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Identification of *Streptococcus faecalis* and *Streptococcus faecium* and Susceptibility Studies with Newly Developed Antimicrobial Agents

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Identification and susceptibility studies were performed on 301 blood and urine *Streptococcus faecalis* and *Streptococcus faecium* isolates. Strep Trio-Tubes S4, S5, and S3 (Carr-Scarborough Microbiologicals, Inc.) were compared with conventional methods for accuracy and rapidity. Of 282 isolates identified as *S. faecalis*, 98% were identified by species in 4 h with Trio-Tubes; the same percentage of isolates analyzed by conventional methods were identified in 24 h. All 14 *S. faecium* isolates (approximately 5% of the total number of isolates) were identified by Trio-Tubes in 24 h. In vitro MIC susceptibility testing of the isolates was performed by the Dynatech 2000 microdilution technique (Dynatech Laboratories, Inc.). Several newly developed antimicrobial agents, including imipenem (a carbapenem) and some of the quinolone drugs, i.e., CI-934, ciprofloxacin, A-56619, A-56620, amifloxacin, norfloxacin, and enoxacin, were tested, as were ampicillin, erythromycin, and vancomycin. Both ampicillin and vancomycin showed good activity against *S. faecalis*, with MICs for 90% of isolates tested (MIC_{90}s) of 1 and 2 μg/ml, respectively; with *S. faecium*, ampicillin exhibited an MIC_{90} of 16 μg/ml and vancomycin exhibited an MIC_{90} of 2 μg/ml. Of the newer antimicrobial agents, imipenem and CI-934 exhibited the greatest activity against *S. faecalis* strains, with MIC_{90}s of 2 and 0.5 μg/ml, respectively. MBCs against the isolates were determined with CI-934, with 90% of *S. faecalis* strains showing MBCs of 1 μg/ml or less.

Because of the greater resistance of *Streptococcus faecalis* and *Streptococcus faecium* to antimicrobial agents as compared with the resistance of other streptococci, rapid identification and knowledge of susceptibility patterns of these two species are important in the institution of appropriate early treatment of infection. Several methods which have been described for identification of these organisms make take from 4 to 48 h (2, 7). This study evaluated Strep Trio-Tubes S4, S5, and S3 for identification of *S. faecalis* and *S. faecium* and compared them with conventional methods for accuracy and time to identification. The in vitro activities of current antimicrobial agents, i.e., ampicillin, erythromycin, and vancomycin, commonly used in the treatment of enterococcal infections were determined by MIC microdilution susceptibility studies. In addition to these drugs, MIC testing was performed with several newer antimicrobial agents, including imipenem (a broad-spectrum carbapenem drug), and seven new quinolone drugs: CI-934, enoxacin, ciprofloxacin, amifloxacin, norfloxacin, A-56619, and A-56620. Imipenem and the quinolones have been shown to have various degrees of activity against enterococci (1, 3, 5, 6, 8, 10, 11). MBCs were determined with CI-934, the quinolone which exhibited the greatest degree of activity against the enterococcal isolates.

MATERIALS AND METHODS

Bacterial strains. A total of 301 enterococcal isolates from blood and urine samples were collected at Evanston Hospital, Evanston, Ill., over a period of 5 months. All isolates were subcultured on sheep blood agar plates and incubated in 5% CO_2 at 35°C for 24 h before identification and susceptibility studies.

Identification. Identification of *S. faecalis* and *S. faecium* was determined with Strep Trio-Tubes S4, S5, and S3, and conventional methods based on the Facklam scheme for differentiation of group D streptococci (7). Gram-positive cocci, morphologically consistent with enterococci, were inoculated into conventional media consisting of bile esculin agar, 6.5% NaCl agar, arginine decarboxylase, and mannitol, as well as arabinose and sorbitol heart infusion broths (Remel, Lenexa, Kans.). Those isolates which were (i) NaCl tolerant, (ii) positive in bile esculin agar, arginine, mannitol, and sorbitol, and (iii) negative in arabinose were identified as *S. faecalis*. Isolates which were (i) NaCl tolerant, (ii) positive in bile esculin agar, arginine, mannitol, and arabinose, and (iii) negative in sorbitol were identified as *S. faecium*. No isolates of *Streptococcus avium* or *Streptococcus durans* were recovered during the study. All media were maintained at 35°C in a non-CO_2 incubator, and results were recorded after 24 and 48 h of incubation. In some instances additional Remel carbohydrates (including sucrose, lactose, raffinose, and inulin) were used to aid in a definitive identification.

Strep Trio-Tubes are recommended for presumptive identification of streptococcal groups A, B, and D, as well as viridans group streptococci. Each Strep Trio-Tube consists of three medium-containing tubes, with two microtubes held in place by medium in the butt of the carrier tube. Trio-Tube S4 contains esculin hydrolysis medium-PYR (L-pyrrolidonyl-β-naphthalamide), arginine-starbose (combination of starch and sorbose), and sodium hippurate. The microtubes and carrier tubes were inoculated with a heavy inoculum (approximately 10 colonies) and maintained at 35°C in a non-CO_2 incubator for 4 h. After incubation,

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reagents were added to the appropriate tube, with PYR test reagent (*p*-dimethyl-amino-cinnamaldehyde) and ninhydrin used for the hippurate test. Trio-Tube S5, containing sodium pyruvate, sorbitol, and raffinose, was incoculated and incubated as above, with acid production recorded at 4 and 24 h. *S. faecalis* is esculin, arginine, pyruvate, and sorbitol positive, whereas *S. faecium* is esculin and arginine positive but pyruvate and sorbitol negative (*S. durans* also exhibits similar reactions). After the initial 4-h incubation, if an identification with S4 and S5 tubes indicated either *S. faecium* or *S. durans*, the S3 tube containing mannitol, inulin, and lactose was inoculated and incubated for 24 h. Most *S. faecium* organisms are mannitol positive; however, a few strains may be negative, whereas most *S. durans* strains are mannitol negative; however, a few strains may be positive. The Trio-Tube system involves a numerical coding system, the numbers of which are determined by positive test results and are interpreted by a listing present in the package insert. A chart is also enclosed indicating the percentage of positive test results for each reaction by species.

Susceptibility studies. (i) Sources and storage of antimicrobial agents. Antimicrobial reference powders are obtained from the following sources: A-56619 and A-56620, Abbott Laboratories, North Chicago, Ill.; ciprofloxacin, Miles Pharmaceuticals, West Haven, Conn.; norfloxacin and imipenem-cilastatin, Merck Sharp & Dohme, Rahway, N.J.; amifloxacin, Sterling-Winthrop Research Institute, Rensselaer, N.Y.; enoxacin, Warner-Lambert, Ann Arbor, Mich.;/Parke-Davis, Div./, CI-934, Warner-Lambert Morris Plains, N.J.; ampicillin, Wyeth Laboratories, West Chester, Pa.; and erythromycin and vancomycin, Eli Lilly & Co., Indianapolis, Ind. All antimicrobial powders were stored desiccated at 2 to 8°C. All stock solutions and microtiter plates were kept at −70°C for a maximum of 3 months.

(ii) Antimicrobial susceptibility testing. The MIC of each antimicrobial agent was determined by the broth microdilution method (16). Antimicrobial agents were diluted in calcium- and magnesium-supplemented Mueller-Hinton broth (CSMHB) (Difco Laboratories, Detroit, Mich.). The dilution ranged from 0.12 to 16 μg/ml. The inoculum was prepared from 24-h growth on sheep blood agar plates, which was inoculated into CSMHB and incubated at 35°C for 2 to 4 h. A multipoint inoculator (Dynatech Laboratories, Inc., Alexandria, Va.) was used to deliver the inoculum. Each well, containing 0.1 ml of an antimicrobial agent in CSMHB, received an inoculum of 10^4 CFU, resulting in a final concentration of 10^5 CFU/ml. MIC plates were incubated at 35°C in ambient air. The MIC, defined as the lowest concentration of an antimicrobial agent that inhibited visible growth of the test organism, was determined after incubation for 24 h. Antimicrobial agent quality control was determined with each batch of MIC plates and once a week thereafter with *Staphylococcus aureus* ATCC 29213, *S. faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853. The MICs and MBCs for organisms tested against CI-934 were determined by the macro tube dilution method (14). Four to five colonies from 18- to 24-h growth on sheep blood agar plates were inoculated into CSMHB, incubated for 2 to 4 h at 35°C on a shaker, and diluted to give a final inoculum of 1×10^5 to 9×10^5 CFU/ml. MICs were determined after 20 h of incubation, when all tubes showing inhibition were vortexed and reincubated at 35°C for an additional 4 h. MBCs were determined by plating 0.1-ml samples from all dilutions demonstrating inhibition of growth after the reincubation period onto antimicrobial agent-free sheep blood agar plates. The MBC was defined as the lowest concentration of the agent which reduced the inoculum by 99.9% after incubation for 24 h at 35°C.

RESULTS

A total of 301 isolates were compared by conventional methods and Trio-tubes, and 296 isolates were identified as either *S. faecalis* or *S. faecium*. Of the 296 isolates, 282 (95%) were identified as *S. faecalis* and 14 (5%) were identified as *S. faecium*. Of the *S. faecalis* isolates, 98% were identified with Trio-Tubes S4 and S5 after 4 h of incubation; 2% required incubation for 24 h. Of the *S. faecalis* isolates identified by conventional methods, 98% required 24 h for identification and 2% required 48 h. All *S. faecium* isolates were identified by S4, S5, and S3 Trio-Tubes after incubation for 24 h. With isolates which were finally identified as *S. faecium*, the initial 4-h reading indicated either *S. faecium* or *S. durans*; the S3 Trio-Tube, incubated for an additional 24 h, was required for species identification. All 14 isolates of *S. faecium* were identified at 24 h with Trio-Tubes, and 13 of 14 were identified at 24 h with conventional methods, with 1 isolate identified at 48 h. Because of strain variability, especially with respect to the production of acid in mannitol broths, the use of the S3 tube for differentiation of *S. durans* from *S. faecium* should be restricted because it might be inappropriate. However, *S. durans* is commonly isolated from milk and dairy products and is rarely isolated from specimens of human origin. Further biochemical testing would be appropriate in laboratories that commonly isolate *S. durans* strains.

Although additional carbohydrates were used, five nonhemolytic isolates remained unidentified by either system. All five isolates exhibited similar biochemical reactions. Esculin, PYR, arginine, hippurate, pyruvate, mannitol, and lactose were positive with Trio-Tubes; there were negative reactions in starbose, sorbitol, raffinose, and inulin. Bile esculin agar, NaCl, arginine, mannitol, sucrose, and lactose were positive by conventional methods, and arabinose, sorbitol, raffinose, and inulin remained negative. According to the percentage tables included with the Trio-Tube identification system, 98% of *S. faecalis* isolates are pyruvate and sorbitol positive. Our isolates were sorbitol negative. *S. faecium* is pyruvate and sorbitol negative. Pyruvate was not tested in our conventional scheme. The need for further identification methods to elucidate the species of these five enterococcal isolates points out that the Trio-Tubes are somewhat limited, as was the conventional methods used, in their ability to distinguish all enterococcal strains. Strep Trio-Tubes are recommended for the presumptive identification of groups A, B, and D and viridans group streptococci; thus further testing with certain aberrant strains can be anticipated.

The results of the microdilution susceptibility studies can be seen in Table 1. A total of 260 *S. faecalis* and 12 *S. faecium* isolates were tested. Ampicillin and vancomycin exhibited the greatest activity against *S. faecalis*, with an ampicillin MIC_{90} of 1 μg/ml and a vancomycin MIC_{90} of 2 μg/ml. Erythromycin exhibited a MIC_{90} of >16 μg/ml for *S. faecalis*. For *S. faecium*, the MIC_{90}s were 2 μg of vancomycin per ml, 16 μg of ampicillin per ml, and >16 μg of erythromycin per ml.

CI-934, the most active quinolone tested, showed MIC_{90}s for *S. faecalis* and *S. faecium* strains at concentrations of 0.5 and 1 μg/ml, respectively. The MIC_{90} of ciprofloxacin for *S. faecalis* strains was 2 μg/ml. For *S. faecalis* strains, the

order of MIC_{90}s among the other quinolone antimicrobial agents was 4 μg/ml (norfloxacin and A-56620) > 8 μg/ml (A-56619 and enoxacin) > 16 μg/ml (amifloxacin).

With *S. faecium*, ciprofloxacin showed a MIC_{90} of 4 μg/ml, norfloxacin exhibited 8 μg/ml, A-56619 and A-56620 showed 16 μg/ml, and amifloxacin exhibited >16 μg/ml.

Imipenem had a MIC_{50} for the S. *faecalis* isolates of 1 μg/ml and a MIC_{90} of 2 μg/ml; however, imipenem was much less active against *S. faecium*, with a MIC_{90} of >16 μg/ml. CI-934, the only antimicrobial agent tested for MBC activity, exhibited MBC_{90}s of 1 and 2 μg/ml, respectively, against *S. faecalis* and *S. faecium* strains.

DISCUSSION

Rapid identification to species level and susceptibility testing of enterococci in the clinical laboratory is not routinely necessary; however, in some instances, it could aid the physician in the choice of appropriate antimicrobial therapy. The Trio-Tubes provide a system for species identification of enterococcal isolates which is accurate, rapid, and more cost effective than the conventional methods used. The cost of each Trio-Tube is one-third the cost of each carbohydrate heart infusion broth. For most isolates, identification of *S. faecalis* and preliminary identification of *S. faecium* can be obtained in 4 h. With S. *faecium*, a more resistant species, rapid identification of this organism could be very important in the management of patient therapy (4), especially when this organism is isolated from a significant blood culture. Species identification of enterococci has also been found to be of epidemiological importance and has been used to establish awareness of nosocomial outbreaks (4).

The MIC_{90}s of ampicillin (1 μg/ml), vancomycin (2 μg/ml), and erythromycin (>16 μg/ml) against *S. faecalis* are similar to those reported in the literature (1, 11, 17). The MIC_{90} obtained for ampicillin (16 μg/ml) with respect to *S. faecium* demonstrates that it is a more resistant organism.

A number of MIC in vitro studies have been published showing the quinolones, which are derivatives of nalidixic acid, to have a broad range of activity against bacteria, including gram-positive as well as gram-negative organisms (5, 6, 8, 10). As can be seen from our data, the MICs of the seven quinolones tested for enterococci are in general agreement with the results of those studies, as well as several others that recently have been presented, (C. L. Combee and E. M. Britt., Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 747, 1985; W. Mandell and H. C. Neu, 25th ICAAC, abstr. no. 748, 1985; S. G. Kelley, 25th ICAAC, abstr. no. 756, 1985; D. Felmingham, M. D. O'Hare, G. L. Ridway, and R. N. Gruneberg, 25th ICAAC, abstr. no. 750, 1985).

TABLE 1. Comparative activities of 11 antimicrobial agents against strains of *S. faecalis* and *S. faecium*

Organism (no. of isolates)	Agent	MIC (μg/ml)		
		Range	50%	90%
S. faecalis (260)	Ampicillin	0.5–2.0	1.0	1.0
	Erythromycin	≤0.12–>16.0	1.0	>16.0
	Vancomycin	0.5–4.0	2.0	2.0
	Imipenem	0.25–4.0	1.0	2.0
	CI-934	≤0.12–1.0	0.5	0.5
	Ciprofloxacin	0.25–4.0	1.0	2.0
	A-56619	0.5–8.0	4.0	8.0
	A-56620	0.5–8.0	2.0	4.0
	Amifloxacin	2.0–16.0	8.0	16.0
	Norfloxacin	1.0–16.0	4.0	4.0
	Enoxacin[a]	2.0–16	8.0	8.0
S. faecium (12)	Ampicillin	≤0.12–16.0	2.0	16.0
	Erythromycin	≤0.12–>16.0	2.0	>16.0
	Vancomycin	0.25–2.0	1.0	2.0
	Imipenem	0.5–>16.0	4.0	>16.0
	CI-934	0.25–2.0	0.5	1.0
	Ciprofloxacin	0.25–16.0	2.0	4.0
	A-56619	1.0–16.0	8.0	16.0
	A-56620	0.5–>16.0	4.0	16.0
	Amifloxacin	4.0–16.0	16.0	≥16.0
	Norfloxacin	1.0–8.0	4.0	8.0

[a] A total of 120 isolates were tested with enoxacin.

For all but two of the quinolones tested, CI-934 and ciprofloxacin, the MIC_{90}s for the *S. faecalis* strains tested were greater than or equal to 4 μg/ml. Because of high obtainable levels of the drugs in urine samples and the broad activity gainst gram-negative bacilli, many of the quinolones could be used as single antimicrobial agents in the treatment of mixed urinary tract infections; however, the activity against enterococci is not great enough to use alone in the treatment of severe systemic infections, e.g., bacterial endocarditis (6, 10). Perhaps the quinolones will be advantageous in treating urinary tract infections because of their activity against enterococci and staphylococci, two groups which commonly occur as superinfecting organisms (8). There have been reports of an increase in nosocomial infections with enterococci, possibly due to the widespread use of broad-spectrum cephalosporins (J. M. L. Hughes, D. R. Olson, T. G. Emori, W. R. Jarvis, D. H. Culver, and C. Thornsberry, 25th ICAAC, abstr. no. 1029, 1985; A. J. Morrison, Jr. and R. P. Wenzel, 25th ICAAC, abstr. no. 946, 1985). Because of their activity against the enterococci, the quinolones may be useful in preventing nosocomial infections due to these organisms.

Of the 11 antimicrobial agents tested against *S. faecalis* strains, the most potent was CI-934, a quinolone specifically developed for increased activity against gram-positive cocci (3). It also exhibited good activity against *S. faecium*, although only 12 strains were tested. CI-934, when tested for MBCs against enterococcal isolates, exhibited good bactericidal activity, with MIC_{90}s of 1 μg/ml or less for *S. faecalis* and 2 μg/ml or less for *S. faecium* strains. It appears that this antimicrobial agent may have great potential for use with urinary and systemic infections, but further clinical studies are indicated.

Imipenem, an agent known to have a very broad antimicrobial spectrum, inhibited 90% of the *S. faecalis* urine and blood isolates at 2 μg/ml. This datum is consistent with several studies performed with imipenem against enterococci isolates from urine specimens, as well as isolates from pelvic, intra-abdominal, and soft tissue infections (1, 9, 11, 12, 15). Imipenem shows a promising advantage for use in therapy against enterococcal infections, because it exhibits good activity against these organisms, whereas other beta-lactam antibiotics do not, and can probably be used alone or in combination with gentamicin to increase its bactericidal activity (1, 13).

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