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Multiply High-Level-Aminoglycoside-Resistant Enterococci Isolated from Patients in a University Hospital

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Enterococci isolated from different body sites were tested for high-level gentamicin resistance. A total of 259 enterococcal isolates were screened for resistance (MIC, >2,000 μg/ml) by a broth-tube method. Thirty-nine (15.1%) were found to exhibit resistance and were confirmed by agar screening (1,000 μg/ml) and agar dilution MIC determinations. The majority of isolates also showed high-level resistance to kanamycin and streptomycin. The remaining isolates showed high-level resistance to gentamicin and kanamycin but not streptomycin. Synergy testing of several isolates confirmed the correlation between lack of synergy and high-level resistance. A retrospective clinical review was performed. Most patients had a source of definite or likely infection (79%). Serious infections such as endocarditis or meningitis were not observed during the course of this study. Retrospective clinical data suggest that in cases not involving endocarditis or meningitis, neither infection refractory to therapy nor relapse of infection is a common sequela of infection with gentamicin-resistant enterococci in hospitalized patients.

Enterococci are an important cause of nosocomial infections. They have been found to be the most common isolates in nosocomial urinary tract infections in certain hospitals (6) and are common isolates from abdominal and wound infections (23). Antibiotic resistance among enterococci is a major obstacle to therapy. Studies have shown that enterococci are intrinsically resistant to penicillins, cephalosporins, clindamycin, and aminoglycosides, relative to other streptococci (19, 27, 28). In contrast to uncomplicated enterococcal infections such as urinary tract and wound infections, which respond to ampicillin, serious infections such as endocarditis require combinations of antibiotics which synergistically kill enterococci (8, 10).

In 1970, enterococci for which streptomycin or kanamycin MICs were >2,000 μg/ml were identified (26). Penicillin in combination with the aminoglycoside to which high-level resistance was shown failed to have a synergistic killing effect. Such enterococci were refractory to combination therapy in the rabbit endocarditis model (18) and were responsible for clinical failures in human endocarditis when treatment with penicillin and the relevant aminoglycoside was employed (13, 25). The percentage of enterococci showing high-level resistance to streptomycin and kanamycin has increased; in major United States cities, currently 25 to 55% of isolates are streptomycin resistant and 15 to 50% are kanamycin resistant (16).

In 1979 the first enterococcal isolates highly resistant to gentamicin (gentamicin-resistant enterococci [GRE]) were reported by Horodniceanu et al. from France (9). Published reports in 1983 from Houston (15), Bangkok (22), and Chile (22) and in 1986 and 1987 from Michigan (29, 30) documented a GRE prevalence of 4.5, 14, 15, and 13 and 55%, respectively, among recent clinical isolates. Ten GRE isolates (reported from three centers) were susceptible to <1,000 μg of streptomycin per ml (3, 11, 30), but all other reported isolates were highly resistant to all aminoglycosides tested.

Despite these reports, it is unclear how widespread are multiply high-level-aminoglycoside-resistant enterococci in other areas of the United States. Furthermore, there are few data on the impact of these bacteria upon morbidity and mortality in hospitalized patients. For these reasons, we undertook an investigation to determine the prevalence of GRE in our hospital, to characterize these isolates, and to assess their clinical importance.

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MATERIALS AND METHODS

Enterococcal isolates from patients at the Hospital of the University of Pennsylvania in Philadelphia between February and August, 1985, were screened for high-level gentamicin resistance. From September to December, 1985, only blood isolates were screened. Multiple isolates from a single patient with the same resistance profile were considered as one isolate for analysis in this study.

Antimicrobial agents. Antibiotics used in this study included gentamicin sulfate (Schering Corp., Kenilworth, N.J.), kanamycin sulfate (Bristol Laboratories, Syracuse, N.Y.), and streptomycin sulfate (Eli Lilly and Co., Indianapolis, Ind.).

Screening methods. Mueller-Hinton broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with calcium and magnesium was used to prepare a screening broth. Samples (1 ml) containing 2,000 μg of gentamicin per ml were prepared and kept frozen at −70°C until used. Enterococci were grown in broth (usually Mueller-Hinton, but also Todd-Hewitt, tryptic soy, or thioglycolate) to a turbidity equivalent to a 0.5 McFarland standard. This produces a concentration of approximately 7×10^7 organisms per ml. A portion of the culture was streaked onto a blood agar plate to check for purity. A 1-ml volume of gentamicin broth was inoculated with 7 μl of the culture, producing a final concentration in the test broth of approximately 5×10^5 organisms

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per ml. The tubes were incubated for 18 to 24 h at 35°C in air. Controls consisted of a broth tube without antibiotics for each isolate tested. Control organisms (susceptible and resistant) were tested with each batch. Macroscopic growth of the organism as indicated by visible turbidity in antibiotic broth indicated high-level resistance.

Further testing of isolates. Isolates obtained from the initial screening were tested for high-level resistance by additional methods. Screening for resistance to gentamicin, kanamycin, and streptomycin was performed by streaking portions of colonies onto dextrose-phosphate broth (GIBCO Laboratories, Madison, Wis.) plates containing 1.7% agar (Difco Laboratories, Detroit, Mich.) with or without an appropriate aminoglycoside. Isolates were defined as highly resistant if there was visible growth on plates containing either 1,000 μg of gentamicin or 2,000 μg of kanamycin or streptomycin per ml (20).

MICs were also determined by agar dilution with a Steers replicator. Organisms were grown in dextrose-phosphate broth at 35°C overnight and diluted 1:100 to achieve an inoculum of approximately 10^7 organisms per ml. A growth control plate without antibiotics was included in each run. Plates were read after 24 h of incubation at 35°C in ambient air. Any growth was considered positive and indicated resistance at that concentration.

Identification of isolates. All enterococci isolated from clinical specimens were identified by standard methods including catalase reaction, Gram stain, bile-esculin reaction, and growth in 6.5% NaCl broth (4). They were subsequently identified to the species level with the API-DMS Rapid Strep system (Analytab Products, Plainview, N.Y.). β-Lactamase testing was performed with the chromogenic cephalosporin assay (Nitrocefin; BBL Microbiology Systems).

Clinical review. The hospital charts of all patients with clinical isolates of GRE documented by both broth screening and agar dilution were reviewed. Culture results, demographic data, medication use, and clinical parameters related to outcome of infection were recorded. Previously published definitions of clinical infection and colonization with enterococci were used (17). Clinical infection was said to be "possibly" present if it could not be determined whether a patient's clinical condition was the result of enterococcal infection or other factors. A nosocomial infection was defined as infection acquired greater than 48 h into hospitalization. Neutropenia was defined by an absolute neutrophil count of <1,000/ml within 48 h of the first isolation of GRE. Steroid use was considered a factor if a patient received >10 mg of prednisone or its equivalent per day for at least 2 weeks during the 4 weeks prior to the first isolation of GRE. Antibiotic use was recorded if a patient received ≥48 h of treatment prior to the first positive culture.

RESULTS

A total of 259 isolates of enterococci were tested prospectively for high-level resistance to gentamicin from February to August, 1985. Enterococci were obtained from a variety of clinical specimens including blood, urine, and wounds. Thirty-nine GRE isolates were found by the Mueller-Hinton broth screening method and were confirmed by agar screening and agar dilution MIC determinations. Site-specific resistance rates were: vascular and catheter tip, 3/6 (50%); skin and soft tissue, 19/74 (25.7%); respiratory, 0/4 (0%); blood, 5/24 (20.8%); urine, 11/120 (9.2%); and abdomen and pelvic, 7/55 (13%). Four patients had GRE isolates from two sites, and two patients had GRE isolates from three sites. Only one isolate was considered for subsequent analysis.

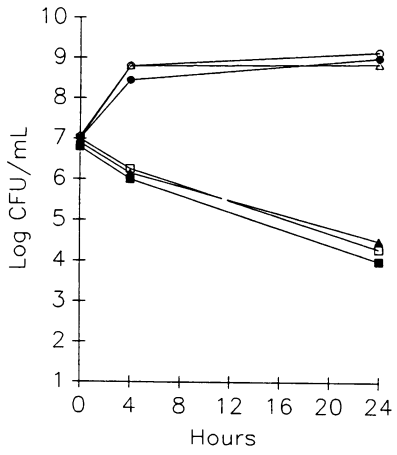

FIG. 1. Effect of antibiotics alone and in combination on high-level-gentamicin-resistant *Streptococcus faecalis* HUP 35. MICs for this isolate were >2,000 μg of gentamicin and streptomycin per ml. Concentrations of antibiotics used: gentamicin, 5 μg/ml; streptomycin, 20 μg/ml; penicillin, 10 U/ml. Symbols: ○, control; ●, streptomycin; △, gentamicin; ▲, gentamicin plus penicillin; □, penicillin; ■, streptomycin plus penicillin.

We determined MICs of gentamicin, kanamycin, and streptomycin for the 39 GRE isolates. Two patterns of high-level aminoglycoside resistance were observed among enterococcal isolates with high-level gentamicin resistance. Twenty-six isolates (66.6%) exhibited resistance against gentamicin, kanamycin, and streptomycin. Thirteen (33.3%) showed resistance to gentamicin and kanamycin but not streptomycin. The combination of streptomycin "susceptibility" (lack of high-level resistance to streptomycin) and high-level gentamicin resistance was present in 33.3% of patients as determined by MIC.

All isolates were identified to species level as *Enterococcus faecalis*. No isolates produced β-lactamase.

Synergy testing was performed on two isolates resistant to high-level gentamicin and streptomycin and on two isolates susceptible to high-level gentamicin and streptomycin. Killing curves for two of the isolates are shown in Fig. 1 and 2. Penicillin in combination with gentamicin or streptomycin did not exhibit synergy against the isolates with high-level gentamicin and streptomycin resistance. Penicillin in combination with gentamicin was not synergistic, but synergy was observed with penicillin in combination with streptomycin against the gentamicin-resistant, streptomycin-susceptible isolates.

Clinical data. The charts from 43 patients with GRE isolates confirmed by agar dilution from both study periods (39 from the initial study period and 4 from the second study period) were reviewed. Forty-three patients had 51 GRE isolates obtained from distinct anatomic sites. Nineteen patients (44%) had skin and soft tissue isolates; 10 (23%) had urine isolates; 10 (23%) had blood isolates; 8 (19%) had abdominal or pelvic isolates; and 4 (9%) had venous catheter tip isolates (>15 colonies). Five patients had isolates from more than one site. Fifty-eight percent of isolates were definitely clinically significant, and 21% were possibly clinically significant. Nine of 43 patients (21%) were colonized only. Of definitely and possibly significant infections, 59% were polymicrobial. Three patients (7%) had community-acquired infection.

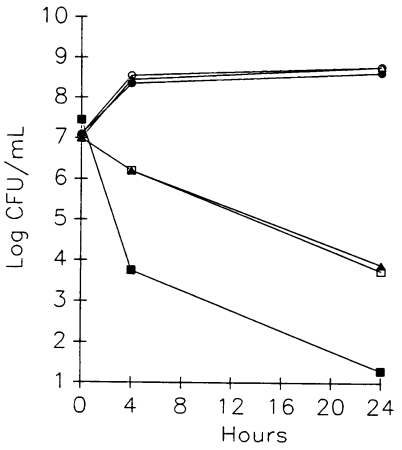

FIG. 2. Effect of antibiotics alone and in combination on high-level-gentamicin-resistant *S. faecalis* HUP 71. MICs for this isolate were >2,000 μg of gentamicin per ml and <2,000 μg of streptomycin per ml. For symbols and antibiotic concentrations used, see the legend to Fig. 1.

There were 25 female and 18 male patients with a mean age of 59 years. Thirty-eight percent had cancer, 17% had debilitating neurological disease, 12% had diabetes mellitus, 5% had collagen-vascular disease, and 5% had end-stage renal disease. There was one intravenous-drug user. Fifty-six percent had had surgery within the previous 4 weeks, 19% used steroids, and 5% were neutropenic. There was no obvious clustering by hospital location or service.

Ninety-one percent of patients received >48 h of antibiotics prior to the first positive culture for GRE. Fifty-five percent of patients received an aminoglycoside, and 50% received gentamicin prior to their first positive culture. The mean number of antibiotics received was 3.1 (range, 0 to 9; based on antibiotic class). Although a wide variety of other antibiotics were used, 52% of patients received a cephalosporin, 36% received clindamycin, 33% received an extended-spectrum cephalosporin, and 26% received trimethoprim-sulfamethoxazole.

Clinical outcome. During the period of investigation, 12 of 43 patients from whom GRE were isolated died (28%), but none of the deaths appeared to be related to enterococcal infection.

Limited information was available on patients with urinary tract isolates. Of 10 patients with urinary isolates, 1 had $<10^4$ CFU/ml, and 9 satisfied culture criteria for urinary infection ($>10^5$ CFU/ml or multiple cultures with $>10^4$ CFU/ml). Follow-up cultures were performed for six of the nine, for three of the patients after they had received either an antienterococcal penicillin or vancomycin and for three after they had received other therapy (cephradine, trimethoprim-sulfamethoxazole, gentamicin, doxycycline, amikacin). Two of these latter three patients had positive cultures 8 and 16 days after the initial positive result. The three patients who received penicillins or vancomycin had negative cultures during and after therapy.

Five patients from the initial study period had bacteremic infection. An additional five patients with bacteremia due to GRE were identified after the initial study period and were included in the clinical analysis. A primary source of infection was identified in 5 of 10 patients.

Of the remaining five patients, four were thought to have clinically significant isolates, and one patient had an isolate with possible significance. Seven of the bacteremic patients had a single positive blood culture, one patient had two positive cultures within 48 h, one patient had three positive blood cultures over a 10-day period, and one patient had three positive cultures over a 6-week period. Both patients with three positive blood cultures had indwelling vascular catheters in situ during bacteremia. They were treated with vancomycin and gentamicin and had negative follow-up blood cultures. Six of the remaining eight patients had follow-up blood cultures 2 to 15 days after the initial positive cultures, and all were negative. Four of the 10 patients died, but in none was enterococcal infection present at the time of death (clinical and microbiologic information). Treatment regimens were ampicillin-gentamicin (three patients); vancomycin-gentamicin (three patients); vancomycin alone (one patient); ampicillin-vancomycin-gentamicin (one patient); vancomycin-amikacin (one patient); and no specific antienterococcal therapy (one patient).

DISCUSSION

The prevalence of high-level gentamicin resistance in enterococci is clearly increasing. Several surveys of clinical isolates between 1970 and 1977 found no high-level gentamicin resistance (1, 2, 20, 21). At the University of Michigan Hospital, rates of gentamicin resistance increased incrementally from 0.4% in 1981 to 13% in 1985 (29). There are now other centers in the United States and elsewhere with rates of 4.5 to 55% (12, 13, 30).

Screening has generally been performed using agar impregnated with 500, 1,000 or 2,000 μg of gentamicin per ml (15, 20, 22, 29), though one center uses broth microdilution and macrodilution methods (11). Sahm and Torres recently reported studies on the use of different screening methods for detecting high-level aminoglycoside resistance in enterococci (24). Although the medium used for screening did not appear to be important for detecting high-level resistance, inoculum size was an important factor. As shown in their study, and with the method used as described in this report, 10^5 CFU/ml is recommended for broth screening methods. We tested for high-level resistance to gentamicin, kanamycin, and streptomycin. Testing of other aminoglycosides such as amikacin or tobramicin has been shown to be a poor predictor of aminoglycoside-penicillin synergy (24).

High-level gentamicin resistance was found in 15.1% of isolates. This rate is higher than those previously reported except at the Veterans Administration Medical Center in Ann Arbor, Mich. (30), and it is higher than any previously noted in a general hospital.

There are several epidemiologic characteristics of patients with GRE in our study which differ from those in other institutions. While all infections in a study by Zervos et al. were nosocomial (29), 7% of infections in our study were community acquired. Since a careful review was performed to exclude recent hospital or chronic care facility residence, we conclude that there is a reservoir of GRE in our community. Three-quarters of patients infected or colonized with GRE in the Zervos study had serious underlying illness, including chronic urologic disease in 31%, cancer in 23%, renal transplantation in 13%, and chronic wound in 8%. Our patients had a somewhat higher incidence of malignancy (38%) and a substantial prevalence of diabetes (12%) and debilitating neurological disease (17%). We found that 79% of our GRE isolates were associated with clinical infection, similar to the rate of 81% reported by Zervos et al. (29). There were also similar rates of steroid use and prior surgery

and similar age and gender profiles. Zervos et al. reported rates of cephalosporin and aminoglycoside use prior to a positive culture for GRE in 94 and 73%, respectively. These rates were higher than those observed in patients colonized or infected with gentamicin-susceptible enterococci. Our rates of antibiotic exposure in colonized and infected patients are similar to those of Zervos et al., supporting the notion that suppression of cephalosporin-susceptible flora and aminoglycoside exposure may be important in colonization and infection with these organisms. This agrees with previous data for high-level streptomycin resistance (20).

The prognosis in infection with GRE is not established. Zervos et al. have reported that 7% of hospitalized medical and surgical patients colonized or infected with GRE had deaths related to enterococci. Criteria relating death to enterococcal infection were broad: death within 72 h of a positive culture from a normally sterile site and a clinical profile consistent with septicemia (30). Ikeda et al. describe one death, in a bacteremic infant with hyaline membrane disease, and two bacteremia cures (11). Two of three patients with urinary infection had posttreatment relapses, and GRE were frequently not eradicated from wounds (11).

We assessed outcome of infection in urinary tract infection and bacteremia. Wound and intraabdominal infections were not examined because the relative role of enterococci in these infections is difficult to assess (12). Microbiologic information relevant to outcome is incomplete owing to the retrospective design of the study. Only one patient had prolonged infection; he had an infected endovascular device, a known correlate of breakthrough bacteremia (7, 14) and relapse (5). Our data suggest, therefore, that infection refractory to therapy and relapse of infection may be relatively uncommon in infection with GRE in hospitalized patients. However, we did not encounter any cases of endocarditis, the condition for which synergistic bactericidal therapy is most likely to be necessary.

At the University of Pennsylvania, several patterns of aminoglycoside resistance were observed. It is particularly noteworthy that while gentamicin resistance was always accompanied by kanamycin resistance, approximately one-fifth of GRE isolates were not highly resistant to streptomycin. Only 10 GRE isolates have been previously reported that were not highly resistant to streptomycin (3, 9, 30). In one hospital, however, these accounted for 14% of isolates (30).

Representative gentamicin-resistant, streptomycin-susceptible isolates in our study were killed synergistically by penicillin plus streptomycin but not by penicillin plus gentamicin. This suggests that in our hospital, and elsewhere, combination therapy with streptomycin may be appropriate in certain serious infections caused by GRE. It emphasizes the need to screen GRE isolates for high-level resistance to kanamycin and especially to streptomycin.

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