Single-Concentration Broth Microdilution Test for Detection of High-Level Aminoglycoside Resistance in Enterococci

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Growth in a single broth microdilution well containing gentamicin at a concentration of 500 µg/ml predicted high-level resistance to gentamicin (MIC, ≥2,000 µg/ml) in 505 of 508 clinical isolates of enterococci. Failure to achieve synergistic killing with the combination of penicillin and an aminoglycoside was demonstrated with 100% specificity in 20 strains which showed resistance to 500 µg of gentamicin per ml. We recommend this procedure be adopted as a routine screening test to detect high-level aminoglycoside resistance in enterococci.

Enterococci are important causes of community-acquired and nosocomial infections (2, 12). Penicillin-and-aminoglycoside combination therapy is recommended for serious enterococcal infections (2, 5). High-level resistance to gentamicin (MIC, ≥2,000 µg/ml) is often associated with resistance to other aminoglycosides, and highly resistant strains show lack of synergistic killing by the combination of penicillin and gentamicin (1, 4). The prevalence of high-level resistance to gentamicin in enterococci has increased since it was first reported in 1979 (1–4). Highly resistant isolates now account for 13% of all enterococcal clinical isolates at the University of Michigan Hospital, Ann Arbor; 55% at the Veterans Administration Medical Center, Ann Arbor, Mich.; 5% at the Yale-New Haven Hospital, New Haven, Conn.; and 45% at the Veterans Administration Medical Center, West Haven, Conn. (12; J. E. Patterson, T. S. Mikesell, C. A. Kauffman, D. R. Schaberl, and M. J. Zervos, Abstr. Annu. Meet. Soc. Microbiol. 1987, C242, p. 363). Because of this increasing problem, screening for high-level resistance in enterococci has been recommended (2, 4, 5, 9, 10, 12; Patterson et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1987). Standard susceptibility testing of enterococci does not, however, discriminate between the usual moderate level of intrinsic resistance seen in most Enterococcus faecalis isolates and high-level resistance. This study evaluated the use of a single-concentration broth microdilution well containing 500 µg of gentamicin per ml for screening of high-level resistance to aminoglycosides and capacity to predict lack of synergistic killing by penicillin-and-aminoglycoside combinations.

The bacteria used were clinical isolates obtained from patients hospitalized at the University of Michigan Hospital, Ann Arbor and West Haven Veterans Administration medical centers, and Yale-New Haven Hospital. The most common sites of isolation were urine (64%), wounds (25%), and blood (8%). Isolates were presumptively identified as enterococci by using bile esculin agar and growth in 6.5 percent NaCl and were tested further by the API-DMS Rapid Strep system (Analytab Products, Plainview, N.Y.) to differentiate species of group D enterococci (6). All isolates were E. faecalis when identified to the species level.

Microdilution testing of the susceptibilities of strains to amikacin (Bristol Laboratories, Syracuse, N.Y.), gentamicin (Schering Corp., Bloomfield, N.J.), kanamycin (Bristol), neomycin (Lederle Laboratories, Pearl River, N.Y.), netilmicin (Schering), spectinomycin (The Upjohn Co., Kalamazoo, Mich.), streptomycin (Sigma Chemical Co., St. Louis, Mo.), and tobramycin (Eli Lilly & Co., Indianapolis, Ind.) was performed using a standardized broth dilution technique (8). Organisms in the log phase of growth were inoculated into Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) supplemented with 50 mg of Ca²⁺ per ml and 25 mg of Mg²⁺ per ml for a final concentration of 5 x 10⁵ CFU/ml. High-level aminoglycoside resistance was defined by an MIC of ≥2,000 µg/ml. A single microdilution well containing gentamicin at a concentration of 500 µg/ml was used to detect high-level resistance; 5,797 isolates were screened. All isolates which showed growth in the 500-µg/ml well were subsequently cultured on brain heart infusion agar containing concentrations of aminoglycosides of 2,000 µg/ml, since this method was previously shown to confirm high-level resistance (4, 5).

Synergy of the combination of penicillin and an aminoglycoside was evaluated by time-kill curves. The methods and criteria for determining synergy were described previously (5, 11). Aminoglycoside concentrations were chosen to reflect clinically achievable concentrations in serum and did not exceed 10 µg/ml for gentamicin and 25 µg/ml for streptomycin when used to test resistant strains. Synergy was defined by a ≥2-log₁₀ reduction in CFU per milliliter at 24 h with the combination of antibiotics compared with penicillin or an aminoglycoside alone. Ten isolates were selected for use as controls in the synergy experiments; for five isolates, gentamicin MICs were ≤64 µg/ml, but these isolates were resistant to high levels of streptomycin. The other five strains were inhibited by concentrations of ≤64 µg of both gentamicin and streptomycin per ml.

Of 508 isolates which showed growth in the single 500-µg/ml-concentration microdilution well, 505 exhibited high-level resistance to gentamicin by agar dilution; for the other 3 isolates, gentamicin MICs ranged from 500 to 1,000 µg/ml. Of the 505 isolates with high-level resistance to gentamicin, 499 also showed high-level resistance to all aminoglycosides except spectinomycin. The other six isolates showed high-
level resistance to amikacin, gentamicin, kanamycin, neomycin, netilmicin, and tobramycin; streptomycin MICs ranged from 125 to 250 μg/ml. For all 508 isolates, spectinomycin MICs ranged from 16 to 64 μg/ml.

Synergy was evaluated with 37 isolates. The 10 strains for which gentamicin MICs were ≤64 μg/ml showed penicillin-gentamicin synergy. There was no synergy of killing when penicillin and streptomycin were tested in combination against the five isolates for which gentamicin MICs were ≤64 μg/ml and streptomycin MICs were ≥2,000 μg/ml. All isolates for which streptomycin MICs were 125 to 250 μg/ml and which showed high-level resistance to amikacin, kanamycin, gentamicin, neomycin, netilmicin, and tobramycin also failed to show synergism by penicillin and streptomycin or gentamicin. Twenty strains for which spectinomycin MICs were ≤64 μg/ml but which showed high-level resistance to all other aminoglycosides were tested with penicillin in combination with gentamicin, streptomycin, or spectinomycin. Each combination failed to show synergism. The positive predictive value of the test for high-level gentamicin resistance was 99.4%. Lack of synergy of penicillin with gentamicin, streptomycin, or spectinomycin was predicted with 100% specificity for the isolates tested.

The results of this investigation show that a single broth microdilution well containing gentamicin at a concentration of 500 μg/ml predicts both resistance at 2,000 μg/ml and lack of synergy of penicillin in combination with gentamicin or streptomycin. Various methods are used in clinical microbiology laboratories for routine susceptibility testing of enterococci, including disk diffusion and standardized microdilution tests. However, neither of these methods screens for high-level resistance to aminoglycosides. Most clinical microbiology laboratories use standardized microdilution systems for routine susceptibility testing. Concentrations of gentamicin for susceptibility testing of enterococci on the microdilution plate usually do not exceed 8 to 64 μg/ml. These levels do not detect high-level resistance, which has a good correlation with lack of synergy of penicillin-and-aminoglycoside combinations. Aminoglycoside disk susceptibility screening for high-level resistance has been described previously (9, 10), but preparation of disks is cumbersome; in addition, there is poor correlation between MIC and zone size with Kirby-Bauer disk diffusion (9). Aminoglycoside disk testing of enterococci is also not recommended by the National Committee for Clinical Laboratory Standards (7). High-level resistance to aminoglycosides has been screened by using a single tube dilution of 2,000 μg of streptomycin per ml, but agar or tube dilution methods are cumbersome to perform as routine screening tests. Tests for synergy of killing are also difficult to perform on a routine basis.

A screening test for high-level resistance in enterococci is important as the prevalence of these clinical isolates increases. In addition to the therapeutic and prognostic implications for patients with serious enterococcal infections, screening has epidemiologic and infection control importance, since these isolates have been reported to be nosocomially transmitted (12). We recommend that screening for high-level aminoglycoside resistance in enterococci be performed by this method, which is well suited for routine susceptibility testing of clinical isolates.

LITERATURE CITED