Evaluation of MicroScan for Identification of Enterococcus Species

DENISE M. TRITZ,* PETER C. IWEN, AND GAIL L. WOODS

Department of Pathology and Microbiology, University of Nebraska Medical Center,
600 S. 42nd Street, Omaha, Nebraska 68198

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Emerging drug resistance of the enterococci necessitates differentiation from group D streptococci and accurate species identification. MicroScan (Baxter Healthcare Corp., West Sacramento, Calif.) has recently developed a microdilution system for identification and antibiotic susceptibility testing of gram-positive cocci. To evaluate the ability of this system to identify Enterococcus species, 100 isolates identified as enterococci by MicroScan were tested by conventional media and 60 isolates of streptococci were tested by MicroScan. Incubation times for conventional and MicroScan methods were 96 and 18 to 24 h, respectively. For 94 strains of enterococci (77 Enterococcus faecalis, 14 Enterococcus faecium, and 3 Enterococcus durans), identification by conventional media and MicroScan agreed. Of the remaining six isolates, four were identified as E. faecalis and two were identified as E. durans by MicroScan, whereas by conventional media the four E. faecalis isolates were identified as Enterococcus solitarius and the two E. durans isolates were identified as Enterococcus hirae. None of the 60 streptococci were identified as enterococci. MicroScan is a reliable method for identification of the commonly encountered enterococcal species E. faecalis and E. faecium; however, modifications of the system are necessary for identification of other Enterococcus species.

The enterococci are catalase-negative, gram-positive cocci that belong to Lancefield group D. These organisms are normal inhabitants of the human gastrointestinal tract and are frequently present in the genitourinary tracts of men and women (9). The enterococci are a common cause of urinary tract infections, are frequently recovered from intra-abdominal abscesses, and are increasingly recognized as an important cause of bacteremia. Enterococcal endocarditis and meningitis occur less commonly (9, 11, 12). Recently, enterococci have become significant nosocomial pathogens, particularly in the elderly (9, 11, 12, 16).

The most important clinical isolates of Enterococcus species include Enterococcus faecalis, E. faecium, and Enterococcus durans. Enterococcus avium and other enterococci probably account for 10% or less of all human enterococcal isolates (5). E. faecalis is the most frequently recovered species, followed by E. faecium (5, 9). Differentiation of the enterococci from the group D streptococci and viridans group streptococci is important because the enterococci are more resistant to antimicrobial agents (4, 9, 17). Recently, strains of enterococci with high-level resistance to aminoglycosides and to penicillin have been reported (3, 13, 14). Vancomycin-resistant strains have also been identified (8). Identification of the species of an enterococcal isolate is also clinically relevant since E. faecium is more resistant than E. faecalis to the more commonly used antimicrobial agents (10, 11).

Several systems for identification of gram-positive cocci are commercially available (1, 2, 6, 7, 10). MicroScan (Baxter Healthcare Corp., West Sacramento, Calif.) has developed a microdilution system which simultaneously provides identification of gram-positive cocci and antimicrobial agent susceptibility results. The purpose of this study was to evaluate the ability of the MicroScan system to accurately identify the Enterococcus species.

**Isolates.** A total of 100 isolates of catalase-negative, gram-positive cocci, initially identified as enterococci by MicroScan, and 60 isolates of streptococci initially identified by conventional methods were evaluated. Most strains were collected from ambulatory and hospitalized patients at the University of Nebraska Medical Center. In addition, R. Facklam (Centers for Disease Control, Atlanta, Ga.) and G. Hall (Cleveland Clinic, Cleveland, Ohio) kindly provided seven and four Enterococcus isolates, respectively.

**MicroScan identification system.** Frozen panels (Gram Positive Breakpoint Combo Panel Type 2; Baxter Healthcare) were thawed and inoculated, using the turbidity standard technique according to the directions of the manufacturer. The panels were incubated for 18 to 24 h at 35°C in ambient air and then read with the MicroScan AutoScan-4 instrument. Organism identification was based on the following test reactions: crystal violet; MicroScan screen; nitrate reduction; PNP-β-D-glucuronide; indoxyl phosphate; Voges-Proskauer; optochin; phosphatase; bile-esculin; pyrrolidonyl-β-naphthylamide; arginine; PNP-β-D-galactopyranoside; urea hydrolysis; raffinose, lactose, trehalose, mannose, sorbitol, arabinose, ribose, inulin, and mannitol fermentation; 6.5% NaCl; bacitracin; and pyruvate utilization.

**Conventional medium identification.** The following tests were performed on isolates identified by MicroScan as enterococci: tolerance to and hydrolysis of bile-esculin; growth in brain heart infusion broth with 6.5% NaCl; deamination of arginine (1%) in Moeller decarboxylase broth; fermentation of 1% lactose, 1% mannitol, 1% sorbose, 1% sorbitol, and 1% arabinose in heart infusion broth base; and determination of motility (media medium S; Difco Laboratories, Detroit, Mich.) and pigmentation after overnight growth on tryptic soy agar. For selected isolates, utilization of pyruvate was tested in 1% pyruvate broth and hydrolysis of sodium hippurate and fermentation of glycero, melibiose, sucrose, and raffinose were also evaluated. The inoculated media were incubated at 35°C in ambient air for up to 96 h. Organism identification was based on reactions outlined by Facklam and Collins (5).

**Identification of the 60 streptococci.** The 60 streptococci were based on latex agglutination (Streptex; Wellcome Diagnostics, Div. Burroughs Wellcome Co., Greenville, N.C.) for isolates of...
groups A, B, C, and G and Streptococcus pneumoniae. Isolates of Streptococcus bovis hydrolyzed bile-esculin, did not grow in 6.5% NaCl broth, and agglutinated with group D typing reagent (Strep test). Viridans group streptococci were identified by colony morphology, hemolytic pattern on sheep blood agar, resistance to optochin, failure to hydrolyze bile-esculin, and failure to grow in 6.5% NaCl broth.

Results. For 94 isolates of enterococci (77 E. faecalis, 14 E. faecium, and 3 E. durans), conventional and MicroScan identifications were in agreement. Four of the six remaining isolates were identified as E. faecalis by MicroScan and as Enterococcus solitarius by the conventional method, and two were identified as E. durans by MicroScan and Enterococcus hirae by conventional media. None of the 60 Streptococcus species tested were identified as Enterococcus by MicroScan.

Ninety-seven enterococci grew in the conventional brain heart infusion broth with 6.5% NaCl after overnight incubation. However, for three isolates, growth was apparent only after incubation for 48 h. Because many laboratories incubate 6.5% NaCl broth for 24 h only, the potential exists for strains that are to be correctly identified. For approximately half the enterococci, fermentation of one of more of the sugars required incubation for more than 24 h. MicroScan 6.5% NaCl and bile-esculin reactions were negative for three and five Enterococcus isolates, respectively, yet for all eight the identification agreed with conventional testing.

The database of the MicroScan system presently includes only four species of enterococci (E. faecalis, E. faecium, E. durans, and E. avium); therefore, any isolates of the eight additional species described by Facklam and Collins (5) would be misidentified. Seven of these eight species could be identified without altering the biochemicals presently on the panel. E. avium ferments arabinose but not raffinose and therefore can be distinguished from Enterococcus raffinosus, which ferments raffinose, and Enterococcus malodoratus and Enterococcus pseudaoavium, which do not ferment arabinose. Lactose fermentation will differentiate E. solitarius (negative) from E. faecalis (positive). Enterococcus gallinarum, Enterococcus cassillexavus, and Enterococcus munditii could be identified by supplementing the panel with a motility test with or without medium to determine pigment production. The addition of sucrose to the panel would aid in the identification of E. hirae, since 75 to 85% of E. hirae strains ferment raffinose and sucrone (5), and E. durans does not ferment either sugar.

In conclusion, the MicroScan is a reliable method for identification of the more common clinical isolates of Enterococcus species (i.e., E. faecalis and E. faecium). Additional modifications of the database of the system and panel tests would enable identification of at least 11 of the 12 Enterococcus species described by Facklam and Collins (5). The accompanying antibiogram, although of benefit, may require additional testing to ensure detection of high-level aminoglycoside resistance (15).

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LITERATURE CITED