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NOTES

Isolation of a β-Lactamase-Producing, Aminoglycoside-Resistant Strain of *Enterococcus faecium*

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β-Lactamase-producing, aminoglycoside-resistant strains of *Enterococcus faecalis* have been isolated from different geographic areas and are endemic at our institution. We report the isolation of a β-lactamase-producing, aminoglycoside-resistant strain of *E. faecium*. The β-lactamase was plasmid mediated and transferable with high frequency into a plasmid-free *E. faecalis* recipient strain. MICs suggested that the *E. faecium* strain also contained intrinsic (chromosomal) resistance to penicillins.

Enterococci are now recognized as important causes of nosocomial infections (5, 11) and are becoming increasingly resistant to antimicrobial agents, including beta-lactams, vancomycin, and high levels of aminoglycosides (5). In 1983, the first enterococcus isolate producing a β-lactamase was described (6). Subsequently, isolates of β-lactamase-producing enterococci have been reported from different geographic areas (3, 8, 9), and all have been identified as *Enterococcus faecalis*. In the present study, we report the first known isolation of a β-lactamase-producing, aminoglycoside-resistant *E. faecium* strain.

Two strains of enterococci, E259A and E259B, and *Candida albicans* were isolated from the urine of a patient at the Veterans Affairs Medical Center; all isolates were present at greater than 100,000 CFU/ml. The enterococci were identified by the method of Facklam and Collins (1). Test results of both E259A and E259B were positive for mannitol, sorbitol, and arginine production and negative for motility and sorbose and pigment production. In addition, E259A grew in medium containing tellurite and, unlike E259B, failed to form acid in arabinose broth. E259A and E259B were nonhemolytic and alpha-hemolytic on sheep blood agar, respectively. On the basis of these results, E259A and E259B were identified as *E. faecalis* and *E. faecium*, respectively.

Recently, Vincent et al. (10) reported that pigment production and motility may be misleading criteria for definitive identification of *E. gallinarum* and *E. casseliflavus* and that the vancomycin MICs for these organisms were usually 4 to 32 μg/ml. They also stated that the type strain of *E. gallinarum* (ATCC 35038) was not *E. gallinarum* and that it should not be used as the reference strain. We tested the following ATCC type strains as control organisms for the reactions described above: *E. faecalis* ATCC 19433, *E. faecium* ATCC 19434, *E. casseliflavus* ATCC 25788, and the newly deposited type strain *E. gallinarum* ATCC 49573 (replacing ATCC 35038). *E. solitarius* (CDC885-78) was kindly provided by Richard Facklam. With one exception, all control organisms, including *E. gallinarum* (motile), yielded the proper phenotypic reactions (1). The one exception was *E. casseliflavus*, which was nonmotile, as was also reported by Vincent et al. (10). The vancomycin MIC for both E259A and E259B was 0.5 μg/ml (see below). These data support the identification of E259A and E259B as *E. faecalis* and *E. faecium*, respectively.

Antibiotic susceptibility testing was performed by using standardized broth microdilution procedures (7). *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 were used as standard control organisms. Antimicrobial agents tested included ciprofloxacin (Miles Pharmaceuticals, West Haven, Conn.), clavulanate (SmithKline Beecham Pharmaceuticals, Philadelphia, Pa.), and penicillin, ampicillin, vancomycin, tetracycline, gentamicin, and streptomycin (Sigma Chemical Co., St. Louis, Mo.). Nitrocefin discs (Cefinase; BBL Microbiology Systems, Cockeysville, Md.) were used to detect β-lactamase. MICs of various antibiotics against E259A and E259B are given in Table 1. Both E259A and E259B produced β-lactamases and were resistant to high levels of gentamicin and streptomycin. However, the MICs of penicillin, ampicillin, and ampicillin-clavulanate for E259B were 64 times greater than the MICs for E259A. Presumably, the β-lactamases and aminoglycoside resistance were plasmid mediated (4) and the increased resistance to penicillins seen with E259B was intrinsic chromosomal resistance, which is more commonly found in *E. faecium* than *E. faecalis* (5). Interestingly, clavulanate, a β-lactamase inhibitor, decreased the MIC by only one dilution, a phenomenon seen with other β-lactamase-producing *E. faecalis* organisms isolated at our institution (4).

The conjugal transfer of β-lactamase determinants and antibiotic resistance markers was performed by using a filter mating technique (2) with *E. faecium* E259B as the donor and *E. faecalis* OG1-RF (a plasmid-free, β-lactamase nonproducer resistant to rifampin and fusidic acid at 200 and 100 μg/ml, respectively) as the recipient. Donor and recipient cells were grown overnight in broth, mixed in a 1:1 ratio of donor and recipient cells, collected on a 0.45-μm-pore-size nitrocellulose filter, and incubated overnight on blood agar at 37°C. Cells were resuspended in saline and spread on agar containing gentamicin (500 μg/ml), rifampin (100 μg/ml), and fusidic acid (20 μg/ml).

Several transconjugates (recipient mating frequency, 4×10^{-6}) were subcultured on selective medium, and one was

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TABLE 1. MICs of various antibiotics against E259A, E259B, OG1-RF, and the transconjugate[a]

Antibiotic	MIC (μg/ml) for:			
	E259A	E259B	OG1-RF	Transconjugate
Penicillin	8	512	2	4
Ampicillin	4	256	1	2
Ampicillin-clavulanate[b]	2	128	1	1
Gentamicin	>1,024	>1,024	32	>1,024
Streptomycin	>1,024	>1,024	128	>1,024
Ciprofloxacin	0.5	4	2	2
Vancomycin	0.5	0.5	1	2
Tetracycline	128	0.25	1	1

[a] E259A and E259B are patient isolates of *E. faecalis* and *E. faecium*, respectively, OG1-RF is the *E. faecalis* recipient, and the transconjugate is OG1-RF mated with E259B.
[b] Ampicillin-clavulanate was used in a 2:1 ratio.

selected for additional testing. The transconjugate was identified as *E. faecalis* and produced a β-lactamase. The transconjugate was resistant to high concentrations of aminoglycosides, and the ampicillin MIC for the transconjugate decreased one dilution with the addition of clavulanate (Table 1). When the inoculum of the transconjugate was increased from 5×10^5 to 5×10^7 CFU/ml (macrodilution broth method), the ampicillin-clavulanate MIC remained unchanged (1 μg/ml). Plasmid DNA was demonstrated as described previously (4). *E. faecalis* E259A, *E. faecium* E259B, and the transconjugate contained a comigrating plasmid band, and the recipient, *E. faecalis* OG1-RF, contained no plasmids (data not shown).

Several unsuccessful attempts were made to cure *E. faecium* E259B of its plasmid(s) by growing it at an elevated temperature (45°C) and in the presence of ethidium bromide or novobiocin (both at 1 μg/ml). No plasmid-free cells were found.

We have isolated a β-lactamase-producing, aminoglycoside-resistant strain of *E. faecium*. β-Lactamase-producing, aminoglycoside-resistant isolates of *E. faecalis* are endemic in our hospital, and at one time they accounted for 11% of all enterococci isolated in the microbiology laboratory (4). Because a relatively large number of patients have been either colonized or severely infected with β-lactamase-producing, aminoglycoside-resistant *E. faecalis* (80 patients in 25 months) and because β-lactamases are plasmid mediated (4), the isolation of a β-lactamase-producing, aminoglycoside-resistant *E. faecium* strain was antipicated. This finding further underscores the difficulty of treating patients infected with *E. faecium*, an organism that is generally more resistant to antimicrobial agents than is *E. faecalis*. Vancomycin remains a reasonable choice for empiric therapy for patients infected with β-lactamase-producing, aminoglycoside-resistant *E. faecium* strains.

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