococci with a daptomycin MIC of >4 µg/ml by Microscan and interpreted as nonsusceptible, 9 out of 10 Enterococcus faecalis isolates and 44 out of 50 Enterococcus faecium isolates had an MIC of ≤4 µg/ml by Etest and were therefore considered susceptible to daptomycin. The MIC results obtained by Etest are shown in Table 1. Overall, only 3 Staphylococcus aureus (all MRSA), 1 Enterococcus faecalis, and 6 Enterococcus faecium (all VRE) isolates were confirmed as nonsusceptible by Etest, indicating a poor correlation of the MIC results between these two methods. This study...
TABLE 1 Number of isolates at each MIC by Etest

<table>
<thead>
<tr>
<th>MIC (μg/ml) by Etest</th>
<th>Staphylococcus aureus (23)</th>
<th>Enterococcus faecium (50)</th>
<th>Enterococcus faecalis (10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>16</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>28</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

The shaded area indicates the range of nonsusceptible MIC values, which includes an MIC of >1 μg/ml for Staphylococcus aureus and an MIC of >4 μg/ml for Enterococcus faecium and Enterococcus faecalis. All isolates tested here were interpreted as nonsusceptible by Microscan.

Alternative methods are usually available in clinical laboratory settings, but this study did not include isolates with a daptomycin MIC of >1 μg/ml whereas all Staphylococcus aureus isolates tested by us had an MIC of >1 μg/ml by Microscan.

The MIC variation obtained with different methods and the concomitant problem with reproducibility may, in part, be related to the MIC distribution of the wild-type strains, which is close to the MIC breakpoint, particularly for E. faecium. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) reports that the wild-type distribution for daptomycin and E. faecium has a mode of 2 μg/ml and that 1,012 (8.1%) of 12,502 isolates have a daptomycin MIC of 4 μg/ml when tested with a variety of MIC methods, including reference methods (12). A doubling dilution error (i.e., plus or minus one double dilution from the correct MIC) considered inherent in susceptibility may result in interpreting a susceptible organism as nonsusceptible.

The high rate of major errors (false nonsusceptible results) in daptomycin testing by Microscan underscores the need for confirming MIC results by a second method. Although the Etest also gives false nonsusceptible results, it performs better than Microscan when BMD results were used as a reference and it is therefore a practical alternative for confirmation of nonsusceptible results obtained by Microscan. Isolates that test as daptomycin nonsusceptible by Microscan but susceptible by Etest should be reported as susceptible.

Siemens has recognized the potential for elevated daptomycin MIC results on Microscan panels for Enterococcus faecalis, and has distributed a technical support bulletin (6) informing Microscan users of the higher MICs to daptomycin obtained by Gram-positive panels compared to those obtained by reference methods for Enterococcus faecalis. The bulletin recommends that users follow CLSI guidelines and confirm nonsusceptible isolates by a second method. However, as shown in this study, Microscan users should be aware that the problem also affects the susceptibility MIC results for Staphylococcus aureus and Enterococcus faecalis.

The confirmatory second method recommended by CLSI guidelines is preferable a reference method, such as disk diffusion or agar or broth dilution. However, disk diffusion is not reliable for testing daptomycin (4) and agar and broth microdilution testing is not widely available in clinical laboratories. Therefore, most laboratories have to rely on automated susceptibility systems or the Etest for determining susceptibility to daptomycin, as these alternative methods are usually available in clinical laboratory settings. However, susceptibility testing of daptomycin by Etest may also be problematic. Previous studies demonstrating a poor correlation among different methods for determining susceptibility against daptomycin have reported that the Etest shows higher MIC results than the BMD reference method (7–9). The higher MIC result by Etest than by BMD as found by these investigators is in agreement with the findings of our study, which, in addition, shows that Microscan yields even higher MIC values. Etest lot-related MIC variation has also been reported (10). Although we did not test different lots, we observed Etest MIC variation (0.5 to 1 doubling dilution) for some isolates when comparing our results to those obtained by Siemens and the reference laboratory. Other investigators (11) have reported a good correlation among reference BMD, Microscan, and Etest for daptomycin MIC results in Staphylococcus aureus, but this study did not include isolates with a daptomycin MIC of >1 μg/ml whereas all Staphylococcus aureus isolates tested by us had an MIC of >1 μg/ml by Microscan.

No financial support was received for this study.
REFERENCES


