Evaluation of the Vitek GPS-TA Card for Laboratory Detection of High-Level Gentamicin and Streptomycin Resistance in Enterococci

BEVERLY METCHOCK* AND JOHN E. McGOWAN, JR.

Department of Pathology and Laboratory Medicine, Emory University School of Medicine, and Grady Memorial Hospital, Atlanta, Georgia 30335

Received 18 April 1991/Accepted 23 September 1991

The Vitek GPS-TA card (Vitek Systems, Hazelwood, Mo.) was compared with single-concentration broth microdilution and disk diffusion methods using high-content disks for the detection of high-level resistance to gentamicin and streptomycin in 99 isolates of enterococci (81 Enterococcus faecalis isolates and 18 Enterococcus faecium isolates). The GPS-TA card accurately detected high-level resistance to gentamicin, but not streptomycin, in E. faecalis. When streptomycin is being considered for therapy, either disk diffusion or time-kill studies should be used to confirm susceptible results obtained by Vitek testing. Additional studies are needed to determine the best method for testing E. faecium isolates.

Bactericidal therapy is recommended for serious enterococcal infections such as sepsis and endocarditis (3). Synergistic bactericidal activity against enterococci can be achieved by combining a cell wall-active agent, such as penicillin, ampicillin, or vancomycin, with an aminoglycoside or aminocyclitol. However, enterococci exhibiting high-level resistance (MIC of >2,000 μg/ml) to the aminoglycoside or aminocyclitol show lack of synergistic killing (3, 4, 5, 9, 12). Because high-level resistance in enterococci is increasingly being reported, screening of enterococci from blood samples and from other normally sterile sites for high-level resistance to streptomycin and gentamicin is recommended (3, 4, 9, 11, 12).

Laboratories therefore require an accurate, reliable, and easy-to-perform screening test for predicting high-level resistance in enterococcal isolates, especially those from serious infections. Standard susceptibility testing does not discriminate between high-level aminoglycoside resistance and the usual level of intrinsic resistance to aminoglycosides seen in most enterococcal isolates (7). Time-kill studies, the most reliable method for determining synergy, are too cumbersome and time-consuming for routine use in most clinical microbiology laboratories. A commercially available frozen microtiter test system (Microscan; Baxter Healthcare Corp., W. Sacramento, Calif.) has been evaluated previously and found to be insensitive (11).

Recently, several practical methods have been described for testing isolates of Enterococcus faecalis for high-level resistance. These include broth microdilution testing using single concentrations of aminoglycosides prepared in-house (11, 12), and agar disk diffusion testing using high-content aminoglycoside disks prepared in-house (8, 10, 11). Vitek Systems, Inc., (Hazelwood, Mo.) has recently introduced a susceptibility card, GPS-TA, to test for high-level resistance to streptomycin and gentamicin in enterococci on their automated system. This report evaluates these three newer methods.

(Part of this research was presented at the 90th Annual Meeting of the American Society for Microbiology, Anaheim, Calif., 13 to 17 May 1990.) Ninety-five isolates of Enterococcus spp. from patients at Grady Memorial Hospital in the period from 1 October 1988 through 30 June 1989 were examined. Of these 95 isolates, 54 were from blood samples, 22 were from other usually sterile sites (8 from peritoneal fluid, 3 from pleural fluid, 3 from bones, 3 from bladders, 2 from cerebrospinal fluid, 2 from the heart valves, 1 from a hip), 10 from clean-catch or catherized urine samples, and 9 from other sites. Multiple isolates from the same patient were tested only if they were of different species. Four strains of enterococci with known susceptibility patterns were obtained from the Antimicrobics Investigations Branch, Centers for Disease Control, Atlanta, Ga.: two Enterococcus faecium strains showing high-level resistance to streptomycin but not gentamicin, one E. faecium strain showing high-level resistance to gentamicin and streptomycin, and one E. faecalis strain showing high-level resistance to gentamicin and streptomycin. All isolates were stored at -70 °C in defibrinated sheep blood prior to testing. Isolates were subcultured at least twice onto Trypticose soy agar with 5% sheep blood (Carr-Scarborough Microbiologicais, Atlanta, Ga.) and incubated at 35 °C in air overnight before testing. The isolates were initially identified as Enterococcus spp. by conventional biochemical tests (2) and later were identified to the species level by a commercially available product (Vitek GPI) according to the manufacturer’s directions.

Each isolate was screened for high-level resistance to gentamicin and streptomycin by three methods: Vitek GPS-TA card, broth microdilution, and disk diffusion with high-content disks. In all disk diffusion and broth microdilution studies, the isolates were incubated for 24 h at 35 °C in air. Antimicrobial agent powders were obtained from Sigma Chemical Co. (St. Louis, Mo.). High-level resistance was defined as high-level resistance by any method. Discrepant results between methods were resolved by time-kill studies.

Susceptibility of the isolates to 500 μg of gentamicin per ml and 2,000 μg of streptomycin per ml was determined by using the Vitek GPS-TA card. The inoculum was prepared directly from overnight growth on sheep blood agar in 0.45% saline according to the manufacturer’s instructions.

For broth microdilution, an inoculum (3 × 10^8 to 7 × 10^7 CFU/ml) was prepared in cation-adjusted Mueller-Hinton broth directly from overnight growth on a sheep blood agar plate; 50 μl of inoculum was added to 50 μl of each antimicrobial agent dispensed in microtiter plates (Dynatech

* Corresponding author.
Laboratories, Inc., Alexandria, Va.) to give a final concentrations of 500 and 2,000 μg of gentamicin per ml and 2,000 μg of streptomycin per ml. A drug-free growth control well was also included.

Susceptibility to high levels of gentamicin and streptomycin was determined on Mueller-Hinton agar by standard disk diffusion methods (6), with disks containing 120 μg of gentamicin and 300 μg of streptomycin (10). Isolates were defined as having high-level resistance if the diameter of the zone of inhibition was equal to or less than 10 mm (11).

Discrepancies between results, which was defined as when any isolate did not show agreement with all three screening methods, were resolved by time-kill studies done in cation-adjusted Mueller-Hinton broth inoculated with log-phase-growth cells adjusted to give an inoculum of about 7.5 × 10^6 CFU/ml. Antimicrobial agents were tested in clinically achievable concentrations (gentamicin, 10 μg/ml; streptomycin, 25 μg/ml; ampicillin, 5 μg/ml; vancomycin, 10 μg/ml) both singly and in combinations of aminoglycoside plus ampicillin and of aminoglycoside plus vancomycin. Vancomycin was tested only for those E. faecalis isolates resistant to ampicillin (MIC of >8 μg/ml). In time-kill studies, the isolates were incubated for 24 h at 35°C in air. At 0, 4, and 24 h after inoculation and incubation, colony counts were determined on sheep blood agar. By using the viable counts determined at each time interval, a 24 h time-kill curve was established. Synergy for an aminoglycoside-ampicillin or aminoglycoside-vancomycin combination was defined as ≥100-fold increase in killing compared with the killing caused by the most active agent alone.

E. faecalis GMH34, which produces aminoglycoside phosphotransferase [APH(2')] aminoglycoside acetyltransferase [AAC(6')] aminoglycoside-modifying enzymes and shows high-level resistance to gentamicin and streptomycin, and E. faecalis ATCC 29212, which does not show high-level resistance to either drug, were used as test organisms for quality control for all methods of testing.

Of the 99 enterococci tested, 81 were E. faecalis isolates and 18 were E. faecium isolates. Forty-four demonstrated high-level resistance to both gentamicin and streptomycin, 23 demonstrated high-level resistance to gentamicin only, and 20 demonstrated high-level resistance to streptomycin only. E. faecalis isolates were more likely to show high-level resistance to gentamicin (36 of 81) than to streptomycin (20 of 81).

High-level resistance to gentamicin was detected in 37 of 99 enterococcal isolates (Table 1), 36 of 81 (44.4%) E. faecalis isolates and 1 of 18 (5.6%) E. faecium isolates. Microdilution with 500 and 2,000 μg of gentamicin per ml and disk diffusion detected all isolates with high-level resistance to gentamicin (100% sensitivity), and Vitek detected 37 (97.3%) of the gentamicin-resistant isolates. The E. faecium isolate with known high-level resistance to gentamicin was not detected by the Vitek GPS-TA card. For disk diffusion, the range of zone diameters for susceptible isolates was 16 to 24 mm (mode, 20 mm). All resistant isolates showed no zone of inhibition. No isolates were falsely characterized as having high-level resistance to gentamicin by any method.

High-level resistance to streptomycin was detected in 34 of 99 enterococcal isolates (Table 1), 20 of 81 (24.6%) E. faecalis isolates and 14 of 18 (77.8%) E. faecium isolates. Disk diffusion detected all isolates with high-level resistance to streptomycin (100% sensitivity), microdilution detected 31 of 34 (91.2%), and Vitek detected 27 of 34 (79.2%). For disk diffusion, the range of zone diameters for susceptible isolates was 15 to 22 mm (mode, 17 mm). Most (33 of 34) resistant isolates showed no zone of inhibition; one isolate had a zone of 10 mm. Five isolates of E. faecium and two isolates of E. faecalis showing high-level resistance to streptomycin by time-kill studies were not detected by Vitek. For three of these E. faecium isolates, high-level resistance was detected only by disk diffusion (one with a zone diameter of 10 mm). No isolates were falsely characterized as having high-level resistance to streptomycin by any method.

For the detection of high-level resistance to gentamicin in E. faecalis, disk diffusion with high-content aminoglycoside disks, broth microdilution with either 500 and 2,000 μg of gentamicin per ml and the Vitek GPS-TA panel were all suitable tests. All three methods detected all 36 E. faecalis isolates showing high-level resistance to gentamicin. Only one E. faecium (obtained from the Centers of Disease Control) showed high-level resistance to gentamicin. Both microdilution and disk diffusion detected this high-level resistance, the Vitek GPS-TA card did not. Studies of additional isolates are needed to determine the best method(s) for detecting high-level resistance to gentamicin in E. faecium.

For the detection of high-level resistance to streptomycin in E. faecalis, disk diffusion and microdilution were the best methods. Both detected all 20 isolates showing high-level resistance to streptomycin. Vitek did not detect 2 of 20 (10%) of these isolates. For E. faecium isolates, disk diffusion was the best method for the detection of high-level resistance to streptomycin.

Overall, disk diffusion with high-content aminoglycoside disks appeared to be the most reliable method among those evaluated for the detection of high-level resistance to gentamicin and streptomycin in enterococci. Vitek accurately detected high-level resistance to gentamicin in E. faecalis. However, 10% of E. faecalis isolates with high-level resistance to streptomycin were not detected by Vitek; this false positive rate could be reduced by increased sensitivity in the disk diffusion method.

**TABLE 1. Detection of high-level resistance to gentamicin and streptomycin in 99 isolates of Enterococcus spp. by three methods**

<table>
<thead>
<tr>
<th>Test method</th>
<th>E. faecalis and E. faecium</th>
<th>Gentamicin</th>
<th>E. faecalis and E. faecium</th>
<th>Streptomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microdilution</td>
<td>37 (100)</td>
<td>36 (100)</td>
<td>1 (100)</td>
<td>31 (91.2)</td>
</tr>
<tr>
<td>Disk diffusion</td>
<td>37 (100)</td>
<td>36 (100)</td>
<td>1 (100)</td>
<td>34 (100)</td>
</tr>
<tr>
<td>Vitek GPS-TA</td>
<td>36 (97.3)</td>
<td>36 (100)</td>
<td>0</td>
<td>27 (79.2)</td>
</tr>
<tr>
<td>Total*</td>
<td>37</td>
<td>36</td>
<td>1</td>
<td>34</td>
</tr>
</tbody>
</table>

* Total resistant after discrepancies between results by different methods resolved by time-kill studies.
appearance of susceptibility is of concern because it might result in the use of streptomycin when it is not indicated. Recent changes in the Vitek GPS-TA card and software may rectify the false susceptibility problems seen with the version of the product used in this evaluation. Until these changes are evaluated, however, an alternative method (disk diffusion, agar screen (9), or time-kill studies) should be used to confirm susceptible results obtained by Vitek testing when high-level resistance to gentamicin is present and streptomycin is being considered for therapy. Vitek did not detect 5 of 14 E. faecium isolates with high-level resistance to streptomycin and the single E. faecium isolate with high-level resistance to gentamicin. Since gentamicin resistance is now being reported more frequently in this species (1), testing of additional E. faecium isolates with high-level aminoglycoside resistance is necessary to determine the best system for detecting resistance in this species.

REFERENCES