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NOTES

Characterization and Comparison of Two Penicillinase-Producing Strains of *Streptococcus (Enterococcus) faecalis*

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We identified two β-lactamase-positive enterococci. One strain was high-level (MIC, >2,000 μg/ml) gentamicin resistant; the other was not (MIC, 12.5 μg/ml). β-Lactamase production was extrachromosomally mediated in both strains, and both strains showed an inoculum effect reversed by β-lactamase inhibitors. The strain lacking high-level gentamicin resistance showed synergistic killing with a combination of penicillin, clavulanic acid, and gentamicin.

Penicillinase production in enterococci was first described by Murray and Mederski-Samoraj in 1983 (7), and since that time only one other penicillinase-producing enterococcal isolate has been reported (6). We screened for penicillinase production in enterococci from diverse geographic areas. The phenotypic and transfer properties of two β-lactamase-positive (Bla+) strains are compared in this study.

A total of 320 enterococcal clinical isolates were collected and screened for penicillinase activity. The isolates were from diverse geographic areas, including West Haven Veterans Administration Medical Center, West Haven, Conn.; Yale-New Haven Hospital, New Haven, Conn.; the laboratory of Vincent T. Andriole, Yale University School of Medicine, New Haven, Conn.; Hutzel Hospital, Detroit, Mich.; Ann Arbor Veterans Administration Medical Center and University of Michigan Hospital, Ann Arbor; University of California-Davis Medical Center, Los Angeles; Mt. Sinai Hospital, New York, N.Y.; University of North Carolina-Chapel Hill Medical Center, Chapel Hill; Washington University Medical Center, St. Louis, Mo.; University of Virginia Medical Center, Charlottesville; University of Manitoba Health Sciences Centre, Winnipeg, Manitoba, Canada; Cleveland Clinic, Cleveland, Ohio; Dallas Veterans Administration Medical Center, Dallas, Tex.; Little Rock Veterans Administration Medical Center, Little Rock, Ark.; Topeka Veterans Administration Medical Center, Topeka, Kans.; and Bay Pines Veterans Administration Medical Center, Bay Pines, Fla. Identification was confirmed in our laboratory using bile-esculin agar and growth in 6.5% NaCl. Strains were identified to the species level with the API-DMS Rapid Strep system (Analytab Products, Plainview, N.Y.) (8). Screening for penicillinase production was done using the chromogenic cephalosporin nitrocefin (Glaxo Laboratories, Middlesex, England) in a 50-μg/ml solution (9). For screening, 0.15 ml of nitrocefin solution was placed in a microdilution well, and several colonies from an overnight culture of the organism incubated on brain heart infusion agar (Difco Laboratories, Detroit, Mich.) were added to the well with a sterile wooden applicator. Positive strains were also assayed by microiodometry (3).

Mueller-Hinton broth (Difco) supplemented with 50 mg of Ca^{2+} per liter and 25 mg of Mg^{2+} per liter was used to determine broth tube dilution MICs. Sulbactam (SB; Pfizer Inc., New York, N.Y.) and clavulanic acid (CA; Beecham Laboratories, Bristol, Tenn.) were used with penicillin (PC) (Sigma Chemical Co., St. Louis, Mo.) or ampicillin (AM) (Sigma), according to manufacturer specifications. Synergy was defined as a ≥2-$\log_{10}$ reduction in growth as evaluated by time-kill curves at 24 h of incubation (11).

Transferability of resistance markers was examined using filter matings as described by Forbes and Schaberg (1), with the following modifications. Rifampin (Merrell Dow, Cincinnati, Ohio)- and fusidic acid (Sigma)- resistant *Streptococcus (Enterococcus) faecalis* JH2-2 was used as the susceptible plasmid-free recipient strain. Erythromycin (EM; Sigma; 25 μg/ml), tetracycline (TC; Sigma; 10 μg/ml), and streptomycin (SM; Sigma; 1,000 μg/ml) were used to select for transfer to JH2-2 of β-lactamase production from the strain lacking high-level gentamicin (GM) resistance. GM (Schering Corp., Bloomfield, N.J.) (100 μg/ml) was used to select for transfer to JH2-2 of β-lactamase production from the GM-resistant strain. Filter matings using PC (10 μg/ml) for selection were also attempted with both strains. The WH257 bacterial mixture was incubated on a filter for 8 h; the WH245 bacterial mixture was incubated for 8 and 24 h. Transfer frequency was expressed as number of transconjugants per recipient.

S. faecalis cells were lysed, and purified plasmid DNA was isolated by the method of Yagi et al. (12). DNA was analyzed by agarose gel electrophoresis as described by Meyers et al. (5). Plasmid molecular weight estimation was made by previously published methods (10).

Of the 320 isolates screened, 2 were Bla+; Bla+ strains *S. faecalis* WH245 and WH257 were originally isolated in the microbiology laboratory of the West Haven Veterans Administration Medical Center. Both WH245 and WH257 were identified as *S. faecalis*. The GM MIC for *S. faecalis* WH245 was 12.5 μg/ml; the strain was resistant to SM (MIC, >2,000 μg/ml), EM (MIC, >100 μg/ml), and TC (MIC, 100 μg/ml) at a 10^5 inoculum. *S. faecalis* WH257 was resistant to SM and GM (MICs, >2,000 μg/ml), EM (MIC, >100 μg/ml), and TC (MIC, 50 μg/ml). The MICs of PC and AM alone and in

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TABLE 1. Susceptibilities of penicillinase-producing *S. faecalis* strains

Strain	Inoculum (CFU/ml)	MIC (μg/ml)[a]					
		PC	PC + CA	PC + SB	AM	AM + CA	AM + SB
WH245	10^5	6.25	1.6	1.6	3.1	1.6	1.6
	10^7	31	3.1	1.6	6.25	1.6	0.8
	10^8	31	3.1	3.1	16	1.6	6.25
WH257	10^5	6.25	1.6	3.1	1.6	1.6	1.6
	10^7	16	1.6	3.1	6.25	1.6	1.6
	10^8	250	6.25	12.5	6.25	1.6	3.1

[a] CA was used in a concentration of 4 μg/ml at each PC and AM concentration; SB was used in a 1:1 concentration with PC and AM.

combination with CA and SB at the 10^5, 10^7, and 10^8 inocula are shown in Table 1.

β-Lactamase inhibitors CA and SB reversed the inoculum effect when combined with PC or AM and lowered the MICs at the 10^5 inoculum. Synergy occurred when PC, CA, and GM were used in combination against the strain lacking high-level GM resistance (WH245) compared with PC and CA without GM (Fig. 1). No synergy was demonstrated against the high-level GM-resistant strain (WH257) with a combination of PC, CA, and GM compared with PC and CA alone.

No transconjugants were obtained using PC for selection in filter matings with either WH245 or WH257. WH257 transferred β-lactamase with high-level GM resistance to JH2-2 at a frequency of 2.4×10^{-4}. Fifty-two transconjugants were screened for β-lactamase; 100% were positive. Incubation for 24 h of the WH245 and JH2-2 bacterial mixture and the use of EM, TC, and SM for selection resulted in transfer of EM, SM, and TC resistances in various combinations from WH245 to JH2-2. β-Lactamase production was transferred with each of these resistances with a frequency ranging from 1.4×10^{-7} to 7.5×10^{-10}. Bla+ transconjugants per number of transconjugants screened using EM, TC, and SM for selection in filter matings were, respectively, 4 of 104, 9 of 18, and 37 of 52.

The WH257 resistance plasmid was further characterized by molecular weight. Plasmid pYN104 was 34 kilobases in size and mediated EM, high-level GM and SM resistances, and β-lactamase production.

Therapy of serious enterococcal infections is often difficult because of the intrinsic relative resistance of these organisms to penicillins and aminoglycosides and complete resistance to cephalosporins. Enterococcal isolates with high-level resistance to all clinically used aminoglycosides are being increasingly recognized from diverse geographic areas (4, 13; J. E. Patterson, T. S. Mikesell, C. A. Kauffman, D. R. Schaberg, and M. J. Zervos, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, C242, p. 363); strains with this level of aminoglycoside resistance do not exhibit synergy of killing with a penicillin-and-aminoglycoside combination.

Penicillinase production is a relatively new mechanism of resistance in enterococci and was first recognized by Murray and Mederski-Samoraj in 1983, when they reported transferable penicillinase production in an *S. faecalis* strain from Houston, Tex. (7). A Bla+ enterococcal blood isolate was reported from Pennsylvania (2, 6). Both of these isolates showed high-level GM resistance, and both showed an inoculum effect with PC and AM. The MICs for these two organisms determined in brain heart infusion broth were much higher at the 10^7 inoculum than for our strains tested in Mueller-Hinton broth; this may be due to the effect of the latter medium, which does not allow for as vigorous growth as the former, or to strain variation. One of our strains was high-level GM resistant; the other lacked this trait. The latter strain is the first reported penicillinase-producing enterococcus that is not high-level GM resistant and demonstrates the ability of β-lactamase production to transfer without the presence of a high-level GM resistance plasmid. The transfer frequency of β-lactamase production when the strain lacking high-level GM resistance was the donor was 10^3 to 10^5 less than when the high-level GM-resistant strain was the donor. Previously characterized Bla+ GM resistance plasmids reported by Murray et al. transferred with a frequency of 10^{-4} and 10^{-5} (6). The Bla+ GM resistance plasmid pYN104 is physically distinct from the two previously characterized enterococcal Bla+ plasmids, based on restriction enzyme patterns (6).

Because of the low MIC at a standard inoculum (10^5), these organisms would not have been detected unless β-lactamase screening was done. The detection of the strains has therapeutic implications in serious enterococcal infections, since synergistic killing of the strain lacking high-level GM resistance was demonstrated in vitro with a combination of PC, CA, and GM. Detection of β-lactamase may also be important for therapy of Bla+ GM-resistant strains. Two preliminary studies of enterococcal endocarditis in animal models using the previously published Bla+ GM-resistant strains have been done. One study concluded that AM in combination with SB and GM was more effective therapy in vivo than AM combined with either the β-lactamase inhibi-

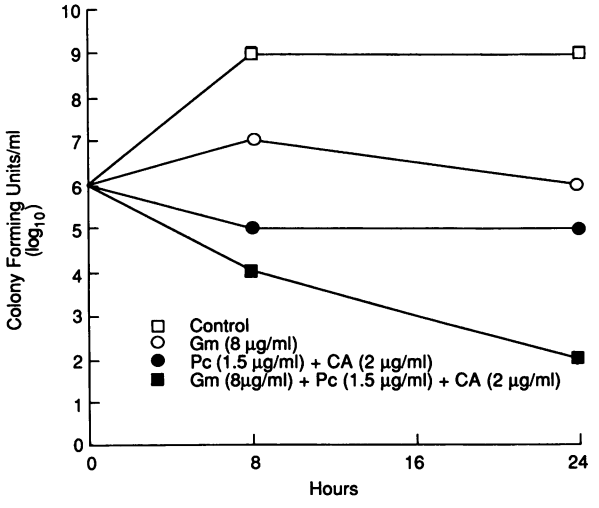

FIG. 1. Effects of GM, PC, and CA alone and in combination against *S. faecalis* WH245.

tor or GM alone (R. G. Hindes, S. H. Willey, B. E. Murray, G. M. Eliopoulos, and R. C. Moellering, Jr., Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 275, 1986). The other study showed that a combination of PC and CA was more effective in lowering vegetation counts than therapy with PC alone (2).

Penicillinase-producing enterococci have now been isolated from diverse geographic areas in the United States—Texas, Pennsylvania, and Connecticut. The trait is extrachromosomally mediated in the four strains studied so far and thus has the ability to disseminate further.

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