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Ampicillin-Resistant Enterococcal Species in an Acute-Care Hospital

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A prospective review of all enterococcal isolates for 13 months showed that 9.0% were resistant to ampicillin (MIC, ≥16 μg/ml; zone diameter, <15 mm), as determined by the Vitek system, disk diffusion, microdilution MIC testing, and macrodilution MIC testing. All were β-lactamase negative. A total of 19 and 3 resistant isolates were from urine and intravascular sites, respectively. Ampicillin-resistant enterococci appear to be a growing clinical problem.

MICs of ampicillin against enterococci usually range from 1 to 8 μg/ml, although MICs for *Enterococcus faecium* may be as high as 32 μg/ml (2, 6, 13, 14). From the 1960s to the early 1980s, there was no change in the susceptibility of enterococci to either penicillin or ampicillin (6). Resistance of isolates to ampicillin by β-lactamase production (8, 10) or unknown mechanisms (1) has been discovered recently.

In 1987, our laboratory changed from the Kirby-Bauer test to the Vitek automated system (Vitek Systems, Hazelwood, Mo.) for susceptibility testing, and ampicillin-resistant enterococci were frequently noted. A retrospective study of the first 6 months of 1987 showed that 19.6% of total enterococcal isolates were ampicillin resistant (zone diameter, <15 mm, as determined by the Kirby-Bauer test). Uncertain as to whether this result was due to methodology or a change in susceptibility, we studied all the enterococcal isolates to determine the incidence of ampicillin resistance by using four different methods. Ampicillin-resistant isolates also were tested for β-lactamase production, high-level gentamicin resistance, and susceptibility to other antibiotics possibly effective for the treatment of enterococcal infections.

From July 1987 through August 1988, all clinical enterococcal isolates identified at the Martinez, Calif., Veterans Administration Medical Center microbiology laboratory as resistant to ampicillin by the Vitek system were saved for further study. Isolates were identified as enterococci by growth on bile-esculin agar and in 6.5% NaCl or by the automated Vitek system and were identified to the species level by the Vitek system on the basis of biochemical reactions. Isolates classified by the Vitek system as *E. avium* were identified by Marc Zervos (William Beaumont Hospital, Royal Oak, Mich.) as *E. raffinosus* by the method of Facklam and Collins (3), since these isolates were arginine negative, arabinose positive, and raffinose positive.

All isolates found resistant to ampicillin (MIC, ≥16 μg/ml) by the Vitek system were tested by Kirby-Bauer disk diffusion and the Beckman system (microdilution MIC technique) (SmithKline Beckman Co., Carlsbad, Calif.), with an inoculum of 5×10^6 CFU/ml. Isolates for which MICs were determined to be ≥16 μg/ml by Vitek and Beckman testing and with a Kirby-Bauer zone size of <15 mm were considered ampicillin resistant and underwent broth macrodilution MIC testing in cation-supplemented Mueller-Hinton broth (50 mg of Ca^{+2} and 25 mg of Mg^{+2} per liter) by standard techniques (5) with an inoculum of 5×10^5 CFU/ml. Five isolates of *E. raffinosus* were tested in brain heart infusion broth as described by Murray et al. (8) because of poor growth in Mueller-Hinton broth. Laboratory standard ampicillin powder (Sigma Chemical Co., St. Louis, Mo.) was used. Strain ATCC 29212 was used as a concurrent control.

All ampicillin-resistant isolates were tested by Kirby-Bauer disk diffusion to determine zone sizes with ciprofloxacin (5-μg disk; BBL Microbiology Systems, Cockeysville, Md.), vancomycin (30-μg disk; BBL), ampicillin-sulbactam (20-μg disk; BBL), and teicoplanin (30-μg disk; BBL) and by broth macrodilution to determine MICs of daptomycin (Lilly

TABLE 1. Susceptibilities of *E. raffinosus* and *E. faecium* to ampicillin

Organism (*n*)	MIC (μg/ml)[a] as determined by:						Zone size (mm)[b] as determined by Kirby-Bauer testing
	Macrodilution testing		Beckman system		Vitek system		
	50%	90%	50%	90%	50%	90%	
E. raffinosus (9)	16	32	16	16	≥16	≥16	8.8 ± 1.05 (no zone to 14)
E. faecium (19)	64	64	>16	>16	≥16	≥16	6 ± 0 (no zone)

[a] 50% and 90%, MIC for 50 and 90% of the isolates, respectively.
[b] Reported as mean ± standard error of the mean (range). Four *E. raffinosus* isolates had no zone of inhibition, and all *E. faecium* isolates had no zone of inhibition. For calculations, no zone of inhibition was considered to be 6 mm.

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TABLE 2. Susceptibilities of ampicillin-resistant enterococci as determined by Kirby-Bauer disk diffusion or broth macrodilution

Organism (*n*)	Zone size (mm)[a] with:			MIC (μg/ml)[b] of daptomycin	
	Ciprofloxacin	Vancomycin	Teicoplanin	50%	90%
E. raffinosus (9)	18.6 ± 0.6 (14–20)	18.6 ± 0.4 (18–20)	16.2 ± 0.4 (15–18)	1	2
E. faecium (19)	13.5 ± 0.3 (12–18)	17.2 ± 0.4 (14–19)	14.8 ± 0.3 (14–18)	2	4

[a] Determined by Kirby-Bauer disk diffusion and reported as mean ± standard error of the mean (range). Zone sizes indicating antibiotic resistance were as follows: ciprofloxacin, ≤15 mm; vancomycin, ≤9 mm; teicoplanin, ≤10 mm.
[b] See Table 1, footnote *a*.

Pharmaceuticals, Indianapolis, Ind.). β-Lactamase production was tested with a nitrocefin disk and powder by standard techniques (8, 10). High-level gentamicin resistance was determined in a single microdilution well containing 500 μg of gentamicin (Sigma) per ml as described by Zervos et al. (15).

During the 13-month surveillance period, 28 (9%) ampicillin-resistant enterococcal isolates (MIC, ≥16 μg/ml) were cultured from 310 single patient specimens. A total of 19 (67.9%) isolates were from urine, 3 (10.7%) were from wounds, 2 (7.1%) were from stools, 2 (7.1%) were from blood, 1 (3.6%) was from bile, and 1 (3.6%) was cultured from a central venous line, an arterial line, and a postmortem heart blood culture. A total of 19 (68.4%) isolates were identified as *E. faecium*, and 9 (31.6%) were identified as *E. raffinosus*.

Of 26 evaluable patients, 25 (96%) were male. The mean age was 66.5 years (range, 32 to 91 years). Underlying diseases such as chronic urological abnormalities, wounds, and cancer were present in 19 (73%). A total of 17 patients were infected, and 9 were colonized with enterococci, on the basis of the criteria of Moellering (7). There was a mean of 37.7 days of hospitalization before a positive culture, and the ampicillin-resistant enterococci that were considered true pathogens were acquired nosocomially. Outpatient isolates were rare and only occurred in patients who had been hospitalized frequently. Twenty-five (96%) patients received antibiotics within 3 months before the positive culture.

The isolates were found ampicillin resistant by all four testing methods, indicating good concordance (Table 1). All were found β-lactamase negative and resistant to ampicillin-sulbactam by disk diffusion. Two clinical isolates exhibited high-level gentamicin resistance. Nine isolates exhibited intermediate susceptibility to ciprofloxacin (Table 2). All ampicillin-resistant isolates were susceptible to vancomycin, teicoplanin, and daptomycin (Table 2).

Resistance of enterococci to beta-lactam antibiotics is an emerging problem of clinical importance (1, 8–10, 12). In 1983, Murray and Mederski-Samoraj described one enterococcal strain that was ampicillin resistant because of β-lactamase production (9). Subsequent published reports (8, 10) suggested a low incidence of ampicillin-resistant enterococci, but more recent reports (1, 12) indicate an increased incidence. Boyce et al. reported that the incidence of ampicillin-resistant enterococci at one institution increased from 0.7% for April 1986 to June 1987 to 2.2% for July 1987 to December 1987 (J. M. Boyce, A. A. Medeiros, E. F. Papa, and G. Potter-Bynoe, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1075, 1988) and then to 8% in 1988 (J. M. Boyce, S. M. Opal, G. Potter-Bynoe, and A. A. Medeiros, 29th ICAAC, abstr. no. 657, 1989). We found an incidence of 9% and suggest that the increase was not due to a change in susceptibility testing methodology.

The first ampicillin-resistant isolates described were *E. faecalis* (8, 10). Ampicillin-resistant *E. faecium*, *E. raffinosus*, and *E. gallinarum* were reported subsequently (1, 12). The majority of ampicillin-resistant isolates, particularly those that do not produce β-lactamase, are *E. faecium*. However, *E. raffinosus* represented a relatively large proportion of resistant isolates in our study and other studies (12; Boyce et al., 29th ICAAC). Our other isolates were identified as *E. faecium*, although Vitek identification of *E. faecium* may not be entirely reliable (11).

Investigation of the first ampicillin-resistant isolates demonstrated β-lactamase production mediated by a transferable plasmid (8–10). In contrast, the ampicillin-resistant isolates in our study and other studies (1, 12; Boyce et al., 29th ICAAC, abstr. no. 657, 1989) were β-lactamase negative. Penicillin resistance of β-lactamase-negative *E. faecium* isolates has been attributed to a decreased affinity of penicillin for penicillin-binding proteins (4, 14), which may also have caused resistance in our isolates.

High-level gentamicin resistance (MIC, >2,000 μg/ml) was found in three of four β-lactamase-positive isolates described in early studies (8, 10). High-level gentamicin resistance appeared infrequently in later studies of β-lactamase-negative ampicillin-resistant isolates (1, 12) but did occur. We had clinical isolates from two patients that were each a mixture of ampicillin-resistant *E. raffinosus* and high-level gentamicin-resistant *E. faecalis*.

In view of the experience of our medical center and others, the degree and incidence of resistance of enterococci to beta-lactam agents such as ampicillin should be monitored. Enterococci identified as pathogens should be tested for susceptibility to ampicillin by any of the methods outlined in this paper. Many issues regarding ampicillin-resistant enterococci require more study, including factors that lead to acquisition, modes of transmission, and optimal therapy.

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