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Evaluation of a Commercial Microtiter System (MicroScan) Using Both Frozen and Freeze-Dried Panels for Detection of High-Level Aminoglycoside Resistance in *Enterococcus* spp.

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The MicroScan system was compared with agar dilution screen plates for the detection of high-level aminoglycoside resistance in 182 enterococcal isolates. Both the frozen Gram-Positive Combo Type 2 and the freeze-dried Type 5 panels were evaluated. The specificity of both panels for the detection of streptomycin and gentamicin resistance was 100%. However, the sensitivities for the detection of gentamicin and streptomycin resistance were 84 and 31%, respectively, for the Type 2 panels and 90 and 41%, respectively, for the Type 5 panels. The sensitivities of these panels for the detection of enterococcal high-level aminoglycoside resistance are inadequate for routine use.

Although the enterococcus has often been considered avirulent, when found in the blood it has been associated with a high mortality rate (4, 7). Treatment of enterococcus infections may be difficult because unlike other streptococci, enterococci are resistant to most antimicrobial agents. Enterococci are tolerant to antibiotics that inhibit cell wall synthesis (such as penicillin, ampicillin, and vancomycin) and are resistant to aminoglycosides (MICs ranging from 6 to 64 μg/ml) (1, 9). The combination of these two groups of agents is synergistic and is able to kill the organism (1, 10, 11). However, there is no synergy when the enterococcal strain has high-level aminoglycoside resistance (MIC $\geq$ 2,000 μg/ml) (8, 9). Several reports have noted the increasing prevalence of this type of resistance in North America (3, 6, 8, 12, 17, 18). Since high-level aminoglycoside resistance is plasmid mediated (2, 18), the incidence of these strains is likely to continue to increase. It is therefore recommended that enterococcal isolates from blood and sterile body sites be screened for high-level aminoglycoside resistance (5, 6, 8). Current methods include an agar screen plate, disk diffusion, and broth dilution procedures (13–16, 19).

In order to facilitate the detection of high-level aminoglycoside resistance in *Enterococcus* species, MicroScan (Travenol Laboratories, Mahwah, N.J.) has incorporated two aminoglycoside synergy wells into the frozen Gram-Positive Combo Type 2 panel. One well contains streptomycin at a concentration of 2,000 μg/ml, and the other contains gentamicin at a concentration of 500 μg/ml. The new freeze-dried Gram-Positive Type 5 panel contains gentamicin at an increased concentration of 2,000 μg/ml; the concentration of streptomycin remains unchanged. In this study, we compared the MicroScan synergy wells in the Type 2 and Type 5 panels with agar screen plates for the ability to detect high-level gentamicin and streptomycin resistance in *Enterococcus* species.

A total of 182 *Enterococcus* species isolated from blood and sterile body sites were tested. Isolates were identified by Gram stain, utilization of bile esculin, and growth in the presence of 6.5% NaCl; they were identified to species level by MicroScan. Prior to testing, one colony from each isolate was subcultured onto a blood agar plate and incubated overnight at 35°C. *Enterococcus faecalis* HH22 (gentamicin and streptomycin resistant) (8), UWHC 1921 (gentamicin resistant, streptomycin susceptible), and UWHC 1936 (gentamicin susceptible, streptomycin resistant) (16) were tested as control organisms.

The agar screen plates were prepared in duplicate and contained either brain heart infusion agar (GIBCO Diagnostics, Madison, Wis.) or Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.), each with the following concentrations of antibiotic: 500 and 1,000 μg of gentamicin (Schering Canada) per ml and 1,000 and 2,000 μg of streptomycin (Allen and Hanburys) per ml. All isolates were tested with each concentration of antibiotic and on both media. 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 10^9 CFU/ml. Screen plates were inoculated by using a Steers replicator for a final inoculum of approximately 10^6 CFU. The plates were incubated for 18 to 24 h at 35°C. Growth of two or more colonies was considered resistant. MicroScan Gram-Positive Combo Type 2 and Type 5 panels were inoculated and incubated for 18 h according to the instructions of the manufacturer. The synergy wells were evaluated by visual inspection.

Time-kill studies were performed by the method described by Moellering et al. (10) on the following: all 10 of the isolates with discrepant gentamicin results, 5 of the isolates with discrepant streptomycin results, and the control strains HH22, UWHC 1921, and UWHC 1936. The concentrations of antibiotics used were as follows: gentamicin, 5 μg/ml; streptomycin, 25 μg/ml; and penicillin G, 10 U/ml.

In an attempt to determine the cause of discrepancies between the agar screen plates and the MicroScan panels, we evaluated the use of increased inoculum size and prolonged incubation on the MicroScan Type 2 and Type 5 panels. Thirty isolates with discrepant results were retested with both panel types using the standard inoculum concentration of 10^5 CFU/ml and an increased inoculum concentration of 10^7 CFU/ml. Colony counts were performed to confirm the inoculum used. The synergy wells were read at 18, 24, and 48 h.

The results of susceptibility testing of the 182 enterococcal isolates by agar dilution were as follows: gentamicin resistant only, 26 isolates; streptomycin resistant only, 49 iso-

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TABLE 1. Sensitivity of the MicroScan Type 2 and Type 5 aminoglycoside synergy wells compared with the agar screen method

Aminoglycoside	No. resistant by MicroScan/no. resistant by agar dilution[a] (sensitivity)	
	Type 2 panel	Type 5 panel
Gentamicin	53/63 (84)	57/63 (90)
Streptomycin	27/86 (31)	35/86 (41)

[a] 1,000 μg of aminoglycoside per ml in brain heart infusion agar; resistance is defined as the presence of two or more colonies.

lates; gentamicin and streptomycin resistant, 37 isolates; and gentamicin and streptomycin susceptible, 70 isolates. Thus, 63 isolates were gentamicin resistant and 86 isolates were streptomycin resistant. Of the 182 isolates tested, 173 were *E. faecalis* and 9 were *E. faecium*. None of the *E. faecium* isolates were gentamicin resistant, and three were streptomycin resistant. The number of *E. faecium* strains was too small to evaluate separately, and their data were therefore included with the *E. faecalis* results.

Of the 63 isolates found to be gentamicin resistant by agar dilution, 53 were also found to be resistant by the Gram-Positive Type 2 panels, and 57 were resistant by the Gram-Positive Type 5 panels. The sensitivity for detecting gentamicin resistance was about the same for both types of panels and was higher than that for detecting streptomycin resistance (Table 1). The Type 2 panels detected only 27 of 86 streptomycin-resistant isolates, and the Type 5 panels detected 35 resistant isolates. The synergy wells of both the Type 2 and the Type 5 panels were 100% specific. Varying the concentration of gentamicin (500 or 1,000 μg/ml) and streptomycin (1,000 or 2,000 μg/ml) in the agar screen plates yielded identical results. The use of either brain heart infusion agar or Mueller-Hinton agar also had no effect on the results. The time-kill studies confirmed the agar screen results in each case.

The use of prolonged incubation and a higher inoculum was evaluated for both the MicroScan Type 2 and Type 5 panels with 30 isolates which had discrepant results. Prolonged incubation did not improve the performance of either panel type. However, an increase in inoculum concentration to 10^7 CFU/ml did increase the sensitivity of detecting gentamicin and streptomycin resistance, with no loss in specificity. With the increased inoculum, the sensitivities of detecting gentamicin resistance were 100 and 85% with the Type 2 and Type 5 panels, respectively. For detection of streptomycin resistance, the sensitivities were 82 and 57% with the Type 2 and Type 5 panels, respectively.

In order to determine appropriate antimicrobial therapy for severe enterococcal infection, a screening method that can rapidly and accurately detect high-level aminoglycoside resistance is desirable. Several methods for the detection of high-level aminoglycoside resistance in enterococci have been described, including agar dilution (14, 16), broth dilution (19), and disk diffusion (13, 15, 16). MicroScan has incorporated high-level aminoglycoside synergy wells into their Gram-Positive Type 2 and Type 5 panels. Our evaluation of these panels indicates that they do not predict high-level aminoglycoside resistance with adequate sensitivity. These results corroborate the findings of two previous studies (16; M. L. Wilson, J. S. Kuhns, B. E. Dunn, R. T. Ellison, and L. B. Reller, Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 689, 1989), in which a smaller sample of enterococcal strains was tested with the Type 2 panels only. Even though the gentamicin concentration in the Type 5 panels was increased to 2,000 μg/ml from 500 μg/ml, there was no significant improvement in the detection of gentamicin resistance; specificity remained 100%. The concentration of streptomycin remained the same as in the Type 2 panels and demonstrated the same low sensitivity for the detection of streptomycin resistance.

We examined possible reasons for the discrepancy between the agar screen method and MicroScan. It has been suggested that there may be excess amounts of antibiotic in the MicroScan wells (16), but the gentamicin results were the same at concentrations of 500 and 2,000 μg/ml. It is possible that the synergy wells might have benefited from longer incubation in order to make growth detectable, but we found that prolonging incubation to 48 h did not improve the performance of either the Type 2 or Type 5 panels. The inoculum size for the MicroScan panels is 2 logs lower than that used for the agar screen method. We found that increasing the inoculum concentration to 10^7 CFU/ml (final inoculum, 10^5 CFU per well) improved the performance of the Type 2 and Type 5 panels. However, it is uncertain what effect an increased inoculum would have on the identification wells in the panels.

At present, the synergy wells of the MicroScan frozen Type 2 and the freeze-dried Type 5 panels do not have a high degree of sensitivity for detecting gentamicin and streptomycin resistance in enterococci. Therefore, we do not recommend the use of MicroScan for the detection of high-level aminoglycoside resistance in *Enterococcus* spp. and suggest that agar screen plates, broth dilution, or disk diffusion methods be used.

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