Reliability of the E Test for Detection of Ampicillin, Vancomycin, and High-Level Aminoglycoside Resistance in Enterococcus spp.

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By comparison with agar dilution results, the E test was investigated for the ability to detect high-level aminoglycoside (gentamicin and streptomycin), ampicillin, and vancomycin resistance among strains representing six enterococcal species. For ampicillin and vancomycin, disk diffusion results also were obtained. No false high-level aminoglycoside resistance occurred, and no false gentamicin susceptibility was noted. With the high-range streptomycin E test (2,048 μg), 24% of the 38 resistant strains were falsely susceptible. However, these discordances likely could be reconciled by adjustments in incubation duration and by using broth microdilution rather than agar screen breakpoint criteria, or by using the lower-range (1,024-μg) strip. For ampicillin, category results obtained by E test and disk diffusion showed good agreement with agar dilution; E test MICs were generally comparable to agar dilution MICs. The E test was more sensitive than disk diffusion for detecting vancomycin-resistant strains, but for these strains and those exhibiting low-level vancomycin resistance (MIC, 32 to 128 μg/ml), disk diffusion and E test inhibition zones must be interpreted with caution. Given the reliability of E test for detecting resistance to anti-enterococcal agents, the decision to use this method should be based on convenience, cost, testing frequency, and satisfaction with currently used methods.

Combination therapy with a cell wall-active agent (i.e., ampicillin, penicillin, or vancomycin) and an aminoglycoside (gentamicin or streptomycin) is recommended for serious enterococcal infections such as endocarditis (11). Acquired resistance to these antimicrobial agents, which abrogates their effectiveness as components of therapy, is being encountered with increasing prevalence (4, 7, 9, 11, 12). Therefore, any enterococcal isolate for which dual therapy is being considered must be monitored for resistance to these agents. The E test (AB Biodisk, Solina, Sweden), a susceptibility testing technique that combines disk-agar diffusion precision with the precision of quantitative results provided by broth and agar dilution methods, has substantial potential as a means for detecting resistance in enterococci. Briefly, the E test consists of an impervious strip impregnated with a continuous antimicrobial gradient. As is done for disk diffusion testing, the strip is applied to the surface of a Mueller-Hinton agar plate that has been freshly inoculated with the test organism. After incubation at 35°C, the MIC is read at the intersection of growth and the MIC scale of the strip (1, 2).

This study investigated the ability of vancomycin, ampicillin, high-content gentamicin, and high-content streptomycin E test strips to accurately detect enterococcal resistance to these antimicrobial components of combination therapy. Results were compared with those obtained by agar dilution. Additionally, susceptibility testing to ampicillin and vancomycin were also done by disk-agar diffusion. Inocula for each susceptibility testing method were prepared after subculturing each isolate twice on Trypticase soy agar supplemented with 5% sheep blood (BBL Microbiological Systems, Cockeysville, Md.). E test strips, provided by AB Biodisk, Solna, Sweden, included ampicillin (MIC range, 0.016 to 265 μg/ml), vancomycin (MIC range, 0.016 to 265 μg/ml), gentamicin (two ranges: 0.064 to 1,024 and 0.125 to 2,048 μg/ml), and streptomycin (two ranges: 0.064 to 1,024 and 0.125 to 2,048 μg/ml). Unless otherwise stated, E tests were inoculated, incubated, and read according to the manufacturer’s recommendations. A common inoculum used for the E test was also used for inoculation in ampicillin and vancomycin agar dilution and disk agar dilution tests performed according to recommended procedures (13, 14). Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) supplemented with gentamicin or streptomycin (range, 250 to 2,000 μg/ml) were used to establish high-level aminoglycoside resistance as described previously (17). Cefine disks (BBL) were used to test all isolates for β-lactamase production (12).

The enterococci included single patient clinical isolates; β-lactamase-producing Enterococcus faecalis isolates were kindly provided by Mark Zervos (Division of Infectious Diseases, Wayne State University School of Medicine). All organisms were identified according to the conventional scheme of Facklam and Collins (6).

E test detection of high-level gentamicin and streptomycin resistance is shown in Table 1. Results were obtained with isolates representing six different species known to exhibit high-level resistance and included E. faecalis, E. faecium, E. gallinarum, E. casseliflavus, E. avium, and E. raffinosus (18). High-level aminoglycoside breakpoints proposed by the National Committee for Clinical Laboratory Standards (NCCLS) (15) for agar testing were used to classify each isolate as resistant or susceptible. Although use of Mueller-Hinton agar differs from the use of brain heart infusion agar proposed by NCCLS, a previous study has shown that the same accuracy is achieved with the use of either medium (18). No false resistance occurred with either the low (1,024-
Aminoglycoside  

<table>
<thead>
<tr>
<th>MIC (µg/ml)</th>
<th>Aminoglycoside</th>
<th>Agar screen&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Breakpoint designation</th>
<th>No. of strains in each E test MIC (µg/ml) category&lt;sup&gt;b&lt;/sup&gt;</th>
<th>E test MIC distribution&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Disk diffusion inhibitory zones (range in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤500</td>
<td>Gentamicin</td>
<td>S</td>
<td>32</td>
<td>≤128-512</td>
<td>0.25 (1), 0.75 (4), 1 (5), 1.5 (1), 8 (2)</td>
<td>14-27</td>
</tr>
<tr>
<td>500</td>
<td></td>
<td>R</td>
<td>38</td>
<td>&gt;512</td>
<td>16 (3), 24 (1), 64 (1)</td>
<td>6</td>
</tr>
<tr>
<td>500</td>
<td></td>
<td></td>
<td></td>
<td>&gt;1,024</td>
<td>64 (1), 96 (1), 128 (1)</td>
<td>6</td>
</tr>
<tr>
<td>&gt;2,000</td>
<td></td>
<td></td>
<td></td>
<td>&gt;2,048</td>
<td>≥256, ≥256 (7)</td>
<td></td>
</tr>
<tr>
<td>≤2,000</td>
<td>Streptomycin</td>
<td>S</td>
<td>32</td>
<td>≤128-512</td>
<td>0.25 (1), 0.38 (1), 0.5 (2), 0.75 (3), 1 (2), 1.5 (2), 2 (1), 4 (2)</td>
<td>18-26</td>
</tr>
<tr>
<td>&gt;2,000</td>
<td></td>
<td>R</td>
<td>38</td>
<td>&gt;512</td>
<td>4 (1), 6 (6), 8 (3)</td>
<td>17-22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;1,024</td>
<td>4 (1), 24 (1), 32 (1), 48 (2), 64 (3)</td>
<td>14-17</td>
</tr>
<tr>
<td>≥256</td>
<td></td>
<td></td>
<td></td>
<td>&gt;2,048</td>
<td>64 (1), 256 (1), ≥256 (4)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Determined after 24 h of incubation.
<sup>b</sup> Mueller-Hinton agar; MIC breakpoint criteria for S (not high-level resistant) and R (high-level resistant) as proposed by NCCLS (15).
<sup>c</sup> E test MIC data obtained with 1,024-µg strip not underlined; 2,048-µg strip data underlined.

µg) or high (2,048-µg)-range gentamicin strips. For one strain, the agar MIC was 500 and the E test MIC was 512 µg/ml; the agar and E test MICs for all others were <512 µg/ml. Similarly, no false susceptibility was noted with the gentamicin E test, as MICs for all 38 resistant strains were >512 and >1,024 µg/ml. When tested with the high-range strip, MICs for 29 isolates were >2,048 µg/ml. These findings are consistent with previous reports by Sanchez et al. (21, 22) who found absolute category agreement between agar and E test results for high-level gentamicin resistance testing.

False streptomycin resistance was not observed with either the low- or high-range E test strips, as MICs for all 32 susceptible strains were <1,024 µg/ml (Table 1). False susceptibility results did occur when E test results were evaluated according to the proposed NCCLS breakpoint criteria of >2,000 µg/ml for agar screens (15). After 24 h of incubation with the high-range strip, 9 (24%) of the 38 resistant strains were falsely classified as susceptible, as they failed to give E test MICs of >2,048 µg/ml, four of which gave MICs ≤1,024 µg/ml. When we prolonged incubation to 48 h before interpretation, which previously has been shown to enhance resistance detection in some enterococcal strains (17), E test MICs for four of these strains changed to >2,048 µg/ml, one increased from 768 to 1,536 µg/ml, and the other four MICs remained unchanged (one at 1,024 and three at 2,048 µg/ml). Similar difficulties were reported by Sanchez et al. (22). In their study, 9 (17%) of 52 strains for which streptomycin agar MICs were >2,000 µg/ml gave E test MICs <2,048 µg/ml. False streptomycin susceptibility is a problem commonly encountered with other streptomycin screening methods and, in some instances, appears to be strain related (10, 17). Therefore, whether recalibration of the high-range (2,048 µg) strip (22) would resolve false susceptibility is uncertain. Alternatively, the currently available strip could be used with the interpretive breakpoint of >1,000 µg/ml proposed by NCCLS for both microdilution screens being applied, rather than the 2,000-µg/ml breakpoint proposed for agar screens (15). With the broh criteria, 37 of 38 (97%) of the streptomycin-resistant strains would have been correctly detected after 48 h of incubation. Another option would be to use the lower-range (1,024 µg) strip and classify any strain for which the MIC was >1,024 µg/ml as resistant. In our study, such an approach resulted in all 38 streptomycin-resistant strains being detected at 24 h (Table 1).

Amoxicillin E test and disk-agar diffusion results obtained with resistant and susceptible strains, as determined by agar dilution, are shown in Table 2. The 39 non-β-lactamase-producing strains tested included 36 E. faecium strains, two E. raffinosus strains, and one E. gallinarum strain. Results obtained with six-β-lactamase-producing E. faecalis isolates are not shown. For these six strains, amoxicillin resistance due to β-lactamase production was not detectable by routine agar dilution (MIC range, 1 to 2 µg/ml) or disk diffusion (zone range, 18 to 23 mm) methods, nor was it detectable by E test (MIC range, 0.75 to 2 µg/ml). These results are consistent with what is known about the inability of nonen-
zymatic testing methods to detect this resistance (12). For non-β-lactamase-producing isolates, no false resistance was noted and the MICs obtained with the 13 susceptible strains were generally comparable with agar dilution MICs. One of the 26 resistant strains was falsely susceptible by E test (MIC, 4 µg/ml), but for most other isolates MICs were within one twofold dilution of agar MICs. This overall good agreement in category results between the E test and conventional dilution techniques is consistent with reports from previous studies (8, 16, 21). In contrast to the report by Sanchez et al. (21), in which nearly 50% of E test ampicillin MICs were more than fourfold higher than agar MICs, the MICs in our study were not notably greater than those obtained by agar dilution. The reason(s) for these differences is not known and requires further study.

By disk-agar diffusion one of the 13 susceptible strains was falsely resistant (MIC, 8 µg/ml; zone, 14 mm), but false susceptibility did not occur with any of the 26 resistant strains (Table 2). The strain that gave a false-susceptible result by E test had a ampicillin inhibition zone of 11 mm. Although there was good agreement between disk diffusion and agar dilution category results, the inhibition zone diameters do not provide any indication regarding the level of ampicillin resistance. Zone diameters for E test ampicillin MICs were generally comparable with agar dilution MICs for these methods. Enterococcal isolates that were resistant to disk diffusion had inhibition zones of 16 mm (intermediate) and 17 mm (susceptible) at 24 h. After 48 h incubation the intermediate strain remained so and the other isolate exhibited a haze of growth up to the disk. These results indicate that whether the E test disk diffusion is being used, interpretation of results must be done with extreme caution, as detection of low-level vancomycin resistance is problematic for these methods. Enterococcal isolates not clearly susceptible should be considered for testing with prolonged incubation to 48 h or for testing by a conventional dilution method.

All of the highly vancomycin-resistant (MIC, ≥256 µg/ml) enterococci were detected by E test (Table 2). The E test MIC for one strain was 64 µg/ml at 24 h, but the strain showed an intermediate level of resistance in the agar screen (MIC, >2,000 µg/ml). Similar previous studies with a limited number of strains reported that the E test successfully detected vancomycin resistance, but the actual level of resistance of these isolates was not stated (8, 21). Also (shown in Table 2), false susceptibility by disk diffusion did not occur with this organism group. All six isolates gave 6-mm zones; four showing good growth and two exhibiting a lesser haze of growth up to the disk.

In summary, E tests for gentamicin, streptomycin, ampicillin, and vancomycin provide a reliable testing approach for identifying enterococcal resistance to these components of combination therapy. The false susceptibility encountered with streptomycin is not unexpected, as similar problems exist with other screening methods (17). Furthermore, the problem with the high-content (2,048-µg) strip appears to be resolvable by increasing incubation to 48 h for strains giving borderline results and by using broth microdilution (MIC, >1,000 µg/ml) rather than agar screen (MIC, >2,000 µg/ml) interpretive criteria. Alternatively, the low-content (1,024-µg) strip could be used. The E test is as reliable as disk diffusion for ampicillin testing. However, whether the potential advantage over disk testing of providing the level of resistance will be realized must await more extensive comparisons that use a greater number of strains with MICs in the 8- to 128-µg/ml range. The E test appears to be more sensitive than disk diffusion for detecting strains intermedi-
ate to vancomycin, but for these strains, and those exhibiting low-level resistance, disk diffusion and E test inhibition zones must be inspected and interpreted with extreme caution. Finally, given that the reliability of the E test for detecting resistance to anti-enterococcal agents compares favorably with results reported for other commercially available methods (10, 17, 24), the decision for use in a particular laboratory should be based on convenience, cost, testing frequency, and satisfaction with currently used methods.

REFERENCES


