

## Revised Approach for Identification and Detection of Ampicillin and Vancomycin Resistance in *Enterococcus* Species by Using MicroScan Panels

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The frequency of antimicrobial agent-resistant enterococci is increasing, making accurate identification and screening for susceptibility essential. We evaluated the ability of MicroScan Positive Breakpoint Combo Type 6 panels (Dade MicroScan Inc., West Sacramento, Calif.) to identify *Enterococcus* species and to detect ampicillin and vancomycin resistance. A total of 398 well-characterized *Enterococcus* isolates from two institutions were inoculated into MicroScan panels, into conventional biochemical assays, and into ampicillin and vancomycin agar dilution media. Resistance was verified by the broth macrodilution method. MicroScan panels accurately detected resistance to ampicillin in 132 of 132 enterococcal isolates, while three isolates for which the MICs were  $<16 \mu\text{g/ml}$  were classified incorrectly by MicroScan panels as resistant. No beta-lactamase-producing enterococci were detected. All 64 isolates showing resistance to vancomycin (MICs  $\geq 32 \mu\text{g/ml}$ ) were correctly classified by MicroScan panels. Seven isolates for which the vancomycin MICs were 8 and  $16 \mu\text{g/ml}$  were incorrectly classified as susceptible by MicroScan panels, while eight isolates for which the MICs were  $4 \mu\text{g/ml}$  were incorrectly labeled as intermediate. Fourteen of these 15 isolates were subsequently identified as motile enterococci. Overall, there were three major errors in susceptibility testing for ampicillin and 15 minor errors for vancomycin. Conventional testing confirmed the identity of 181 *Enterococcus faecalis* isolates, 157 *E. faecium* isolates, and 60 isolates of other species; however, 56 of these 60 isolates were misidentified by the MicroScan panels. After recognition of this problem, a revised approach which included tests for pigment, motility, and sucrose fermentation was devised. In combination with these additional assays, the conventional MicroScan panels accurately identified the 56 originally misidentified isolates. In summary, the ability of MicroScan panels to detect vancomycin and ampicillin resistance in enterococci was confirmed. Our study found that the inability of MicroScan panels to identify enterococci other than *E. faecalis* and *E. faecium* can be compensated for by the addition of standard assays.

Currently, 14 species of enterococci recovered from humans have been identified (6, 7, 9, 17, 18). *Enterococcus faecalis* accounts for 80 to 90% of enterococcal infections, with *E. faecium* being responsible for 10 to 15% (18). The number of other species is generally reported at less than 5%, although this number may be higher since methods to identify enterococci other than *E. faecalis* and *E. faecium* are not widely utilized. Additionally, enterococci are significant nosocomial pathogens and have the capacity to develop and transfer antimicrobial resistance (5, 12, 14, 19). Enterococci resistant to high levels of aminoglycosides and the beta-lactam antimicrobial agents—and, more recently, the glycopeptides, including vancomycin and teicoplanin—have emerged (1, 2, 4, 5, 14, 19, 21). Currently, there are no known effective agents to treat infections caused by vancomycin-resistant enterococci; prevention and early detection are the best approaches to control (5, 10, 19).

Automated systems, including the Vitek and MicroScan systems, have been developed to identify and to determine the antimicrobial susceptibility of enterococci (1, 2, 18, 22, 23, 25–28). Previous studies have shown conventional MicroScan panels to be reliable in the identification of *Enterococcus* spe-

cies, even though the data bank includes only *E. faecalis*, *E. faecium*, *E. durans*, and *E. avium* (24). There are, however, conflicting reports on the reliability of the MicroScan system to detect ampicillin- and vancomycin-resistant strains of *Enterococcus* species (13, 22, 23, 27).

Recently, the Food and Drug Administration expressed concerns with the ability of both of the conventional MicroScan Dried Positive panels to detect ampicillin and vancomycin resistance in strains of enterococci. MicroScan personnel subsequently requested that their panels not be used to detect ampicillin and vancomycin resistance in enterococci until their performance could be verified. MicroScan reported acceptable performance results with enterococcal challenge strains from the Centers for Disease Control and Prevention, and clearance was granted from the Food and Drug Administration to remove the ampicillin and vancomycin limitation for these panels (results on file at MicroScan).

The purpose of this study was to evaluate the ability of the conventional MicroScan Positive Breakpoint Combo Type 6 panel to identify *Enterococcus* species and to detect vancomycin and ampicillin resistance. After recognition of a problem in identifying enterococci, we devised a modified approach whereby the MicroScan panels, in combination with supplemental testing, were successful in identifying *Enterococcus* species not included in the present data management system.

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TABLE 1. Performance of conventional MicroScan Positive Breakpoint Combo Type 6 panels for detection of ampicillin and vancomycin resistance in *Enterococcus* species

Antibiotic	MIC <sup>a</sup> (μg/ml)	Total no. of isolates	No. with MicroScan panel result <sup>b</sup>			NCCLS classification	No. incorrectly identified
			S	I	R		
Vancomycin	<8	315	307	8	0	S	8 <sup>c</sup>
	8	17	6	11	0	I	6 <sup>d</sup>
	16	2	1	1	0	I	1 <sup>e</sup>
	≥32	64 <sup>f</sup>	0	0	64	R	0
Ampicillin	<8	258	256		2	S	2 <sup>g</sup>
	8	8	7		1	S	1 <sup>h</sup>
	16	18	0		18	R	0
	≥32	114	0		114	R	0

<sup>a</sup> Determined by the broth macrodilution method by using NCCLS procedures.

<sup>b</sup> Abbreviations: S, susceptible; R, resistant; I, intermediate.

<sup>c</sup> Seven isolates were *E. gallinarum* and one was *E. casseliflavus*; the MICs for all were 4 μg/ml.

<sup>d</sup> Four isolates were *E. gallinarum*, one was *E. faecalis*, and one was *E. casseliflavus*.

<sup>e</sup> Isolate was *E. gallinarum*.

<sup>f</sup> Sixty isolates identified as *E. faecium* (52 *vanA* and 8 *vanB*) and 4 identified as *E. faecalis* (3 *vanB* and 1 *vanA*).

<sup>g</sup> One isolate each of *E. faecium* and *E. faecalis*; the MICs for both were 4 μg/ml.

<sup>h</sup> Isolate was *E. raffinosus*.

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#### MATERIALS AND METHODS

**Organisms.** Three hundred ninety-eight enterococcal isolates were evaluated, including 370 randomly selected clinical isolates of enterococci identified in the Clinical Microbiology Laboratory at the University of Nebraska Medical Center (195 from blood, 94 from urine, and 81 from tissue and other body fluids). An additional 28 well-characterized *Enterococcus* isolates (4 *E. faecium vanB* isolates, 4 *E. faecium vanA* isolates, 4 *E. faecalis vanB*, isolates, 1 *E. faecalis vanA* isolate, 9 *E. gallinarum vanC1* isolates, and 6 *E. casseliflavus* isolates) were kindly supplied by Daniel F. Sahn (Jewish Hospital of St. Louis, St. Louis, Mo.). Only one isolate per patient was tested. The isolates included were pyrrolidonyl arylamidase-positive, catalase-negative, and Gram stain-positive specimens which had been stored frozen for up to 2 years at -70°C. A bacitracin-susceptible, beta-hemolytic, pyrrolidonyl-arylamidase-positive isolate was identified as a group A streptococcus and excluded from evaluation. Prior to testing, the isolates were passed twice on sheep blood agar to ensure a pure culture.

**MicroScan panels.** Conventional MicroScan panels (Positive Breakpoint Combo Type 6; Dade MicroScan Inc., West Sacramento, Calif.) were inoculated with fresh isolates by the turbidity standard technique. The panels were incubated for a full 24 h at 35°C in ambient air and read with the MicroScan autoSCAN-4 reader. All procedures were performed according to the manufacturer's directions.

**Conventional biochemicals.** The abbreviated conventional biochemical identification scheme of Facklam and Collins was used as a basis for species identification (9). Briefly, tests for the following were performed on all enterococcal isolates: tolerance to and hydrolysis of bile-esculin; growth in brain heart infusion broth with 6.5% NaCl; deamination of arginine (1%) in Moeller decarboxylase base; fermentation of 1% lactose, 1% mannitol, 1% sorbose, 1% sorbitol, 1% glycerol, 1% sucrose, 1% raffinose, 1% ribose, and 1% arabinose in heart infusion broth; and motility at 30°C and pigmentation (detected by swabbing sheep blood agar following a 48-h incubation). For selected isolates, the utilization of pyruvate was tested in 1% pyruvate broth and the ability to grow and reduce tellurite (indicated by blackening of the medium) in Todd-Hewitt broth supplemented with 0.05% potassium tellurite and to ferment 1% inulin was tested. Unless specified, the inoculated media were incubated at 35°C in ambient air for up to 7 days. Modifications to Facklam and Collins' identification scheme included the following differentiations: lactose-negative strains of *E. faecalis* (tellurite reduction and ribose fermentation positive) from *E. solitarius* (negative for both), yellow-pigmented and motile *E. casseliflavus* (ribose fermentation positive) from yellow-pigmented and motile *E. flavescens* (ribose fermentation negative), and *E. hirae* (pyruvate utilization negative) from *E. dispar* (pyruvate utilization positive) (1, 2, 6, 9, 17, 18, 25). When identification discrepancies between MicroScan panels and conventional biochemicals occurred, the following MicroScan panel biochemical results were determined: lactose, ribose, arabinose, and raffinose fermentations and pyruvate utilization.

**Agar dilution method.** The agar dilution procedure of the National Committee for Clinical Laboratory Standards (NCCLS) using the direct colony suspension method for preparing the inoculum was used with the following modifications: Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) was supplemented with 4 μg of either vancomycin (Sigma Chemical Co., St. Louis, Mo.) or ampicillin (Sigma) per ml, and a 0.001-ml calibrated loop was used to place an aliquot of the organism on the surface of the media for an inoculum of approximately 10<sup>5</sup> CFU per spot (15). The plates were incubated at 35°C in ambient air and read at 24 and 48 h, and any growth or haze was considered to indicate resistance to the antibiotic used. All resistant isolates on the agar screen plate and any isolates with discrepant agar dilution method and MicroScan panel results were subsequently retested by the broth macrodilution method to verify resistance.

**Broth macrodilution method.** All enterococcal isolates resistant (intermediate) to ampicillin and/or vancomycin as determined by the agar dilution method and/or MicroScan panels were subsequently retested by the broth macrodilution method according to NCCLS recommendations (15). A standardized inoculum was prepared by making a saline suspension of colonies from an 18- to 24-h growth on sheep blood agar adjusted to match the 0.5 McFarland turbidity standard and then diluting the suspension 1:200 in Mueller-Hinton broth. The final inoculum in the growth control well contained approximately 5 × 10<sup>5</sup> CFU/ml (range of 3 × 10<sup>5</sup> to 7 × 10<sup>5</sup> CFU/ml). *E. faecalis* ATCC 29212 was used as a control organism; the ampicillin MIC for this organism is 0.5 to 2.0 μg/ml, and the vancomycin MIC for this organisms is 1.0 to 4.0 μg/ml. Isolates were considered resistant to ampicillin if the MIC was ≥16 μg/ml and resistant to vancomycin if the MIC was ≥32 μg/ml (all isolates for which the vancomycin MIC was 8 to 16 μg/ml were classified as intermediate as recommended by the NCCLS). A minor error in testing was defined as an MIC of <8 μg/ml for vancomycin classified by the MicroScan panel as intermediate or an MIC of 8 to 16 μg/ml for vancomycin classified by the MicroScan panel as susceptible. A major error was considered to be an MIC for ampicillin of ≤8 μg/ml classified by the MicroScan panel as resistant. The broth macrodilution results were also utilized to resolve discrepancies between MicroScan panels and agar dilution testing.

**Beta-lactamase detection.** Beta-lactamase production was detected by the chromogenic cephalosporin (nitrocefin) disc method (BBL Microbiology, Cockeysville, Md.).

#### RESULTS

All 132 isolates resistant to ampicillin by the broth macrodilution method were correctly identified by MicroScan panels, while 3 isolates (MIC <16 μg/ml) were reported incorrectly by MicroScan panels as resistant to ampicillin (Table 1). No beta-lactamase-producing enterococci were identified. Additionally, all isolates (64 of 64) resistant to vancomycin were correctly classified by MicroScan panels. Seven isolates for which the vancomycin MICs were 8 and 16 μg/ml (intermediate) were incorrectly identified as susceptible by MicroScan panels. Eight motile *Enterococcus* isolates were classified incorrectly by MicroScan panel as intermediate; the vancomycin MICs for these isolates were 4 μg/ml. Thus, a total of 15 discrepancies in vancomycin susceptibility results between MicroScan panels and the broth macrodilution test were identified. Of 315 isolates susceptible to vancomycin, 32 grew on the agar screen

TABLE 2. Ability of conventional MicroScan Positive Breakpoint Combo Type 6 panels to identify *Enterococcus* species<sup>a</sup>

MicroScan panel identification	No. of isolates correct/no. tested	No. of errors (correct identification)
<i>E. faecalis</i> <sup>b</sup>	181/181	None
<i>E. faecium</i>	157/202	27 ( <i>E. gallinarum</i> <sup>c</sup> ) 18 ( <i>E. casseliflavus</i> <sup>c</sup> )
<i>E. avium</i>	1/7	6 ( <i>E. raffinosus</i> <sup>d</sup> )
<i>E. durans</i>	3/7	4 ( <i>E. hirae</i> <sup>e</sup> )
Group D <i>Enterococcus</i>	0/1	1 ( <i>E. casseliflavus</i> <sup>e,f</sup> )

<sup>a</sup> Species of *Enterococcus* known to be isolated from human sources which were not detected in this study included *E. solitarius*, *E. flavescens*, *E. mundtii*, *E. malodoratus*, *E. pseudoavium*, and *E. dispar*. Asaccharolytic variants of *E. faecalis* also were not detected.

<sup>b</sup> Twenty-one isolates did not ferment lactose, but all fermented ribose and reduced tellurite.

<sup>c</sup> All isolates exhibited motility at 30°C, with *E. casseliflavus* having a distinct yellow pigment.

<sup>d</sup> All isolates were raffinose fermentation positive on the MicroScan panel.

<sup>e</sup> One isolate was raffinose fermentation positive by the MicroScan panel, and all fermented both raffinose and sucrose in conventional biochemical tests.

<sup>f</sup> Negative for arginine dihydrolase.

plate containing 4 µg of vancomycin per ml. As determined by the broth microdilution method, the MICs for all of these isolates were 2 to 4 µg/ml, and all but three *E. faecalis* isolates were identified as motile enterococci.

Three hundred ninety-eight *Enterococcus* isolates were identified by conventional biochemicals as 181 *E. faecalis* isolates (21 failed to ferment lactose), 157 *E. faecium* isolates, 27 *E. gallinarum* isolates, 19 *E. casseliflavus* isolates, 6 *E. raffinosus* isolates, 4 *E. hirae* isolates, 3 *E. durans* isolates, and 1 *E. avium* isolate (Table 2). Fifty-six enterococcal isolates from species not included in the data management system were misidentified by MicroScan panels. All six *E. raffinosus* isolates were misidentified as *E. avium* by MicroScan panels, and all four *E.*

*hirae* isolates were incorrectly identified as *E. durans*. Forty-five of the motile enterococcal isolates detected (27 *E. gallinarum* isolates and 18 *E. casseliflavus* isolates) were identified by MicroScan panels as *E. faecium*, with 1 *E. casseliflavus* isolate identified as a group D *Enterococcus* species. A new approach to identify the 14 clinical species of enterococci known to be isolated from human sources by utilizing conventional biochemical assays, as suggested by other investigators, is outlined in Table 3 (1, 2, 6, 9, 18, 25). Fifty-three of the misidentified isolates were identifiable through a combination of results from the MicroScan panels and tests for motility and pigment production. Additional testing for sucrose fermentation was required to identify the three *E. hirae* isolates which were raffinose fermentation negative as indicated by MicroScan panels.

## DISCUSSION

The ability to accurately identify enterococci at the species level is important not only for epidemiological purposes but also to recognize species such as *E. faecium* and the motile enterococci, which tend to show resistance to antimicrobial agents commonly used for therapy. Automated systems, such as the Vitek system and both the conventional and rapid MicroScan systems, are reported to provide accurate identification for both *E. faecalis* and *E. faecium*; however, they are not considered reliable for the identification of the other *Enterococcus* species (1, 2, 18, 24, 26). In addition, using automated systems to perform susceptibility testing of enterococci for antimicrobial agents, such as ampicillin, vancomycin, and the aminoglycosides, has had conflicting results (13, 21–23, 26, 27). This study evaluated the ability of the conventional MicroScan Positive breakpoint Combo Type 6 panels to identify enterococci and to detect resistance to both ampicillin and vancomycin.

The results of the present study showed that 132 of 132

TABLE 3. Revised approach to identification of *Enterococcus* species using the MicroScan Positive Breakpoint Combo Type 6 panel results along with supplemental testing<sup>a,b</sup>

MicroScan panel identification	Panel results	Supplemental test result(s) required	Reference identification
<i>E. faecalis</i>	Lac <sup>+</sup> , rib <sup>+</sup> or lac <sup>+</sup> , rib <sup>-</sup>	None	<i>E. faecalis</i>
	Lac <sup>-</sup> , rib <sup>+</sup>	None	<i>E. faecalis</i>
	Lac <sup>-</sup> , rib <sup>-</sup>	None	<i>E. solitarius</i> <sup>c</sup>
<i>E. faecium</i>	All results	Mot <sup>-</sup> , yel pig <sup>-</sup>	<i>E. faecium</i>
		Mot <sup>+</sup> , yel pig <sup>+</sup>	<i>E. casseliflavus</i> or <i>E. flavescens</i> <sup>c,d</sup>
		Mot <sup>+</sup> , yel pig <sup>-</sup>	<i>E. gallinarum</i>
		Mot <sup>-</sup> , yel pig <sup>+</sup>	<i>E. mundtii</i> <sup>c</sup>
<i>E. avium</i>	Ara <sup>+</sup> , raff <sup>+</sup>	None	<i>E. raffinosus</i>
	Ara <sup>+</sup> , raff <sup>-</sup>	None	<i>E. avium</i>
	Ara <sup>-</sup> , raff <sup>+</sup>	None	<i>E. malodoratus</i> <sup>c</sup>
	Ara <sup>-</sup> , raff <sup>-</sup>	None	<i>E. pseudoavium</i> <sup>c</sup>
<i>E. durans</i>	Raff <sup>+</sup> , prv <sup>-</sup>	None	<i>E. hirae</i>
	Raff <sup>+</sup> , prv <sup>+</sup>	None	<i>E. dispar</i> <sup>c</sup>
	Raff <sup>-</sup> , prv <sup>+</sup>	None	<i>E. faecalis</i> <sup>c,e</sup>
	Raff <sup>-</sup> , prv <sup>-</sup>	Suc <sup>-</sup>	<i>E. durans</i>
		Suc <sup>+</sup>	<i>E. hirae</i>

<sup>a</sup> Symbols: +, positive result; -, negative result. Abbreviations: lac, lactose fermentation; rib, ribose fermentation; mot, motility; yel pig, yellow pigment; ara, arabinose fermentation; raff, raffinose fermentation; prv, pyruvate utilization; suc, sucrose fermentation.

<sup>b</sup> In cases of questionable identification, i.e., group D *Enterococcus* and *Streptococcus* species, additional conventional biochemical tests may be warranted.

<sup>c</sup> Tentative identification based on conventional biochemical testing from other investigators (1, 2, 6, 9, 18, 25).

<sup>d</sup> Evaluation of MicroScan result for ribose fermentation to differentiate *E. casseliflavus* (positive) from *E. flavescens* (negative).

<sup>e</sup> Asaccharolytic variants.

ampicillin-resistant enterococcal isolates were detected by conventional MicroScan panels. One isolate for which the ampicillin MIC was 8  $\mu\text{g/ml}$ , which is at the high ends of the susceptible category as described by the NCCLS, and two isolates for which the MICs were 4  $\mu\text{g/ml}$  were classified by MicroScan panels as resistant to ampicillin. The overall performance (sensitivity of 100% and specificity of 98.9%) was similar to those reported by Holmsen et al., who also showed that *Enterococcus* isolates for which the MICs were 8  $\mu\text{g/ml}$  were classified as resistant by the MicroScan Rapid Positive MIC panels (11). Additionally, Louie et al. showed that the MicroScan Positive MIC Type 6 panels demonstrated poor sensitivity (83.3%) for detection of ampicillin resistance when readings relied on the Walkaway System (13).

Since no beta-lactamase-positive enterococci were identified in this study, the reliability of conventional MicroScan panels for detecting strains which produce this enzyme is not known. Following multilaboratory testing of various *Enterococcus* isolates, Tenover et al. demonstrated that MicroScan panels using the autoSCAN-4 reader correctly detected susceptibility to ampicillin when the MIC was  $\leq 4$  or  $>256$   $\mu\text{g/ml}$  but were unable to identify a beta-lactamase-producing strain in a majority of cases (23). The NCCLS recommends that all clinically significant ampicillin-susceptible enterococci be tested for beta-lactamase production by using a direct, nitrocefin-based test (15). Currently, the identification of these strains is considered rare, and the cost-effectiveness of this approach has been challenged (1, 18, 20).

Vancomycin resistance in enterococci has recently become a clinical problem due to a lack of alternative therapies (4, 5, 14, 19). Three major phenotypes of vancomycin-resistant enterococci have been described (12, 28). These are the *vanA* strains, which show high-level vancomycin resistance (MICs of  $>32$   $\mu\text{g/ml}$ ) and resistance to teicoplanin; *vanB* strains, which have variable resistance to vancomycin (MICs of 4 to  $\geq 128$   $\mu\text{g/ml}$ ) and susceptibility to teicoplanin; and *vanC* motile strains, which show intrinsic resistance to low levels of vancomycin (MICs of 2 to 32  $\mu\text{g/ml}$ ) and susceptibility to teicoplanin. From studies of vancomycin resistance, Willey et al. showed the MicroScan Positive MIC Type 6 panels to have a sensitivity and specificity of 99 and 96%, respectively, when the visual inspection method of detection is used (27). In addition, other studies have shown these panels to be reliable except in cases in which low-level vancomycin resistance (MICs of 4 to 8  $\mu\text{g/ml}$ ) was present (22). Tenover et al. showed in multilaboratory testing, using MicroScan panels and the autoSCAN-4 reader and enterococci for which the vancomycin MICs were 16 to 64  $\mu\text{g/ml}$ , that laboratories had only 36.8 to 57.9% agreement with the reference MIC result (23). In our study, 15 isolates had vancomycin results misclassified by the MicroScan system; 8 as intermediate, with MICs being 4  $\mu\text{g/ml}$ , and 7 as susceptible, with MICs being 8 and 16  $\mu\text{g/ml}$ , for an overall sensitivity and specificity of 91.6 and 97.5%, respectively. Of these, 14 were identified as motile enterococci and 1 as *E. faecalis* (*vanB* phenotype) with a vancomycin MIC of 8  $\mu\text{g/ml}$ . All isolates for which the vancomycin MICs were  $\geq 32$   $\mu\text{g/ml}$  were classified as resistant to vancomycin by the MicroScan panel. This group included 53 *vanA* enterococcal isolates (52 *E. faecium* isolates and 1 *E. faecalis* isolate) and 11 *vanB* enterococcal isolates (8 *E. faecium* isolates and 3 *E. faecalis* isolates).

Since low-level vancomycin resistance has been described as intrinsic to certain motile species of enterococci, proper identification of these organisms may help in recognizing this low-level vancomycin resistance characteristic (12). However, identification of the *vanB* enterococci for which the vancomycin MICs are in the range of 8 to 16  $\mu\text{g/ml}$  has proved to be a

challenge for the MicroScan system and other automated systems. The present study showed that the conventional MicroScan panel misclassified 7 of the 19 isolates for which the MICs were 8 to 16  $\mu\text{g/ml}$ . Similar results were found by Tenover et al. when they evaluated automated systems (22).

Currently, the NCCLS recommends testing all clinically significant enterococci with an agar screen plate containing 6  $\mu\text{g}$  of vancomycin per ml or by using the disc diffusion method (10, 15, 21). We evaluated the use of an agar dilution plate containing 4  $\mu\text{g}$  of vancomycin per ml as a supplemental test to the MicroScan panel in order to detect enterococci with low-level resistance to vancomycin. Enterococci which grew on this vancomycin-agar screen medium were retested by the disc diffusion method as described by the NCCLS to verify resistance. Additionally, all *E. faecium* bacteria which grew on the screen plate were tested for motility and pigment production. In this study, all motile enterococci grew on the agar screen medium. This medium appears to be useful to identify motile species for which the MICs are less than 4  $\mu\text{g/ml}$ , which was the case with six isolates of *E. casseliflavus*, for which the MICs were 2  $\mu\text{g/ml}$ . Three false-resistant *E. faecalis* isolates which grew on the vancomycin screen medium were verified as susceptible by the broth microdilution method (MICs of 2  $\mu\text{g/ml}$ ).

Of the 398 *Enterococcus* isolates tested, MicroScan panels correctly identified 342 of an error rate of 8.6%, which is similar to that reported by others (1, 24). The 56 misidentified species were not included in the present MicroScan data management system. All of the misidentified species were identifiable by results of supplemental tests for motility, pigment production, and/or sucrose fermentation in combination with panel results. The six *E. raffinosus* isolates were distinguished from *E. avium* isolates by their ability to ferment raffinose on the MicroScan panel. The 27 *E. gallinarum* and 19 *E. casseliflavus* isolates were correctly identified through the use of a motility test and determination of pigment production. One of four *E. hirae* isolates was distinguished from *E. durans* isolates by the ability to ferment raffinose on the MicroScan panel. It has previously been shown that 75 to 85% of *E. hirae* strains ferment both raffinose and sucrose whereas *E. durans* does not ferment either sugar (24). The addition of sucrose fermentation testing helped distinguish three of four *E. hirae* isolates from the raffinose-negative *E. durans* isolates. Identification of other characteristics may be needed to identify some enterococci since nonmotile, nonpigmented *E. casseliflavus* bacteria and motile, pigmented *E. flavescens* bacteria have been described (3, 7, 8, 12, 25). DNA-based assays have been developed to identify these species; however, Cartwright et al. have shown using DNA amplification testing that tests for pigmentation and motility were sufficiently reliable for identification of these species (3, 5, 11). Six of the 14 *Enterococcus* were not included in this study; additional studies are needed to evaluate our revised approach for identification for these other species.

The results of this study confirm that the conventional MicroScan Positive Breakpoint Combo Type 6 panels are not reliable in identifying species of enterococci other than *E. faecalis* and *E. faecium*. However, supplemental testing in combination with MicroScan panel biochemical results was useful to correctly identify these species. Recognition of resistance to both ampicillin and vancomycin was acceptable with these panels. This study demonstrated that an agar screen plate containing 4  $\mu\text{g}$  of vancomycin per ml was useful to identify *Enterococcus* species with low-level vancomycin resistance. The clinical significance of detecting these enterococcal isolates is not known, even though serious infections caused by the low-

level vancomycin-resistant motile enterococci have been reported (16).

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#### REFERENCES

- Bryce, E. A., S. J. V. Zemcov, and A. M. Clarke. 1991. Species identification and antibiotic resistance patterns of the enterococci. *Eur. J. Clin. Microbiol. Infect. Dis.* **10**:745-747.
- Buschelman, B. J., M. J. Bale, and R. N. Jones. 1993. Species identification and determination of high-level aminoglycoside resistance among enterococci: comparison study of sterile body fluid isolates, 1985-1991. *Diagn. Microbiol. Infect. Dis.* **16**:119-122.
- Cartwright, C. P., F. Stock, G. A. Fahle, and V. J. Gill. 1995. Comparison of pigment production and motility tests with PCR for reliable identification of intrinsically vancomycin-resistant enterococci. *J. Clin. Microbiol.* **33**:1931-1399.
- Centers for Disease Control and Prevention. 1993. Nosocomial enterococci resistant to vancomycin; United States, 1989-1993. *Morbidity and Mortality Weekly Report*. **42**:597-599.
- Centers for Disease Control and Prevention. 1995. Recommendations for preventing the spread of vancomycin resistance; recommendations of the hospital control practices advisory committee. *Morbidity and Mortality Weekly Report*. **44**(RR-12):1-3.
- Collins, M. D., V. M. Rodrigues, N. E. Pigott, and R. R. Facklam. 1991. *Enterococcus dispar* sp. nov., a new *Enterococcus* species from human sources. *Lett. Appl. Microbiol.* **12**:95-98.
- Donabedian, S., J. W. Chow, D. M. Shlaes, M. Green, and M. J. Zervos. 1995. DNA hybridization and contour-clamped homogeneous electric field electrophoresis for identification of enterococci to the species level. *J. Clin. Microbiol.* **33**:141-145.
- Dutka-Malen, S., S. Evers, and P. Courvalin. 1995. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. *J. Clin. Microbiol.* **33**:24-27.
- Facklam, R. R., and M. D. Collins. 1989. Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. *J. Clin. Microbiol.* **27**:731-734.
- Free, L., and D. F. Sahn. 1995. Investigation of the reformulated Remel Synergy Quad plate for detection of high-level aminoglycoside and vancomycin resistance among enterococci. *J. Clin. Microbiol.* **33**:1643-1645.
- Holmsen, K., S. Cullen, L. Nea, F. Julio, M. McCreary, D. Ballou, and B. Vanderhoof. 1995. Detection of ampicillin resistance in enterococci using MicroScan® Rapid Pos MIC panels (RPM), abstr. C-267, p. 47. In *Abstracts of the 95th General Meeting of the American Society for Microbiology 1995*. American Society for Microbiology, Washington, D.C.
- Leclercq, R., S. Dutka-Malen, J. Duval, and P. Courvalin. 1992. Vancomycin resistance gene *vanC* is specific to *Enterococcus gallinarum*. *Antimicrob. Agents Chemother.* **36**:2005-2008.
- Louie, M., A. E. Simor, S. Szeto, M. Patel, B. Kreiswirth, and D. E. Low. 1992. Susceptibility testing of clinical isolates of *Enterococcus faecium* and *Enterococcus faecalis*. *J. Clin. Microbiol.* **30**:41-45.
- Montecalvo, M. A., H. Horowitz, C. Gedris, C. Carbonaro, F. C. Tenover, A. Issha, P. Cook, and G. P. Wormser. 1994. Outbreak of vancomycin-, ampicillin-, and aminoglycoside-resistant *Enterococcus faecium* bacteremia in an adult oncology unit. *Antimicrob. Agents Chemother.* **38**:1363-1367.
- National Committee for Clinical Laboratory Standards. 1993. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Publication M7-A3, 3rd ed. National Committee for Clinical Laboratory Standards, Villanova, Pa.
- Patterson, J. E., A. H. Sweeney, M. Simms, N. Carley, R. Mangi, J. Sabetta, and R. W. Lyons. 1995. An analysis of 110 serious enterococcal infections; epidemiology, antibiotic susceptibility, and outcome. *Medicine (Baltimore)* **74**:191-199.
- Pompei, R., F. Berlutti, M. C. Thaller, A. Ingiani, G. Cortis, and B. Dainelli. 1992. *Enterococcus flavescens* sp. nov., a new species of enterococci of clinical origin. *Int. J. Syst. Bacteriol.* **42**:365-369.
- Ruoff, K. L., L. de la Maza, M. J. Murtagh, J. D. Spargo, and M. J. Ferrano. 1990. Species identities of enterococci isolated from clinical specimens. *J. Clin. Microbiol.* **28**:435-437.
- Spera, R. V. Jr., and B. F. Farber. 1994. Multidrug-resistant *Enterococcus faecium*: an untreatable nosocomial pathogen. *Drugs* **48**:678-688.
- Steffe, C. H., R. M. Morrell, and B. L. Wasilauskas. 1995. Routine testing for beta-lactamase positive enterococci: is it really necessary? A paradigm shift in the clinical detection of rare organisms, abstr. C-119, p. 21. In *Abstracts of the 95th General Meeting of the American Society for Microbiology 1995*. American Society for Microbiology, Washington, D.C.
- Swenson, J. M., N. C. Clark, M. J. Ferraro, D. F. Sahn, G. Doern, M. A. Pfaller, L. B. Reller, M. P. Weinstein, R. J. Zabransky, and F. C. Tenover. 1994. Development of a standardized screening method for detection of vancomycin-resistant enterococci. *J. Clin. Microbiol.* **32**:1700-1704.
- Tenover, F. C., J. M. Swenson, C. M. O'Hara, and S. A. Stocker. 1995. Ability of commercial and reference antimicrobial susceptibility testing methods to detect vancomycin resistance in enterococci. *J. Clin. Microbiol.* **33**:1524-1527.
- Tenover, F. C., J. Tokars, J. Swenson, S. Paul, K. Spitalny, and W. Jarvis. 1993. Ability of clinical laboratories to detect antimicrobial agent-resistant enterococci. *J. Clin. Microbiol.* **31**:1695-1699.
- Tritz, D. M., P. C. Iwen, and G. L. Woods. 1990. Evaluation of MicroScan for identification of *Enterococcus* species. *J. Clin. Microbiol.* **28**:1477-1478.
- Vincent, S., R. G. Knight, M. Green, D. F. Sahn, and D. M. Shales. 1991. Vancomycin susceptibility and identification of motile enterococci. *J. Clin. Microbiol.* **29**:2335-2337.
- Willey, B. M., B. N. Kreiswirth, A. E. Simor, Y. Faur, M. Patel, G. Williams, and D. E. Low. 1993. Identification and characterization of multiple species of vancomycin-resistant enterococci, including an evaluation of Vitek software version 7.1. *J. Clin. Microbiol.* **31**:2777-2779.
- Willey, B. M., B. N. Kreiswirth, A. E. Simor, G. Williams, S. R. Scriver, A. Phillips, and D. E. Low. 1992. Detection of vancomycin resistance in *Enterococcus* species. *J. Clin. Microbiol.* **30**:1621-1624.
- Zabransky, R. J., A. R. Dinuzzo, and G. L. Woods. 1995. Detection of vancomycin resistance in enterococci by the Alamar MIC system. *J. Clin. Microbiol.* **33**:791-793.