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Ampicillin-Resistant *Enterococcus raffinosus* in an Acute-Care Hospital: Case-Control Study and Antimicrobial Susceptibilities

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A prospective study identified 9 (32%) of 28 ampicillin-resistant (MIC ≥ 16 μg/ml) enterococcus isolates as *Enterococcus raffinosus*. A case-control study found no significant differences with respect to underlying diseases, catheterization, or surgery between patients with ampicillin-resistant *E. raffinosus* and those with ampicillin-susceptible *Enterococcus* spp. Prior treatment with antibiotics and prolonged hospitalization were more frequent among patients with ampicillin-resistant *E. raffinosus*. Patients with the same strain (determined by plasmid analysis) were frequently hospitalized concurrently.

From the 1960s to the early 1980s, susceptibility of enterococci to ampicillin and penicillin did not change (6). Resistance of a few *Enterococcus faecalis* isolates to ampicillin by β-lactamase production (10, 13) was reported in the 1980s. *Enterococcus faecium*, *Enterococcus raffinosus*, and *Enterococcus gallinarum* were reported subsequently (2, 16), with ampicillin resistance probably due to decreased penicillin-binding affinity of penicillin-binding proteins (3, 17). In recent reports, *E. raffinosus* has constituted a relatively large proportion of resistant isolates (1, 16), and resistance to penicillins might be a feature of *E. raffinosus* (4).

A prospective study of ampicillin-resistant enterococcal clinical isolates at the Martinez Veterans Administration Medical Center revealed that 28 (9%) of 310 enterococcal isolates were ampicillin resistant (MIC ≥ 16 μg/ml) (12). Of these 28 isolates, 9 (32%) were *E. raffinosus*. Little has been reported about the risk factors for acquisition of ampicillin-resistant enterococci, including *E. raffinosus*. We report a case-control comparison of patients with ampicillin-resistant *E. raffinosus* (ARER) with patients with ampicillin-susceptible enterococcal isolates to determine factors important for acquisition of ARER. Plasmid analysis was done to study nosocomial transmission.

We reviewed microbiology laboratory records to identify patients with ARER between July 1987 and August 1988. Enterococci were identified to the species level as previously described (12). Ampicillin susceptibility testing was done with the Vitek System, disk diffusion, and microdilution and macrodilution MIC techniques (12). Isolates with ampicillin MICs of ≥16 μg/ml and zone sizes of <15 mm were considered resistant. *E. faecalis* ATCC 29212 was used as a concurrent control. Microdilution MIC testing was done with ampicillin-sulbactam (2:1 ratio by weight; Pfizer, New York, N.Y.), ciprofloxacin (Miles, West Haven, Conn.), daptomycin (Lilly, Indianapolis, Ind.), clindamycin (Sigma, St. Louis, Mo.), fosfomycin (Sigma), streptomycin (Lilly), gentamicin (Schering, Kenilworth, N.J.), and vancomycin (Lilly). β-Lactamase production (detected with nitrocefin disks and powder) and high-level gentamicin resistance (MIC ≥ 2,000 μg/ml) were detected as described previously (10, 13, 19).

Each patient was matched by date of initial isolation of ARER with two control patients from whom ampicillin-susceptible (MIC ≤ 8 μg/ml) enterococci had been cultured. We retrospectively examined the medical records for age, sex, underlying disease, bladder and vascular catheterization, exposure to antibiotics, patient ward location, hospital service, and invasive procedures. Nosocomial infection was defined as the isolation of *Enterococcus* spp. after at least 72 h of hospitalization. Urinary tract infection was diagnosed when $\geq 10^3$ CFU/ml were cultured from a clean-voided or catheterized specimen from a patient with pyuria and with a clinical setting consistent with infection (8). Ulcers and wounds were considered infected if there was purulent drainage. Intra-abdominal sites were considered infected if the patient had fever and leukocytosis with purulent drainage from intra-abdominal drains. Bacteremia was defined as at least one positive blood culture and clinical findings consistent with infection. Statistical comparisons were made with the Student *t* test or Fisher exact test, with $P \leq 0.05$ considered significant.

The plasmid content of *E. raffinosus* was established by methods previously described and used as a marker of strain identity (18). Strains were also compared for molecular relatedness of chromosomal DNA by pulsed-field electrophoresis (11). Estimates of molecular weights of DNA were made by comparing the migration distances of *Eco*RI, *Hin*dIII, and *Sfi*I bacteriophage λ DNA fragments.

From July 1987 through August 1988, culture specimens from nine patients at the Martinez Veterans Administration Medical Center yielded ARER (12). The nine isolates comprised 32% of all ampicillin-resistant isolates and 3% of all enterococcal isolates during that period. Sites included urine (four patients), wounds (two patients), peritoneal fluid (one patient), bile (one patient), and blood (one patient).

All ARER were β-lactamase negative and resistant to ampicillin-sulbactam, clindamycin, and fosfomycin but susceptible to vancomycin, daptomycin, and ciprofloxacin. No isolates exhibited high-level gentamicin or streptomycin resistance.

Characteristics of patients and controls are listed in Table 1. There were no statistically significant differences in clin-

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TABLE 1. Clinical characteristics of patients from whom ARER and ampicillin-susceptible *Enterococcus* spp. were isolated

Characteristic[a]	Value for or number of patients infected with:	
	ARER (n = 9)	Susceptible enterococci (n = 18)
Age[b]	67.2 ± 3.7 (50–91)	66.7 ± 3.2 (52–94)
No. of males/no. of females	8/1	18/0
Underlying illness	8	13
Hospital service		
Medicine	3	11
Surgery	4	5
Urology	1	2
Other	1	0
Surgical procedures	4	8
Urinary tract instrumentation	9	14
No. of days of hospitalization before isolation[b]	75.4 ± 27.5 (1–291)	28.0 ± 13 (1–241)
Antibiotics		
Penicillins	4	5
Cephalosporins	5	8
Any	8	11

[a] All clinical characteristics had $P > 0.05$.
[b] Mean ± standard error of the mean.

ical characteristics, although patients with ARER were hospitalized longer before culture and had received prior antibiotics more frequently. Two patients and no controls were transferred from nursing homes.

Four (44%) of nine patients were infected with ARER: one isolate was from blood, two were from urine, and one was from bile. Three of these patients had severe underlying diseases and died soon after culture of ARER. The fourth patient, with ARER in the urine, had recurrent urinary tract infections. Five (56%) of nine patients were colonized with ARER: two isolates were from urine, two were from wounds, and one was from peritoneal fluid.

Of 18 controls, 12 (67%) were infected with susceptible enterococci: 8 isolates were from urine, 2 were from blood, 1 was from bile, and 1 was from an abscess. Six patients (33%) were colonized with ampicillin-susceptible enterococci: four isolates were from wounds, and two were from urine.

Seven (78%) of the nine ARER isolates were hospital acquired. Four of these seven patients had prior cultures with ampicillin-susceptible *E. faecalis*. The two patients with community-acquired ARER were colonized and frequently seen in outpatient clinics. Six patients with ampicillin-susceptible enterococci had community-acquired isolates: four of these patients were infected (three isolates from urine and one from an inguinal abscess), and two were colonized (in wounds).

Gel electrophoresis for plasmid analysis (Fig. 1) and pulsed-field electrophoresis for chromosomal relatedness identified three different strains of *E. raffinosus*. Four patients had strain I, four had strain II, and one had strain III.

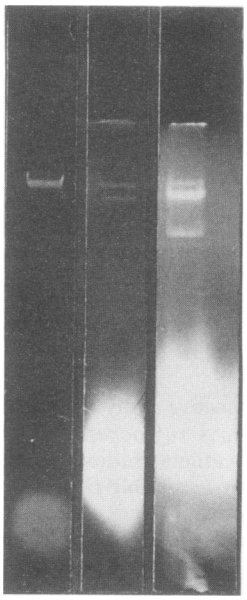

FIG. 1. Agarose gel electrophoresis of plasmid DNA purified by cesium chloride density gradient centrifugation, showing three strain types of *E. raffinosus*.

The four patients with strain I were hospitalized concurrently. Three were on the same surgical ward concurrently. The fourth patient was hospitalized on a different ward on the same surgical floor. Two patients with strain II were hospitalized concurrently in 1987, and the other two were hospitalized concurrently in 1988. The two patients hospitalized concurrently in 1987 were on different wards but were cared for by the same house staff. The two patients hospitalized concurrently in 1988 were on the same ward and cared for by the same nurses and house staff.

Little has been published about *E. raffinosus*. A recent review reported that only 1 (0.3%) of 302 enterococcal isolates was *E. raffinosus* (15). Sapico et al. (16) found that 4 (25%) of 16 ampicillin-resistant, β-lactamase-negative enterococci discovered during an 80-month period were *E. raffinosus*. Boyce et al. (1) found that 6 (23%) of 26 ampicillin-resistant, β-lactamase-negative enterococcal isolates were nosocomially acquired *E. raffinosus*. Plasmid analysis indicated that three patients had the same strain.

A prospective study at our institution revealed that 9% of enterococci were ampicillin resistant, with 32% of resistant isolates identified as *E. raffinosus* (12). All ARER isolates were β-lactamase negative and resistant to ampicillin-sulbactam.

Little is known about risk factors and mechanisms of acquisition of ARER. Acquisition of gentamicin-resistant *E. faecalis* was associated significantly with prior antimicrobial therapy, perioperative antibiotic prophylaxis, prior surgical procedures, and longer hospitalization (18). All enterococcal isolates were nosocomially acquired, and plasmid analysis suggested nosocomial transmission and exogenous acquisition.

We found that patients with ARER were similar to patients with ampicillin-susceptible *Enterococcus* spp. in age and in history of underlying diseases, urinary tract instrumentation, perioperative antibiotic prophylaxis, and prior surgical procedures, although they were more likely to be geographically and temporally clustered on the surgical

service, to have received prior antibiotics, and to be hospitalized longer. Statistical analysis was limited, however, by the small number of patients.

DNA analysis as a marker of strain identity indicates clusters of patients with identical strains, suggesting person-to-person spread or exogenous acquisition via indirect contact transmission. However, no common source was identified.

ARER caused infection, including bacteremia, as well as colonization. Treatment of serious infections requires synergistic combinations of a cell wall-active agent with an aminoglycoside. Ampicillin and gentamicin are not predictably synergistic against ARER (9), and other bactericidal regimens are needed. This need is underscored by the increasing incidence of plasmid-mediated high-level gentamicin resistance (14) and reports of vancomycin-resistant enterococci (5, 7).

All work was performed at the Veterans Administration Medical Center, Martinez, Calif., and William Beaumont Hospital, Royal Oak, Mich.

REFERENCES

1. **Boyce, J. M., S. M. Opal, G. Potter-Bynoe, A. A. Medeiros.** 1989. Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 657.
2. **Bush, L., J. Calmon, C. Cherney, M. Wendeler, P. Pitsakis, J. Poupard, M. E. Levison, and C. C. Johnson.** 1989. High-level penicillin resistance among isolates of enterococci: implications for treatment of enterococcal infections. Ann. Intern. Med. **110**:515–520.
3. **Fontana, R., A. Grossato, L. Rossi, Y. R. Cheng, and G. Satta.** 1985. Transition from resistance to hypersusceptibility to β-lactam antibiotics associated with loss of a low-affinity penicillin-binding protein in a *Streptococcus faecium* mutant highly resistant to penicillin. Antimicrob. Agents Chemother. **28**:678–683.
4. **Grayson, M. L., G. M. Eliopoulos, C. B. Wennersten, K. L. Ruoff, K. Klimm, F. L. Sapico, A. S. Bayer, and R. C. Moellering, Jr.** 1991. Comparison of *Enterococcus raffinosus* with *Enterococcus avium* on the basis of penicillin susceptibility, penicillin-binding protein analysis, and high-level aminoglycoside resistance. Antimicrob. Agents Chemother. **35**:1408–1412.
5. **Johnson, A. P., A. H. C. Uttley, N. Woodford, and R. C. George.** 1990. Resistance to vancomycin and teicoplanin: an emerging clinical problem. Clin. Microbiol. Rev. **3**:280–291.
6. **Kaye, D.** 1982. Enterococci: biological and epidemiological characteristics and in vitro susceptibility. Arch. Intern. Med. **142**:2006–2009.
7. **Leclerq, R., E. Derlot, J. Duval, and R. Courvalin.** 1988. Plasmid mediated resistance to vancomycin and teicoplanin in *Enterococcus faecium*. N. Engl. J. Med. **319**:157–161.
8. **Lipsky, B. A., R. C. Ireton, S. D. Fihn, R. Hackett, and R. E. Berger.** 1987. Diagnosis of bacteriuria in men: specimen collection and culture interpretation. J. Infect. Dis. **155**:847–854.
9. **McCabe, R. E., V. A. Chirurgi, S. Oster, and A. A. Goldberg.** Unpublished data.
10. **Murray, B. E., D. A. Church, A. Wanger, K. Zscheck, M. E. Levison, M. J. Ingerman, E. Abrutyn, and B. Mederski-Samoraj.** 1986. Comparison of two β-lactamase-producing strains of *Streptococcus faecalis*. Antimicrob. Agents Chemother. **30**: 861–864.
11. **Murray, B. E., K. V. Singh, J. D. Heath, B. R. Sharma, and G. M. Weinstock.** 1990. Comparison of genomic DNAs of different enterococcal isolates using restriction endonucleases with infrequent recognition sites. J. Clin. Microbiol. **28**:2059–2063.
12. **Oster, S. E., V. A. Chirurgi, A. A. Goldberg, S. Aiken, and R. E. McCabe.** 1990. Ampicillin-resistant enterococcal species in an acute-care hospital. Antimicrob. Agents Chemother. **34**:1821–1823.
13. **Patterson, J. E., B. L. Masecar, and M. J. Zervos.** 1988. Characterization and comparison of two penicillinase-producing strains of *Streptococcus* (*Enterococcus*) *faecalis*. Antimicrob. Agents Chemother. **32**:122–124.
14. **Patterson, J. E., and M. J. Zervos.** 1990. High-level gentamicin resistance in *Enterococcus*: microbiology, genetic basis, and epidemiology. Rev. Infect. Dis. **12**:644–652.
15. **Ruoff, K. L., L. de la Maza, M. J. Murtagh, J. D. Spargo, and M. J. Ferraro.** 1990. Species identities of enterococci isolated from clinical specimens. J. Clin. Microbiol. **28**:435–437.
16. **Sapico, F. L., H. N. Canawati, V. J. Ginunas, D. S. Gilmore, J. Z. Montgomerie, W. J. Tuddenham, and R. R. Facklam.** 1989. Enterococci highly resistant to penicillin and ampicillin: an emerging clinical problem? J. Clin. Microbiol. **27**:2091–2095.
17. **Williamson, R., C. Le Bouguenec, L. Gutmann, and T. Horaud.** 1985. One or two low affinity penicillin-binding proteins may be responsible for the range of susceptibility of *Enterococcus faecium* to benzylpenicillin. J. Gen. Microbiol. **131**:1933–1940.
18. **Zervos, M. J., S. Dembinski, T. Mikesell, and D. R. Schaberg.** 1986. High-level resistance to gentamicin in *Streptococcus faecalis*: risk factors and evidence for exogenous acquisition of infection. J. Infect. Dis. **153**:1075–1083.
19. **Zervos, M. J., J. E. Patterson, S. Edberg, C. Pierson, C. A. Kauffman, T. S. Mikesell, and D. R. Schaberg.** 1987. Single-concentration broth microdilution test for detection of high-level aminoglycoside resistance in enterococci. J. Clin. Microbiol. **25**:2443–2444.