Assessment of Two Commercial Susceptibility Test Methods for Determination of Daptomycin MICs

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Daptomycin is a lipopeptide antibiotic with activity against several important gram-positive bacterial pathogens, including drug-resistant staphylococci and enterococci. Because the mechanism of action of daptomycin is calcium-dependent depolarization of the cell membrane, susceptibility testing requires medium supplemented with a physiological level of calcium. This study assessed two Food and Drug Administration-cleared commercial test devices for determination of daptomycin MICs, Etest and JustOne. A collection of 220 selected isolates, including Staphylococcus aureus, coagulase-negative staphylococci, Enterococcus faecalis, E. faecium, E. avium, E. durans, E. casseliflavus, and E. gallinarum, were tested by both methods. Included in the collection were 22 S. aureus and 14 Enterococcus sp. isolates that were recovered from patients and were nonsusceptible on the basis of the daptomycin MICs. As the reference method for comparison, all isolates were tested by the Clinical and Laboratory Standards Institute broth microdilution method incorporating cation-adjusted Mueller-Hinton broth with 50 µg/ml calcium. Daptomycin MICs agreed, within 1 twofold dilution, for 97% of the isolates by Etest and for 100% by JustOne. However, daptomycin MICs determined by Etest were 1 dilution lower than the reference MICs for 65% of the Enterococcus sp. isolates tested. This resulted in 28.5% very major (VM) errors (4/14) with enterococci (all E. faecium) but none (0/22) with staphylococci. Use of JustOne yielded MICs that were 1 dilution lower than the reference MICs for 69% of the staphylococci and 25% of the enterococci. This resulted in 13.6% VM errors (3/22) with staphylococci and 14.3% VM errors (2/14) with enterococci. The manufacturer-recommended JustOne inoculum preparation resulted in mean colony counts of only 5 × 10⁴ to 1 × 10⁵ CFU/ml in the wells of the strip. Increasing the inoculum to 3 × 10⁶ to 4 × 10⁶ CFU/ml eliminated two of five VM errors upon retesting. No major interpretative errors occurred with either device. In summary, daptomycin MICs generated by the Etest or JustOne method generally agreed within 1 dilution of the reference daptomycin MICs. However, both devices produced slightly lower MICs that resulted in some VM errors.

MATERIALS AND METHODS

Antimicrobial agents. Daptomycin was tested with each isolate by Etest, Just-One, and the CLSI reference broth microdilution method. The inoculum for the tests was prepared with colonies grown on sheep blood agar plates incubated for 20 to 24 h that were suspended in 0.9% saline to obtain a suspension equivalent to the turbidity of a 0.5 McFarland standard. The same standardized 0.5 McFarland suspension of each strain was used for each of the test methods described below. Linezolid and vancomycin were also tested as control drugs in the reference microdilution panels and by Etest.

Test isolates. A group of 220 isolates was examined for susceptibility to daptomycin by the two commercial methods and the CLSI reference susceptibility test method (5). The collection included 84 S. aureus (65 MRSA), 25 coagulase-negative Staphylococcus sp. (15 methicillin-resistant, coagulase-negative Staphylococcus sp.), 70 Enterococcus faecium (80 VRE), 22 E. faecalis (5 VRE), 3 E. avium, 4 E. durans, 5 E. casseliflavus, and 5 E. gallinarum isolates. This test group was composed primarily of daptomycin-susceptible isolates. However, 22 non-daptomycin-susceptible S. aureus and 14 non-daptomycin-susceptible Enterococcus sp. (13 E. faecium, 1 E. faecalis) isolates from our strain collection or those kindly provided by Cubist Pharmaceuticals (Lexington, MA) supplemented the strain collection. The daptomycin MICs for the nonsusceptible S. aureus isolates ranged from 2 to 16 µg/ml, and those for the nonsusceptible Enterococcus sp. isolates ranged from 8 to >32 µg/ml.

Etest strips. MICs were determined with each isolate by using a single lot of daptomycin Etest strips, and a subset of strains were tested by using two additional lots of Etest strips. The Etest strips contained additional calcium (40 µg/ml), as well as the daptomycin concentration gradient. Etest strips were applied to the surfaces of 150-mm Mueller-Hinton agar plates (BD Diagnostics,
Sparks, MD) that had been inoculated with a swab dipped in the 0.5 McFarland organism suspension. Linezolid and vancomycin Etest strips were included as control drugs. In addition, 50 selected isolates were tested in parallel with Remel (Lenexa, KS) Mueller-Hinton agar plates. Plates were incubated at 35°C in ambient air for 16 to 20 h prior to determination of MICs. The MIC was defined by the intersection of the growth ellipse margin with the Etest strip by using reflected light. In instances where the Etest MIC was read between the usual twofold MIC increments, Etest MICs were rounded to the next higher log2 MICs reflecting light. In instances where the Etest MIC was read between the usual twofold MIC increments, Etest MICs were rounded to the next higher log2 MICs for comparison with the reference MICs.

**Results**

When daptomycin MICs generated by use of the Etest and JustOne methods with the groups of gram-positive bacteria included in this study were compared with the CLSI reference broth microdilution values, the MICs from the two commercial methods generally agreed within a single twofold dilution of the reference values (Table 1). MICs generated with the primary lot of Etest strips tended to be lower with some *Enterococcus* sp. isolates, although 94.6% of the MICs were within 1 dilution of the reference values. Daptomycin MICs generated by the Etest method were somewhat more likely to be lower than the reference values with the nonsusceptible strains of both enterococci and staphylococci (Table 1). Daptomycin MICs determined with the JustOne strips also tended to be lower than the reference values with both the susceptible and nonsusceptible strains of both genera. Despite the trend to lower MICs obtained by the JustOne strip method, all of the MICs were within 1 dilution of the reference values.

When three different lots of daptomycin Etest strips were compared with the same brand and lot of Mueller-Hinton agar, there were slight differences in the MICs, but none was greater than 1 dilution different from the reference MIC (Table 2). Assessment of two lots of daptomycin Etest strips tested on two different brands of Mueller-Hinton agar with *S. aureus* and...
Enterococcus sp. isolates did not reveal any notable shifts of MICs (Table 3). The Etest MICs of the two control drugs (vancomycin and linezolid) were also unaffected by the brand of Mueller-Hinton agar used for testing.

Early in this study, it was noted that the final colony counts of inoculated JustOne microdilution wells were lower than the target values of the CLSI reference broth microdilution method. The mean colony count of 21 Staphylococcus sp. isolates was 1 × 10^5 CFU/ml, and the mean count for 20 Enterococcus sp. isolates was 5 × 10^4 CFU/ml (Table 4). Because these values were 0.5 to 1 log_10 lower than the recommended 5 × 10^5 CFU/ml inoculum density recommended by the CLSI for broth microdilution tests (5), a higher-vol aliquot (50 μl instead of 10 μl) of the 0.5 McFarland inoculum suspension was transferred to 10 ml of cation-adjusted Mueller-Hinton broth to prepare the final inoculum for transfer to Just One wells. The higher inoculum volume resulted in mean final inoculum densities of 3 × 10^5 to 3.7 × 10^5 CFU/ml for the two genera tested (Table 4). Table 5 lists the daptomycin MICs resulting from use of the two JustOne inoculum densities. While the JustOne daptomycin MICs were within a single dilution of the reference MICs with both inoculum preparations, there was greater agreement with the reference MICs obtained with the higher inoculum density.

Table 6 describes the interpretive category errors that resulted from the Etest and standard JustOne test methods. Since there is no intermediate interpretive category for daptomycin, only VM or major category interpretive errors can occur. There were no major errors (false resistance) by either method. However, there were VM errors (false susceptibility) by both methods. With the Etest method, three of the four VM errors involved MICs greater than 1 dilution different from the reference MICs. There were five VM errors resulting from the JustOne tests; none of which was due to an MIC greater than 1 dilution lower than the reference MIC. Repeat testing of the discrepant isolates with the fivefold higher inoculum density corrected one of three errors with S. aureus and one of two errors with Enterococcus sp. (Table 6).

**DISCUSSION**

This study has assessed the use of two different commercial devices for determining daptomycin MICs, one a gradient diffusion strip method (Etest), the other a dried panel broth microdilution strip method (JustOne). Both products incorporated calcium supplementation to allow the calcium-dependent cell membrane depolarization mechanism of daptomycin to be optimized (2, 12). Unfortunately, it became apparent after daptomycin achieved regulatory approval for marketing in the United States that disk diffusion testing of daptomycin did not allow reliable recognition of nonsusceptible strains of staphylococci or enterococci (7, 12a). Thus, disk diffusion testing is not an option for clinical microbiology laboratories at this time (6). Therefore, it is necessary to use an MIC method to determine the daptomycin susceptibility of clinical isolates from serious infections. This study has focused on two potentially convenient MIC devices for testing daptomycin with a collection of staphylococci and enterococci, including strains for which the MICs are elevated.

In general, daptomycin MICs determined with both the Etest and JustOne methods agreed well with the CLSI reference method MICs, although MICs obtained by both methods tended to be slightly lower than the reference values. At the time that daptomycin was approved for clinical use, there were no strains that were recognized as resistant to the drug. Thus, both the FDA-approved drug package insert and the CLSI-approved interpretive criteria include susceptible-only breakpoints of ≤1 μg/ml for staphylococci and streptococci (excluding S. pneumoniae) and ≤4 μg/ml for enterococci (6). Strains for which the MICs exceed those breakpoints are, for now, referred to as nonsusceptible. More recently, strains have been
recognized for which the daptomycin MICs exceed the susceptibility breakpoints, including some from therapy failures (7, 8, 12a). Therefore, in the future, it may be possible to define conventional categories of intermediate and resistant. For now, the lack of an intermediate category means that any category errors between a test method and the reference susceptibility test method can be categorized as either major (false resistance) or VM (false susceptibility). There were no major interpretive category errors encountered in this study. However, there were VM errors associated with both the Etest and JustOne methods. Most, but not all, of the VM errors attributed to the Etest method were within a single dilution of the reference MIC and occurred at the single interpretive breakpoint for each genus (Table 6). The finding that calcium-supplemented Etest daptomycin MICs tended to be lower than reference dilution test values was reported earlier with Iso-SensiStest agar for the Etests (9). All of the VM errors associated with the JustOne method differed by only 1 dilution from the reference MIC. Two of five VM errors with the JustOne strips were eliminated if a higher inoculum density similar to that used in the CLSI reference method was employed. Laboratories could easily accommodate this change by simply transferring a 50-μl aliquot of the 0.5 McFarland suspension rather than 10 μl to the cation-adjusted Muller-Hinton broth at the time of inoculation of the dried microdilution strip. It is not clear why the manufacturer’s standard protocol employs such a low final inoculum density in the panel wells.

The Etest method was slightly more convenient to set up than the JustOne panel. However, daptomycin MICs were considered more difficult to interpret by the Etest as opposed to the JustOne strips. The Etest ellipse margins were especially hazy and poorly demarcated with the enterococcal isolates. The recently modified Etest strips employed in this study performed better, however, than an earlier formulation that we tested in a prior investigation (10). While the JustOne strips took slightly more time to set up, the MICs were simple to interpret.

In summary, clinicians and microbiologists should be aware of the possibility of the emergence of daptomycin resistance (or increases in MICs) during prolonged therapy and closely monitor the susceptibility of persisting isolates that might be recovered during therapy. While a resistant interpretive category has yet to be defined for daptomycin, laboratories should promptly detect and report any nonsusceptible clinical isolates. Both the Etest and JustOne methods represent convenient commercially available test reagents for determining daptomycin MICs. This is especially important in the wake of the removal of disk diffusion testing as an option with this antimicrobial agent.

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REFERENCES


