Comparison of the New MicroScan Pos MIC Type 6 Panel and AMS-Vitek Gram Positive Susceptibility Card (GPS-TA) for Detection of High-Level Aminoglycoside Resistance in Enterococcus Species

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We compared the MicroScan Pos MIC Type 6 panel and AMS-Vitek Gram Positive Susceptibility Card (GPS-TA) to agar dilution screen plates for the detection of high-level aminoglycoside resistance in 182 enterococcal isolates. The specificity of the two commercial systems was 100%, with the exception of one susceptible isolate found to be streptomycin resistant by the Vitek system. The MicroScan and Vitek systems had comparable sensitivities for the detection of gentamicin resistance (90 and 95% respectively) and streptomycin resistance (85 and 78%, respectively). These results suggest that screening tests such as agar dilution screen plates, broth dilution, or disk diffusion should continue to be used to detect high-level gentamicin and streptomycin resistance.

The treatment of bacteremic enterococcal infections is often problematic because of the intrinsic resistance of the organisms to many antimicrobial agents. Enterococci are inhibited but not killed by cell wall-inhibiting antibiotics such as β-lactams or glycopeptides and are inherently resistant to the aminoglycosides (MICs, 6 to 64 µg/ml) (1, 4). A combination of a cell wall-inhibiting agent plus an aminoglycoside has been found to be synergistic and effective for the treatment of serious enterococcal infections (1, 5). However, there is no synergy when an enterococcal strain has high-level aminoglycoside resistance (MIC, ≥ 2,000 µg/ml), and infections due to such organisms may not respond to treatment with such a combination (6, 7).

Since it was first described in 1979, high-level aminoglycoside resistance among enterococci has increased (3, 6, 7, 13). Currently, the methods available for the detection of high-level aminoglycoside resistance include single-concentration agar dilution screen plates, disk diffusion, and broth dilution (8, 10–12, 14). These methods have been found to be reliable but require 18 to 24 h of incubation before results are obtained. There are also several commercial microdilution susceptibility test systems which include wells formulated for the detection of high-level resistance to gentamicin and streptomycin.

A previous evaluation by our laboratory of MicroScan (Travenol Laboratories, Mahwah, N.J.) frozen Gram-Positive Combo Type 2 and freeze-dried Gram-Positive Type 5 panels demonstrated inadequate sensitivity for the detection of high-level aminoglycoside resistance in enterococci. We extended this study to evaluate a new panel, the MicroScan Pos MIC Type 6 panel, and the AMS-Vitek Gram Positive Susceptibility Card (GPS-TA) (Vitek Systems, Hazelwood, Mo.) for the detection of high-level aminoglycoside resistance in enterococci. The new MicroScan Pos MIC Type 6 panel consists of a reformulation of the original MIC Type 6 freeze-dried panel. The new panel contains dextrose phosphate broth to enhance the growth of enterococci. The panel incorporates two aminoglycoside synergy wells, one containing 2,000 µg of gentamicin per ml and the other containing 2,000 µg of streptomycin per ml. The GPS-TA also incorporates two aminoglycoside synergy wells, one containing 500 µg of gentamicin per ml and the other containing 2,000 µg of streptomycin per ml. We evaluated the accuracy of these two methods, as compared with agar dilution screen plates, for detecting high-level aminoglycoside resistance.

A total of 182 clinical enterococcal isolates collected from blood and sterile body sites had been evaluated as part of a previous study (2). The isolates were characterized by Gram stain, bile-esculin hydrolysis, and tolerance in the presence of 6.5% sodium chloride and were identified to the species level by MicroScan. Enterococcus faecalis HH22 (gentamicin and streptomycin resistant and β-lactamase positive), UWHC 1921 (gentamicin resistant and streptomycin susceptible), and UWHC 1936 (gentamicin susceptible and streptomycin resistant) (12) were tested as control organisms.

The agar dilution screen plates, which were found previously to accurately detect high-level aminoglycoside resistance (2), contained brain heart infusion agar (GIBCO Diagnostics, Madison, Wis.) and the following concentration of antibiotic: 1,000 µg of gentamicin (Schering Corp., Montreal, Quebec, Canada) per ml or 2,000 µg of streptomycin (Sigma Chemical Co., St. Louis, Mo.) per ml. All isolates were tested with each antibiotic as previously described (2). Two to four colonies of each isolate were suspended in brain heart infusion broth and incubated overnight at 35°C to achieve an inoculum of 10⁶ CFU/ml. Using a Steers replicator, we inoculated the agar dilution screen plates to a final inoculum of approximately 10⁶ CFU. The plates were incubated for 18 to 24 h at 35°C. The growth of two or more colonies was considered indicative of resistance. The MicroScan Pos MIC Type 6 panel and GPS-TA were inoculated and incubated in accordance with manufacturer instructions. The final inoculum of the MicroScan Type 6 panel was approximately 10² CFU/ml (10⁴ CFU per well), and the final inoculum of the GPS-TA was approximately 10³ CFU/ml (10⁵ CFU per well). The MicroScan Type 6 panel results were available after 18 h of incubation. The GPS-TA results

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were available after 6 h of incubation. In an attempt to determine the cause of discrepancies between the two commercial systems and agar dilution, we evaluated the effects of altering inoculum size and incubation period.

Of the 182 enterococcal isolates evaluated by agar dilution screen plates, 26 were gentamicin resistant only, 49 were streptomycin resistant only, 37 were gentamicin and streptomycin resistant, and 70 were gentamicin and streptomycin susceptible. Therefore, in total 63 isolates were gentamicin resistant and 86 isolates were streptomycin resistant. Of the 182 isolates, 173 were E. faecalis and 9 were Enterococcus faecium. The number of E. faecium isolates was too small to evaluate separately, so the E. faecium results were included with the E. faecalis results.

The results for the 182 enterococcal isolates tested by the two commercial systems are shown in Table 1. The MicroScan and Vitek systems demonstrated comparable sensitivities for the detection of gentamicin and streptomycin resistance. The most notable observation was the improved sensitivity of the MicroScan panel for the detection of streptomycin resistance over that in the original study, which revealed 31 and 41% sensitivities for the MicroScan frozen Gram-Positive Combo Type 2 and freeze-dried Gram-Positive Type 5 panels, respectively (2). Both commercial systems demonstrated 100% specificity, with the exception of one false-negative detection of streptomycin resistance in a susceptible isolate by the Vitek system. The improved sensitivity of the MicroScan panel for the detection of high-level streptomycin resistance is likely attributable to the reformulation of the panel with glucose phosphate broth, which was used specifically to enhance the growth of enterococci.

Previous attempts to improve MicroScan Type 2 and Type 5 panels with an increased inoculum size were successful but not practical (2). Although Sahm et al. (9) found enhanced detection of high-level aminoglycoside resistance with prolonged incubation of MicroScan panels, prolonged incubation of MicroScan panels in our studies did not have a beneficial effect. Increasing the inoculum size of the GPS-TA from 10^2 to 10^3 CFU/ml was unsuccessful. Because of the heavy initial inoculum in the positive control well of the card, subsequent readings of the card indicated no significant increase in growth. Therefore, a final report of “insufficient growth in positive control well” was given. An attempt to increase the incubation time of the card was not possible because of limitations placed on the length of incubation by the software in the Vitek computer program.

Using agar dilution screen plates as the “gold standard,” we found the MicroScan and Vitek systems to be comparable for detecting gentamicin and streptomycin resistance. However, the Vitek system was able to provide results in approximately 6 h, whereas the MicroScan system required overnight incubation. Although both systems demonstrated excellent specificity and improved sensitivities, there is still a need to continue using a more accurate technique, such as agar screen dilution plates, broth dilution, or disk diffusion.

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