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Enterococci Highly Resistant to Penicillin and Ampicillin: an Emerging Clinical Problem?

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Sixteen clinical isolates of ampicillin-resistant enterococci (ARE) were recovered from the microbiology laboratory of a 450-bed rehabilitation medical center from January 1981 to September 1987. These isolates were detected when a disk diffusion test using 10 μg of ampicillin on a blood agar plate revealed no zones of inhibition. Tube macrodilution tests yielded an MIC of ≥16 μg of ampicillin per ml. None of the isolates were penicillinase producers by the chromogenic cephalosporin disk test. Ten isolates were *Enterococcus faecium*, four isolates were *E. raffinosus*, one isolate was *E. gallinarum*, and one isolate was not identified (lost). There were 6 male and 10 female patients. The sources of isolates were urine (n = 7), wound (n = 5), ascitic fluid (n = 2), blood (n = 2), peritoneal catheter tip (n = 1), Bartholin's cyst abscess (n = 1), rectal swab (n = 2), and pancreatic abscess (n = 1). The organism was isolated from multiple sites in 4 patients, was a pure culture isolate in 5 patients, and was part of a polymicrobial flora in 11 patients. Six patients were diabetic, and four had liver cirrhosis. All but four patients had received at least one antibiotic within 3 weeks of ARE isolation. The MICs (micrograms per milliliter) for 50 and 90% of isolates tested, respectively, were as follows: ampicillin, 64 and 64; penicillin, 128 and >128; vancomycin, 1 and 2; gentamicin, 4 and 16; ciprofloxacin, 1.6 and 3.2; imipenem, 128 and >128; and daptomycin (LY146032), 1.6 and 6.4. ARE may be an emerging pathogen in the hospitalized patient population.

In the past, enterococci had shown remarkable stability with regards to in vitro susceptibility to beta-lactam antibiotics. In fact, as recently as 1982, Kaye (13) commented that the susceptibility of enterococci to penicillin G and to ampicillin had not changed in the previous 20 years (31). In 1970, Standiford et al. (30) demonstrated high-level aminoglycoside resistance (MIC, >2,000 μg/ml) among enterococcal strains that failed to show synergistic killing when exposed to combinations of penicillin and streptomycin or kanamycin. Nine years later, Moellering and his group (21) showed that *Enterococcus faecium* strains accounted for the streptomycin- and amikacin-resistant isolates found clinically but that even these strains were still relatively susceptible to gentamicin and were killed readily by the penicillin-gentamicin combination. Sporadic reports of *E. faecium* strains highly resistant to penicillin G started to appear in the literature (1, 7, 9, 18, 34). Some of these strains were found to possess altered penicillin-binding proteins with decreased affinity to penicillin G (18, 34). In 1983, Murray (24) reported a strain of *E. faecalis* highly resistant to penicillin G that produced large amounts of penicillinase. Subsequently, other β-lactamase-producing strains of *E. faecalis* were reported by separate investigators and the presence of a plasmid was demonstrated in two strains (23, 27). Other very disturbing developments that have occurred recently among the enterococci are reports of high-level gentamicin resistance and rare isolations of vancomycin- as well as teicoplanin-resistant *E. faecium* (10, 16, 25, 33, 35).

Since 1981, we have noticed the increasing occurrence of penicillin and ampicillin resistance among our clinical isolates of enterococci. We reviewed our clinical experience with these isolates for a period of 6 years and 8 months and report some of the studies we have done on these isolates.

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MATERIALS AND METHODS

Patients. The clinical records of patients who yielded isolates of ampicillin-resistant enterococci (ARE) from clinical specimens submitted to the Rancho Los Amigos Clinical Microbiology Laboratory from January of 1981 to September of 1987 were reviewed. One patient included in this report was seen by one of us (W.J.T.) in a community hospital nearby. The rest were all seen at Rancho Los Amigos Medical Center.

Methods. The ARE isolates were initially detected when virtually no zone of antibiotic inhibition was detected around a 10-μg ampicillin disk during performance of conventional disk diffusion susceptibility testing. Other clinical isolates generally showed at least 16 mm of inhibition around the ampicillin disk and were considered susceptible (26). Identification of the isolates was based on conventional methods using a bile-escul in medium, 6.5% NaCl broth, and biochemical utilization of sorbitol, pyruvate, sucrose and arabinose, as well as on the ability to hemolyze horse erythrocytes and to liquify gelatin. The isolates were identified at the Centers for Disease Control (Atlanta, Ga.) by one of us (R.R.F.) and classified on the basis of newly established criteria (8). MICs and MBCs were determined by conventional tube macrodilution methods using Mueller-Hinton broth (12) and an inoculum of about 10^5 organisms per ml. The MBC was defined as the lowest antibiotic concentration showing no growth in a 0.01-ml sample. Enterococcal iso-

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TABLE 1. Clinical and microbiologic features of the 16 patients with ARE isolates

Patient no.	Age (yr)/ sex[a]	Enterococcal species isolated	Source	Underlying illness	Antimicrobial agent(s) received within 2 wk prior to ARE isolation[b]
1	51/F	*F. faecium*	Clean-catch urine[c]	Brain tumor	None
2	60/M	*E. faecium*	Foot stump wound after transmetatarsal amputation	Insulin-requiring diabetes mellitus	None
3	86/F	*E. raffinosus*	Stasis dermatitis ulcer	Venous stasis, lower extremities	None
4	63/F	*E. raffinosus*	Clean-catch urine[c]	Alcoholic liver disease with ascites	Dicloxacillin (p.o.), ampicillin, oxacillin (i.v.)
5	71/F	*E. raffinosus*	Bartholin's gland abscess, rectal swab	Rheumatoid arthritis	Oxacillin (i.v.)
6	53/F	*E. faecium*	Ankle disarticulation, stump wound	Insulin-requiring diabetes mellitus	Ampicillin, clindamycin, gentamicin, oxacillin (i.v.)
7	83/M	*E. faecium*	Peritoneal dialysis catheter tip	Non-insulin-requiring diabetes mellitus, renal failure	Imipenem, oxacillin, cefotaxime, clindamycin (i.v.)
8	17/M	Unidentified enterococcus	Catheter-obtained urine[c]	Spinal cord injury with neurogenic bladder	Cefazolin, amikacin, piperacillin, gentamicin, cefotaxime (i.v.)
9	45/F	*E. raffinosus*	Clean-catch urine,[c] rectal swab	Alcoholic liver disease with ascites	Neomycin (p.o.)
10	75/F	*E. faecium*	Clean-catch urine[c]	Motor vehicular accident	Cephapirin (i.v.)
11	64/F	*E. faecium*	Healing knee stump wound	Non-insulin-requiring diabetes mellitus	Clindamycin, cefazolin, cefotaxime (i.v.)
12	66/M	*E. faecium*	Leg stump wound	Insulin-requiring diabetes mellitus	Ampicillin, cefotaxime, clindamycin, gentamicin, imipenem, ceftazidime (i.v.), amphotericin B (wound irrigation)
13	74/F	*E. faecium*	Catheter-obtained urine[c]	Rheumatoid arthritis	Cephapirin, clindamicin, tobramycin (i.v.)
14	49/M	*E. faecium*	Blood, pancreatic abscess, urine[c]	Gallstone pancreatitis, adult respiratory distress syndrome	Chloramphenicol, cefotaxime, cefoperazone, tobramycin (i.v.)
15	47/F	*E. gallinarum*	Ascitic fluid[d]	Alcoholic liver disease with ascites	Cefotaxime (i.v.)
16	60/M	*E. gallinarum*	Blood, ascitic fluid[d]	Alcoholic liver disease with ascites	None

[a] F, Female; M, male.
[b] p.o., Orally; i.v., intravenously.
[c] $>10^5$ CFU/ml.
[d] Ascitic fluid polymorphonuclear count (250/mm^3).

lates for which the MIC was 16 μg or more of ampicillin per ml were arbitrarily considered to be highly resistant.

Laboratory standard powders of the following antibiotics were used in the study: ampicillin and penicillin G (Wyeth Laboratories, Westchester, Pa.); vancomycin, streptomycin, and daptomycin (LY146032) (Eli Lilly & Co., Indianapolis, Ind.); gentamicin (Schering Corp., Kenilworth, N.J.); imipenem (Merck Sharp & Dohme, West Point, Pa.); and ciprofloxacin (Miles Laboratories, West Haven, Conn.).

β-Lactamase production was investigated by using the nitrocefin disk method (2).

RESULTS

Sixteen patients yielded 16 isolates of ARE. The 16 isolates of ARE were identified as 10 isolates of *E. faecium*, 4 isolates of *E. raffinosus*, one isolate of *E. gallinarum*, and one unidentified isolate which was lost before it could be identified. MICs of >16 μg of ampicillin per ml were demonstrated for all 16 isolates by tube macrodilution susceptibility testing. These isolates represented less than 1% of the total enterococcal isolates recovered by our clinical microbiology laboratory. The isolates were recovered from 6 male and 10 female patients, with a mean age of 60 years (range, 17 to 86 years).

Table 1 shows the clinical and microbiologic features of the 16 patients included in the study. It should be noted that one patient (no. 16) yielded ARE (*E. gallinarum*) from both blood and ascitic fluid. Another patient (no. 14) had blood, pancreatic abscess, and urine cultures all positive for *E. faecium*. A third patient (no. 15) yielded *E. gallinarum* from an ascitic fluid culture. These three patients, therefore, yielded ARE from body sites or fluids that are normally sterile and were therefore considered to have clinically significant infections. Seven patients yielded ARE in their urine. Four of the isolates from these bacteriuric patients were *E. faecium*, two were *E. raffinosus*, and one was unidentified. Six of the seven patients were afebrile. Five of these six afebrile and bacteriuric patients had no urinary tract symptoms, with the exception of patient no. 13, who had complained of burning urination. Patient no. 14 had fever and chills, but this patient also had a pancreatic abscess and ARE bacteremia. Five of these patients received no therapy for ARE bacteriuria. One of these patients was transferred to another hospital with no subsequent follow-up, two patients had spontaneous disappearance of ARE without therapy, and two others had no repeat urine cultures taken.

Among the 10 patients who yielded ARE from sources other than urine or rectal swabs (Table 1), 7 demonstrated at

TABLE 2. Antimicrobial susceptibility of the ARE isolates

Antimicrobial agent	MIC (μg/ml)	
	50%	90%
Ampicillin	64.0	64.0
Penicillin G	128.0	>128.0
Vancomycin	1.0	2.0
Gentamicin	4.0	16.0
Imipenem	64.0	128.0
Ciprofloxacin	3.2	6.4
Daptomycin	1.6	6.4

least one of the following three characteristics: (i) fever, (ii) purulent discharge, or (iii) peripheral leukocytosis. These patients were considered to have clinically significant infections.

Five isolates were recovered in pure culture, and eleven were isolated with other microorganisms. Five were isolated with one other organism, three were isolated with two other organisms, and three were isolated with three other organisms. The accompanying organisms were coagulase-negative staphylococcus (n = 4), *Escherichia coli* (n = 4), *Proteus mirabilis* (n = 2), *Pseudomonas aeruginosa* (n = 2), *Klebsiella oxytoca* (n = 2), *Klebsiella pneumoniae* (n = 1), ampicillin-susceptible enterococci (n = 2), *Candida tropicalis* (n = 1), *Candida albicans* (n = 1), and viridans group streptococci (n = 1).

Diabetes mellitus (n = 6; four insulin-dependent isolates) and alcoholic liver disease (n = 4) were the most frequently present underlying diseases.

Twelve of the sixteen patients had received at least one antimicrobial agent for at least 72 h within 3 weeks prior to ARE isolation (Table 1). Ten of these patients received multiple antimicrobial agents. Eleven of the twelve patients who received antimicrobial agents had received at least one beta-lactam agent, and ten had received two or more beta-lactam agents.

Table 2 shows the antimicrobial susceptibility of the 16 isolates to ampicillin, penicillin G, vancomycin, gentamicin, imipenem, ciprofloxacin, and daptomycin. The MBCs of ampicillin and penicillin G for the ARE isolates were generally within one twofold dilution higher than the MICs, and none of the strains were tolerant (MBC, ≥32 times higher than MIC). On the other hand, the vancomycin MBCs for 50 and 90% of the isolates (MBC_{50} and MBC_{90}, respectively) were much higher than the MICs for 50 and 90% of the isolates (MIC_{50} and MIC_{90}, respectively). The MBC_{50} and MBC_{90} were 32 and 128 μg/ml, respectively. Only two isolates were not tolerant to vancomycin. The MBCs of imipenem, ciprofloxacin, and daptomycin were generally one tube dilution higher than the MICs. The MBCs of imipenem for *E. faecium* were generally one or two dilutions higher than for other species. All of 16 isolates, however, were highly resistant to imipenem (MIC, ≥32 μg/ml).

There appeared to be a trend toward increasing numbers of ARE isolations from 1981 to September of 1987. One isolate was recovered in 1981, none were recovered in 1982, three were recovered in 1983, two were recovered in 1984, four were recovered in 1986, and four were recovered by September of 1987.

Isolates from five patients were recovered within 3 days of admission to the study hospitals and were considered to have been acquired from the outside (i.e., other, transferring hospitals). Eleven patients had their isolates recovered many days or weeks after admission and/or had previous negative cultures negative for ARE from the same sites reported by our clinical microbiology laboratory.

There were no clustering of cases and no evidence of person-to-person spread at the time this report was written.

Seven patients received therapy directed at the ARE. Four patients received intravenous vancomycin, which would be considered appropriate therapy for ARE infections. Two of these cases had clinical as well as bacteriologic cures. Two patients were deemed unevaluable (one patient had a healing and clean stump wound with minimal drainage, and another underwent amputation of an infected foot subsequent to ARE isolation). One other patient received erythromycin for a positive *E. raffinosus* culture of a chronic stasis ulcer which remained unchanged after treatment, and no repeat cultures were performed. Three patients received therapy with antimicrobial agents to which the ARE were not susceptible in vitro. These patients (including one with ARE spontaneous bacterial peritonitis treated with sulfatrimethoprim) appeared to do well clinically despite seemingly inappropriate therapy. The other two patients, however, underwent wound debridement (for a stump wound infection) and abscess drainage (for a Bartholin's gland abscess), which may have been primarily responsible for the clinical response.

DISCUSSION

The antimicrobial susceptibility pattern of enterococci appears to be undergoing a state of dynamic change. At least 40% of the strains isolated from 1970 have shown high-level resistance to streptomycin (22). Most of this resistance has been attributed to production of streptomycin-modifying enzymes encoded by plasmid determinants (6, 15). High-level resistance to kanamycin and amikacin and, more recently, to gentamicin has also been reported (11, 19, 25, 35). These aminoglycoside-resistant isolates have been shown to lose the bactericidal synergy demonstrated by their susceptible counterparts with combinations of aminoglycosides and penicillin or vancomycin. Most of these aminoglycoside-resistant isolates have been *E. faecium*.

A cause of serious concern has been the description of β-lactamase production in isolates of *E. faecalis* in recent years (23, 24, 27). Other species of enterococci have not been implicated to this point. This type of antimicrobial resistance had not been described previously in enterococci and has been shown to be plasmid mediated. Even more disturbing, perhaps, has been the report of plasmid-mediated vancomycin resistance in isolates of *E. faecalis* and *E. faecium* (16, 33). Two of these *E. faecium* strains also demonstrated resistance to another glycopeptide antibiotic, teicoplanin (16). No cross resistance, however, was observed with the lipopeptide antibiotic daptomycin.

In the past, there have been sporadic reports of *E. faecium* with relatively high MICs of ampicillin on the basis of diminished penicillin-binding affinity of their penicillin-binding protein (9, 34). High-level penicillin and ampicillin resistance in non-β-lactamase-producing enterococci, however, has still been very unusual. Most of the enterococcal clinical isolates have been susceptible to 4 μg of ampicillin per ml and 8 μg of penicillin G per ml (13, 14, 32).

The emergence of these non-β-lactamase-producing ARE in our institution did not appear to be a point-source outbreak, and there was no strong evidence of person-to-person transmission. The isolation of multiple species of ARE argues against a common-source outbreak. The presence of antibiotic pressures, especially the use of multiple beta-

lactam antibiotics, might have caused the emergence of these ARE. Our observation that 11 patients received a beta-lactam antibiotic shortly before isolation of ARE supports this concept. One of our patients had a CVP catheter tip positive for ampicillin-susceptible *Enterococcus* sp. prior to isolation of ARE. The original isolate, however, was not saved for identification. Emergence of ampicillin resistance in an originally susceptible strain in the face of beta-lactam antibiotic therapy remains a possibility.

There appears to be a trend of increasing incidence of these ARE. Recently, two other institutions have reported the isolation of similar ARE from clinical specimens (4; J. M. Boyce, A. A. Medieros, E. F. Papa, and G. Potter-Bynoe, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1075, 1988). These reports (including ours) are from widely separate parts of the country.

The significance of the isolation of ARE in clinical specimens may be questioned, especially when the enterococcus is part of a polymicrobial flora. However, as seen in this study, ARE was isolated from normally sterile body fluids or sites, including blood, ascitic fluid, and pancreatic tissue. The organism was also isolated in pure culture four times from the urine. We are continuing to monitor infections caused by ARE in our facility.

The possibility that high-level ampicillin resistance in enterococci can become a much more common occurrence is a matter of great concern. Currently, the only established alternative antimicrobial agent for the treatment of infections due to ARE has been vancomycin. If resistance to vancomycin also becomes a problem, our choices would be severely limited. Some possible options include ciprofloxacin (3, 5, 29) and imipenem (5, 7). However, the clinical experience in the treatment of enterococcal infections with these antimicrobial agents is quite limited, and *E. faecium* isolates, unlike *E. faecalis* isolates, are characteristically resistant in vitro to imipenem. Our present report also shows that other non-*E. faecalis* ARE species may also be resistant to imipenem.

Daptomycin, an experimental lipopeptide antibiotic, has shown impressive activity in vitro, as well as in vivo (in experimental animals), against enterococci (20, 28). This antibiotic may be a viable alternative in the treatment of ARE infections in the future, and further studies with this drug are indicated.

It remains to be seen whether these non-β-lactamase-producing ARE are destined to be significant clinical problems nationwide. All of our ARE isolates belonged to species other than *E. faecalis*. It is unclear whether ampicillin resistance in ARE due to mechanisms other than β-lactamase production will remain confined to the non-*E. faecalis* species. We do believe that our experience should prompt other institutions and clinicians to be fully aware of the possible emergence of these organisms in their own institutions. The mechanism(s) of resistance to ampicillin and penicillin in these strains should be elucidated in the future.

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