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Emergence and Nosocomial Transmission of Ampicillin-Resistant Enterococci

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Between 1986 and 1988, the incidence of ampicillin-resistant enterococci increased sevenfold at a university-affiliated hospital. Forty-three patients acquired nosocomial infections with ampicillin-resistant enterococci, most of which were also resistant to mezlocillin, piperacillin, and imipenem. An analysis of plasmid and chromosomal DNAs of isolates revealed that the increase was due to an epidemic of 19 nosocomial infections that yielded closely related strains of *Enterococcus faecium* and to a significant increase in the incidence of nonepidemic, largely unrelated strains of ampicillin-resistant enterococci. The nonepidemic strains were identified as *E. faecium*, *E. raffinosus*, *E. durans*, and *E. gallinarum*. A logistic regression analysis revealed that patients with nonepidemic resistant strains were 16 times more likely than controls to have received preceding therapy with imipenem. In our institution, the increase in the incidence of ampicillin-resistant enterococci appears to be due to the selection of various strains of resistant enterococci by the use of imipenem and to the nosocomial transmission of *E. faecium* and *E. raffinosus*.

Enterococci have become an increasingly important cause of nosocomial infections (10, 14–16, 18, 25, 38, 43). They are now the second most common cause of nosocomial urinary tract and surgical wound infections and are the third most common cause of nosocomial bacteremias (10). Most of these infections are caused by *Enterococcus faecalis*, a species that is usually susceptible to ampicillin, mezlocillin, piperacillin, and imipenem. A few strains of *E. faecalis* produce β-lactamase, rendering them resistant to ampicillin, mezlocillin, and piperacillin (17, 20–22, 24, 29–33), but nosocomial transmission of such strains has occurred infrequently (17, 24, 33).

Other species of enterococci, such as *E. faecium*, may be resistant to imipenem as well as to penicillin, ampicillin, mezlocillin, and piperacillin (11). Such species do not produce β-lactamase and are resistant by virtue of their producing penicillin-binding proteins with a low affinity for beta-lactams (19, 41). Infections caused by these species are much less common than those caused by *E. faecalis*.

In 1987 and 1988, we noted an increase in the frequency of enterococci resistant to penicillin, ampicillin, mezlocillin, piperacillin, and imipenem among patients at a university-affiliated hospital. The increase was due to an epidemic of closely related strains of *E. faecium* which was superimposed on an increasing incidence of nonepidemic, largely unrelated strains of *E. faecium*, *E. raffinosus*, *E. gallinarum*, and *E. durans*. The emergence of nonepidemic strains of ampicillin-resistant enterococci was associated with an increase in the use of imipenem.

MATERIALS AND METHODS

Microbial identification and susceptibility tests. Isolates recovered from clinical specimens were identified as enterococci by the clinical microbiology laboratory at Miriam Hospital on the basis of colony morphology, a positive bile esculin reaction, and growth in 6.5% NaCl broth. The first 24 ampicillin-resistant isolates recovered in 1987 and early 1988 were further identified at the Centers for Disease Control by R. R. Facklam. Twenty-three ampicillin-resistant isolates subsequently recovered during the latter part of 1988 were identified at Miriam Hospital by methods recently described (7).

Standardized disk diffusion antimicrobial susceptibility tests were performed on all enterococci recovered from clinical specimens, and isolates were defined as resistant to ampicillin and penicillin by standard criteria (26). Enterococci categorized as ampicillin resistant or penicillin resistant on the basis of disk diffusion tests were subsequently tested for susceptibility to penicillin and ampicillin by agar dilution methods (27). *E. faecalis* ATCC 29212 was used as a control strain. High-level aminoglycoside resistance was determined by inoculating 10^5 organisms onto Mueller-Hinton agar containing either gentamicin (2,000 μg/ml) or streptomycin (2,000 μg/ml). Isolates were tested for β-lactamase production by placing a heavy suspension of organisms into a microtiter well containing nitrocefin (100 μmol/ml).

Analysis of plasmid and chromosomal DNAs. Plasmid analysis was performed on 46 of the 47 ampicillin-resistant enterococcal isolates by the technique of Anderson and McKay (1). Purified plasmid DNA from 12 *E. faecium* isolates with similar plasmid profiles and from 4 *E. raffinosus* isolates was digested with *Eco*RI restriction endonuclease (New England BioLabs, Beverly, Mass.) in accordance with the manufacturer's recommendations. Restriction fragment analysis of genomic DNA was performed on seven isolates by use of the contour-clamped homogeneous electric field (CHEF) electrophoresis methods previously described by Murray et al. (23).

Epidemiologic investigation. (i) Cases. A case was defined as any patient from whom ampicillin-resistant enterococci were recovered. The hospital record of each case was

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reviewed by an experienced infection control coordinator. Wound infections were defined as the presence of purulent drainage at a surgical site or decubitus ulcer, and urinary tract infections were defined as the presence of pyuria (≥10 leukocytes per high-power field) and $\geq 10^5$ organisms per ml of urine. Infections were defined as nosocomial in origin if they developed 72 h or more after admission. In a few instances, infections present at the time of admission were considered nosocomial if the patient appeared to have acquired the infection as a result of a previous hospitalization during the preceding 2 months. Cases were divided into two major groups (epidemic cases and nonepidemic cases) on the basis of plasmid analysis of ampicillin-resistant enterococcal isolates.

(a) **Epidemic cases.** Nineteen patients with nosocomial infections that yielded strains of ampicillin-resistant *E. faecium* that were sorbitol and sucrose positive and contained plasmids of 66.5, 26.3, 20.1, 4.9, and 3.1 kbp were considered to have closely related epidemic strains of *E. faecium*. For analytic purposes, these 19 patients were considered to represent a single outbreak of *E. faecium*.

(b) **Nonepidemic cases.** An additional 28 patients (24 with nosocomial and 4 with community-acquired infections) with other strains of ampicillin-resistant *E. faecium* and strains of *E. raffinosus*, *E. durans*, and *E. gallinarum* were classified as nonepidemic cases.

(ii) **Control group.** A single control group was used for comparison with epidemic and nonepidemic cases with nosocomial infections. The control group consisted of patients with nosocomial ampicillin-susceptible enterococcal infections. A list of all patients from whom ampicillin-susceptible enterococci were recovered from January 1987 through September 1988 was generated, and a systematic sample of patients (every 25th patient) was selected as controls. Patients who had community-acquired enterococcal infections were excluded from the control group.

(iii) **Chart review.** Charts of epidemic and nonepidemic cases and of controls were reviewed, and the following information was recorded: age, sex, service, ward location at the time of the positive enterococcus culture, all previous ward locations, body sites from which enterococci were recovered, preceding surgical procedures, and preceding antibiotic therapy. Because the epidemic was suspected of being a common-source outbreak, the following additional data were recorded for epidemic cases and controls: preceding exposure to nasogastric and endotracheal tubes, enteral feedings, upper or lower gastrointestinal endoscopy or barium studies, computerized tomographic scans with oral contrast, sucralfate use, and hospital personnel (physicians and nurses who had previously cared for case patients or controls).

Antibiotic use trends. Pharmacy records were reviewed to determine trends in the use of ampicillin, extended-spectrum penicillins (ticarcillin, mezlocillin, piperacillin, and ticarcillin-clavulanic acid), cephalosporins, and imipenem during 1986, 1987, and 1988.

Statistical analyses. We used case-control methods to analyze the relationship between ampicillin-resistant nosocomial infections and suspected risk factors (37). Separate case-control analyses were conducted for the two outbreak groups. For bivariate analyses of dichotomous outcome data, tests of homogeneity of proportions and Fisher's exact tests were performed (8, 37). Continuous variables were compared with the Mann-Whitney U test (42).

Multiple logistic regression was used to assess the independent influence of exposure to selected risk factors on the cumulative incidence of ampicillin-resistant nosocomial infections. Point estimates for exposure to imipenem, ampicillin, and extended-spectrum penicillins, cephalosporins, and an intern (epidemic cases only) were adjusted for age, sex, length of stay prior to development of the nosocomial enterococcal infection, and whether the patient had preceding surgery. Age was approximately normally distributed and was entered as a continuous variable. The distribution of the variable length of stay was negatively skewed. Because the distribution of the length of stay was not normally distributed, the length of stay was entered as a categorical variable: ≤7 days, 8 to 14 days, and 15 or more days. Log likelihood ratio tests were used to determine whether the independent variables and all two-way interaction terms significantly improved the fit of the models (12, 37). All logistic regression analyses were conducted with BMDPLR statistical analysis software (5).

RESULTS

Increasing incidence of ampicillin-resistant enterococci. From April 1986 through December 1988, the number of enterococcal isolates identified by the clinical microbiology laboratory at Miriam Hospital remained relatively stable, with 400 to 450 isolates being recovered from clinical specimens per 6-month period. However, the proportion of enterococci that were found resistant to ampicillin by disk diffusion tests increased from 1% (7 resistant isolates among 623 tested) in 1986 to 1.9% (17 of 891) in 1987 and 7.8% (67 of 853) in 1988 ($P < 0.00000001$). Ampicillin-resistant enterococci were recovered from 9 (0.1%) of 9,650 patients discharged in 1987 but from 44 (0.45%) of 10,270 patients discharged in 1988 ($P = 0.000009$). No changes in susceptibility testing methods occurred during the 3-year period.

Characteristics of ampicillin-resistant enterococci. Ampicillin-resistant enterococcal isolates from 47 of 53 affected patients were available for further testing. Twenty-four ampicillin-resistant isolates from the beginning of the study period were forwarded to the Centers for Disease Control for definitive identification. Identification of subsequent isolates was performed at Miriam Hospital by use of recommended biochemical reactions and growth characteristics (7). Overall, 36 of the 47 isolates were *E. faecium*, 9 were *E. raffinosus*, 1 was *E. durans*, and 1 was *E. gallinarum*. None of the 47 isolates was identified as *E. faecalis*, and none produced β-lactamase.

(i) **Epidemic strains.** *E. faecium* isolates from 12 patients each contained plasmids of 66.5, 26.3, 20.1, 4.9, and 3.1 kbp. These 12 isolates had identical plasmid profiles (Fig. 1) and *Eco*RI restriction endonuclease digestion patterns. *E. faecium* isolates from seven additional patients contained the same 66.5-, 26.3-, 20.1-, 4.9-, and 3.1-kbp plasmids plus an additional 46.8- or 4.6-kbp plasmid (Fig. 1). These seven isolates had restriction endonuclease digestion patterns very similar to those of the other 12 isolates but contained a few extra bands, signifying that additional plasmid DNA was present. Since the 19 isolates all shared five plasmids of the same size and had similar restriction endonuclease digestion patterns, all 19 were considered to be closely related epidemic strains. The likelihood that these 19 strains were closely related was also suggested by the fact that they were more likely than other strains of *E. faecium* to be sorbitol positive (19 of 19 versus 4 of 17; $P = 0.00001$), raffinose positive (9 of 19 versus 1 of 17; $P = 0.008$), and sucrose positive (19 of 19 versus 14 of 17; $P = 0.095$).

Restriction fragment analysis of genomic DNA was per-

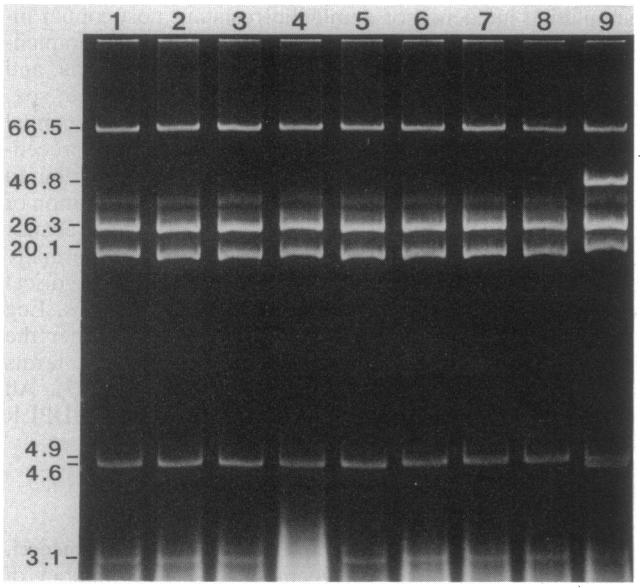

FIG. 1. Agarose gel electrophoresis of plasmid DNA from outbreak strains of *E. faecium*. The molecular sizes of the plasmids are given in the left margin as kilobase pairs. Lanes: 1 to 8, representative isolates with 66.5-, 26.3-, 20.1-, 4.9-, and 3.1-kbp plasmids; 9, isolate with 66.5-, 46.8-, 26.3-, 20.1-, 4.9-, 4.6-, and 3.1-kbp plasmids.

formed on five epidemic strains. Three of the five had the 66.5-, 26.3-, 20.1-, 4.9-, and 3.1-kbp plasmids, one epidemic strain had these five epidemic plasmids plus a 46.8-kbp plasmid, and the remaining epidemic strain had the five epidemic plasmids plus a 4.6-kbp plasmid. All five yielded the same CHEF pattern (Fig. 2).

All 19 epidemic strains of *E. faecium* were found resistant to penicillin, ampicillin, mezlocillin, piperacillin, imipenem, and erythromycin and susceptible to vancomycin by disk

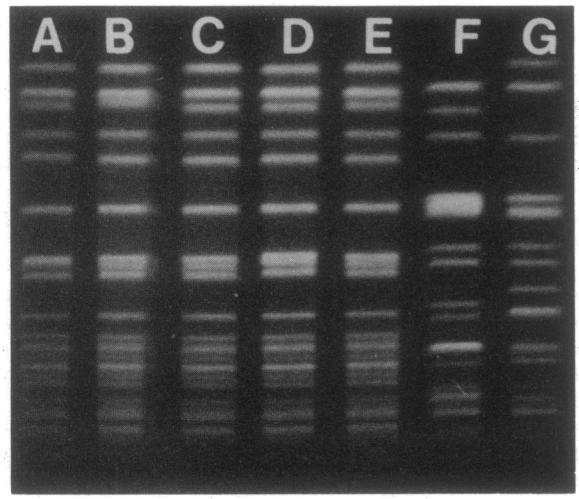

FIG. 2. CHEF electrophoresis of *Sma*I-digested chromosomal DNA from isolates of *E. faecium*. Lanes A through E are epidemic strains containing 66.5-, 26.3-, 20.1-, 4.9-, and 3.1-kbp plasmids. The strain in lane A also contained a 46.8-kbp plasmid, and the strain in lane B also contained a 4.6-kbp plasmid. Lanes F and G are nonepidemic strains of *E. faecium*.

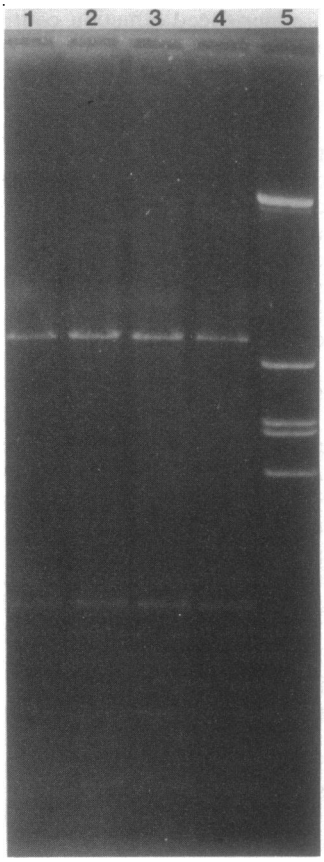

FIG. 3. Gel electrophoresis of *Eco*RI restriction endonuclease digests of four identical isolates of *E. raffinosus* (lanes 1 to 4). Lane 5 contains lambda phage DNA molecular mass standards.

diffusion tests. For all of these strains, penicillin MICs were ≥32 μg/ml and ampicillin MICs were ≥16 μg/ml. All possessed high-level resistance to streptomycin (MIC, ≥2,000 μg/ml) but not to gentamicin.

(ii) Nonepidemic strains. Of the remaining 28 patients, 17 had *E. faecium* isolates, 9 had *E. raffinosus* isolates, and 1 each had *E. gallinarum* and *E. durans* isolates. All 28 isolates had plasmid profiles that differed from those of the *E. faecium* epidemic strains. Two patients had *E. faecium* isolates that contained a single 56-kbp plasmid, and two other patients had isolates that contained plasmids of 36, 4.8, and 4.4 kbp. Four patients developed wound infections that yielded an isolate of *E. raffinosus* containing plasmids of 11.2 and 8.4 kbp. All four *E. raffinosus* isolates had identical restriction endonuclease digestion patterns of plasmid DNA (Fig. 3).

The remaining 20 patients had ampicillin-resistant isolates with the following plasmid contents: 5 *E. raffinosus* isolates, 1 *E. faecium* isolate, and the *E. durans* and *E. gallinarum* isolates were plasmidless, 11 *E. faecium* isolates contained from one to five plasmids ranging in size from 65.4 to 3.4 kbp, and one isolate was not available for testing. Restriction fragment analysis of genomic DNA from two *E. faecium* isolates (one was plasmidless and one contained 63.9- and 40.5-kbp plasmids) yielded CHEF patterns that differed from one another, and neither resembled the CHEF patterns of *E. faecium* epidemic strains (Fig. 2).

All 28 nonepidemic strains were found resistant to peni-

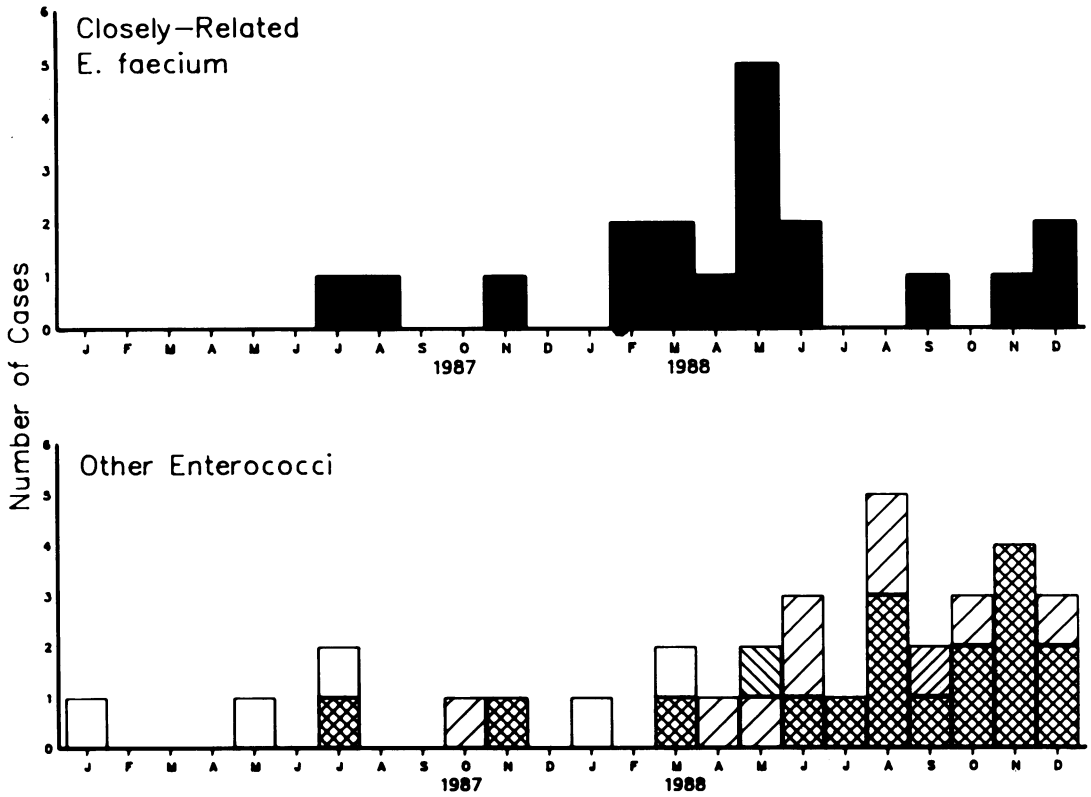

FIG. 4. (Top panel) Nineteen cases caused by closely related strains of *E. faecium*, by date of onset. (Bottom panel) Cases caused by other ampicillin-resistant enterococci, by date of onset. Symbols: □, not identified to the species level; ▩, *E. faecium*; ▨, *E. raffinosus*; ▧, *E. gallinarum*; ▨, *E. durans*.

cillin, ampicillin, and imipenem and susceptible to vancomycin by disk diffusion tests. For all of these strains, penicillin MICs were ≥16 μg/ml (resistant). For 10 of these strains, ampicillin MICs were 4 or 8 μg/ml, and for the remaining 18, ampicillin MICs were 16 to 64 μg/ml. One strain possessed high-level resistance to both gentamicin and streptomycin, 14 possessed high-level resistance to streptomycin, and 13 lacked high-level aminoglycoside resistance.

Cases with epidemic strains. The first recognized case of infection caused by an epidemic strain of *E. faecium* occurred in July 1987, and cases continued to occur through December 1988, with the greatest number of cases occurring in May and June 1988 (Fig. 4). All 19 patients from whom closely related epidemic strains of *E. faecium* were isolated appeared to have acquired the organism in the hospital. Cases occurred on eight wards, but there was a geographic clustering of cases on four wards (4 B, 4 East, 2 West, and intensive care unit), as well as a temporal clustering of cases (Fig. 5).

The characteristics of the 19 patients are shown in Table 1. Two of the 19 patients were bacteremic. One of these patients responded to a 20-day course of vancomycin therapy, and the other patient, who was treated with empiric cefazolin and gentamicin, died before the results of the blood cultures were available. Two patients with suspected intra-abdominal infections had postmortem blood cultures that yielded an epidemic strain. Five patients had urinary tract infections. The clinical significance of the epidemic strains of *E. faecium* recovered from the remaining patients was difficult to assess, since the organism was often recovered in mixed cultures along with other potential pathogens.

The 19 patients involved in the epidemic were hospitalized longer before their first positive culture for enterococci than controls (22 versus 15.9 days; $P = 0.032$) and underwent barium enemas more frequently (5 of 19 versus 1 of 33; $P = 0.02$) than controls (with nosocomial ampicillin-susceptible enterococcal infections). These patients were not significantly different from controls with respect to all other clinical characteristics, previous and current ward locations, exposure to the intensive care unit, diagnostic procedures, and therapeutic modalities examined.

Analysis of personnel to whom the 19 patients were exposed revealed that a greater proportion of the patients (9 of 19, or 47%) than of the controls (6 of 33, or 18%) were exposed to one intern ($P = 0.028$). During the last 5 months of his internship, he saw 9 (82%) of 11 patients hospitalized during this time period but only 4 (27%) of 15 controls ($P = 0.017$). None of the other personnel saw more than five of the patients. Cases associated with the implicated intern were located on six different wards at the time of acquisition of the epidemic strains of ampicillin-resistant enterococci.

The results of the multivariate analyses were similar to those of the bivariate analyses. Patients with epidemic strains of *E. faecium* were significantly more likely to have been exposed to the intern than were controls (odds ratio [OR], 7.26; $P = 0.0461$), after adjustment for age, sex, preceding length of stay, and use of imipenem, ampicillin or extended-spectrum penicillins, and cephalosporins. Furthermore, after adjustment, patients were found to be more likely than controls to have been hospitalized for more than 2 weeks (OR, 28.2; $P = 0.002$) and to have been female (OR, 12.10; $P = 0.013$). The use of imipenem was positively

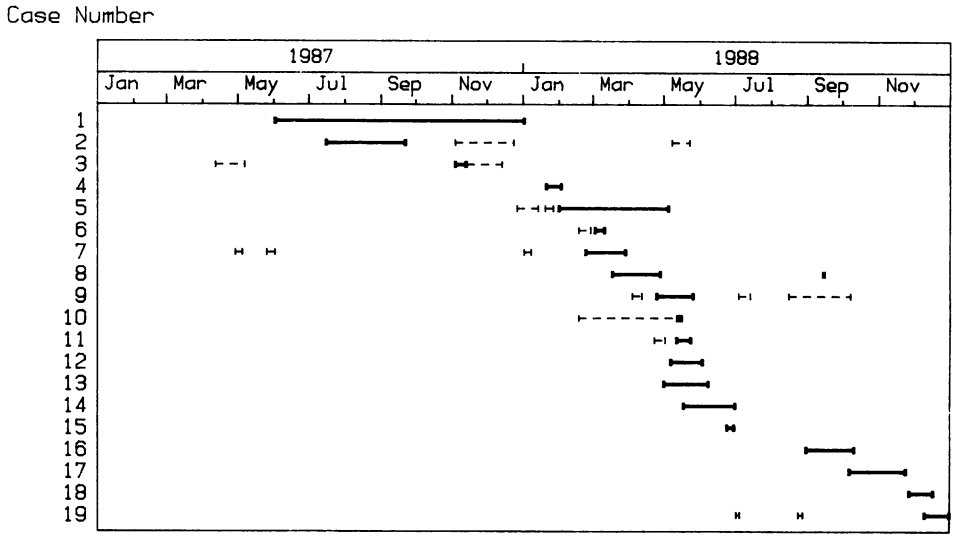

FIG. 5. Dates of hospitalization of 19 patients with outbreak strains of *E. faecium*. Solid lines, hospitalization during which an epidemic strain was isolated; broken line, other hospitalizations.

related to infection, but the association was not statistically significant (OR, 9.8; $P = 0.123$). None of the other independent variables or the two-way interaction terms were statistically significant.

Cases with nonepidemic strains. After excluding the 19 patients with the epidemic strains of *E. faecium*, we compared the incidence of all other ampicillin-resistant enterococci among patients discharged in 1987 and 1988. Resistant strains were recovered from 6 (0.06%) of 9,647 patients discharged in 1987 and from 28 (0.2%) of 10,242 patients discharged in 1988 ($P = 0.0003$).

Twenty-four patients developed nosocomial infections that yielded largely unrelated strains of ampicillin-resistant enterococci. However, four patients developed wound infections that yielded the same strain of *E. raffinosus*. These four patients were clustered in time, and two of the four patients were on the same ward at the same time, suggesting nosocomial transmission.

The characteristics of the 24 patients are given in Table 1. Two patients developed *E. faecium* bacteremia. One of the two patients responded to vancomycin therapy, and the other, who developed transient *E. faecium* bacteremia secondary to an intra-abdominal abscess, subsequently became septic and died despite 10 days of therapy with imipenem. One patient from whom *E. faecium* and other potential pathogens were obtained from a decubitus ulcer developed sepsis and disseminated intravascular coagulation and died despite therapy with imipenem. The cause of death in the latter two patients was not determined. One patient who had persistent growth of *E. raffinosus* and other potential pathogens from an intra-abdominal infection developed septic shock and died despite therapy with imipenem. A postmortem examination revealed findings consistent with sepsis and a myocardial abscess that contained many gram-positive cocci. Postmortem blood cultures yielded only ampicillin- and imipenem-resistant *E. raffinosus*. Seven patients developed nosocomial urinary tract infections. In the remaining patients, ampicillin-resistant enterococci and other potential pathogens were recovered from wound or sputum cultures. All but one of these patients responded to therapy directed against the other pathogens present, suggesting that resistant enterococci were not the primary pathogens.

The bivariate comparisons of the 24 patients with nonepidemic strains and the 33 controls with ampicillin-susceptible enterococci revealed that patients were more likely than controls to have received prior therapy with imipenem (46 versus 6%; $P < 0.001$), to have been hospitalized longer before developing their nosocomial enterococcal infections (26.6 versus 15.9 days; $P = 0.004$), and to have had a longer interval between surgery and their nosocomial enterococcal infections (25.7 versus 10.1 days; $P = 0.007$). To determine whether length of stay was a confounding variable responsible for the association between imipenem use and acquisition of resistant enterococci, we stratified patients and controls into two groups: those with preceding hospital stays of 2 weeks or less and those with preceding stays of more than 2 weeks. The analysis revealed that patients received preceding imipenem therapy significantly more often than controls (stratified Mantel-Haenszel chi-square test; $P = 0.008$). Cases were located on eight wards, and controls were located on nine wards. There were no significant differences between patients and controls with respect to age, sex, service, preceding surgery, body sites positive for enterococci, previous or current ward location, exposure to the intensive care unit, or preceding cephalosporin therapy. There was no significant difference in the frequency of exposure of patients (21%) and controls (18%) to the intern associated with epidemic cases.

The results of the multivariate analyses were again consistent with those of the bivariate analyses. Patients with ampicillin-resistant enterococci were 16 times more likely to have received preceding imipenem therapy than controls (OR, 16.21; $P = 0.008$), after adjustment for age, sex, preceding length of stay, and use of ampicillin or extended-spectrum penicillins and cephalosporins. In addition, patients were nearly seven times more likely than controls to have been female (OR, 6.58; $P = 0.03$) and nearly 20 times more likely to have been hospitalized for more than 2 weeks before developing their nosocomial enterococcal infections (OR, 19.84; $P = 0.0025$). None of the other independent variables or the two-way interaction terms were statistically significant.

The remaining four patients were admitted from nursing homes or long-term care facilities with decubitus or foot

TABLE 1. Characteristics of patients with nosocomial infections yielding ampicillin-resistant enterococci

Characteristic[a]	Ampicillin-resistant enterococci		Ampicillin-susceptible enterococci (n = 33)
	E. faecium outbreak strains (n = 19)	Other strains (n = 24)	
Sex			
Males	7	10	19
Females	12	14	14
Age (yr)			
Mean	72.6	66.2	70.5
Range	35–92	27–92	28–88
Service			
Surgery	14	16	23
Medicine	5	8	10
Body site			
Blood	4	2	1
Wound	7	11	11
Urine	5	7	15
Other	3	4	6
Preceding surgery			
Gastric	5	0	6
Small bowel	7	3	8
Colon	5	4	6
Biliary tract only	0	0	5
Vascular	3	2	4
Other	3	9	12
None	3	8	9
Preceding antibiotics			
Cephalosporins			
Narrow spectrum	6	7	8
Extended spectrum	8	15	19
Broad spectrum	3	4	2
Ampicillin or extended-spectrum penicillins	7	7	7
Imipenem	4	11[b]	2[b]
Time interval (days)			
Surgery to first enterococcus isolation			
Mean	14.2	25.7[c]	10.1[c]
Range	4–34	4–78	3–39
Admission to first enterococcus isolation			
Mean	22.0[d]	26.6[e]	15.9[d,e]
Range	5–87	5–87	4–97

[a] Unless otherwise indicated, data are reported as number of patients.
[b] $P = 0.0004$.
[c] $P = 0.007$.
[d] $P = 0.032$.
[e] $P = 0.004$.

ulcers (three patients) or pneumonia (one patient) that yielded ampicillin-resistant enterococci plus other potential pathogens. Three infections yielded *E. raffinosus*, and one yielded *E. faecium*.

Antibiotic use trends. From January 1986 through December 1988, there was a progressive increase in the use of ampicillin or the extended-spectrum penicillins and of imipenem. The number of grams of ampicillin or extended-spectrum penicillins used increased from 28,400 in 1986 to 41,973 in 1988. Almost all of the increase was due to increased use of mezlocillin and piperacillin. The number of grams of imipenem used increased fivefold from 502 in 1986 to 2,357 in 1988. The number of grams of cephalosporins used increased from 51,113 in 1986 to 58,473 in 1987 but did not increase in 1988.

DISCUSSION

In 1982, the first nosocomial outbreak of ampicillin-resistant *E. faecium* infections was reported (4). More recently, an outbreak of ampicillin- and vancomycin-resistant *E. faecium* infections occurred in England (39, 40). These two epidemics represent the only previously reported nosocomial outbreaks of *E. faecium* infections.

Since 1988, several reports have described the recovery of strains of penicillin- or ampicillin-resistant enterococci other than *E. faecalis* from hospitalized patients (2, 3, 6, 9, 13, 28, 35, 36). Most strains were *E. faecium*, but a few were identified as *E. raffinosus* (3, 9, 28, 36). Although a number of the patients were described as having nosocomial infections (3, 28, 35, 36), in only one institution was nosocomial transmission documented (3). Only one report documented a statistically significant increase in the incidence of resistant *E. faecium* (9).

Our investigation documented that the incidence of ampicillin-resistant enterococci among hospitalized patients increased sevenfold over a period of 2.75 years ($P < 0.00000001$). None of the resistant strains produced a detectable β-lactamase, and none were *E. faecalis*. The strains were identified as *E. faecium* (77%), *E. raffinosus* (19%), and *E. durans* and *E. gallinarum* (2% each). There was no concomitant increase in the incidence of ampicillin-susceptible enterococci isolated from clinical specimens.

The emergence of ampicillin-resistant enterococci at our institution was due to an epidemic of nosocomial infections yielding closely related strains of *E. faecium* which was superimposed on an increase in the incidence of other, largely unrelated strains of ampicillin-resistant *E. faecium*, *E. raffinosus*, *E. gallinarum*, and *E. durans*. Although temporal and geographic clustering of cases provided strong evidence that the epidemic was due to nosocomial transmission of closely related strains of ampicillin-resistant *E. faecium*, restriction endonuclease digestion of plasmid and chromosomal DNAs of *E. faecium* isolates and plasmid analysis of *E. raffinosus* isolates were indispensable in establishing the relatedness of these organisms. Neither antimicrobial susceptibility testing nor biotyping of isolates could distinguish outbreak strains from other strains of *E. faecium* or *E. raffinosus*.

The epidemic may have been due in part to the spread of closely related strains by a house officer, who cared for a significantly larger proportion of case patients than controls. However, the implicated intern identified during our investigation moved to another hospital before the results of the case-control study were available and, as a result, microbiologic confirmation that he served as a common source is lacking. Other investigators have found that health care workers may become transiently colonized with epidemic strains of *E. faecalis* (4, 33, 44). We cannot exclude the possibility that some other common source existed or that closely related strains were transmitted on the hands of several medical personnel.

A majority of nonepidemic cases yielded unrelated strains of *E. faecium*. However, we documented that four patients had wound infections that yielded a single strain of *E. raffinosus*. Temporal and geographic clustering of cases

provided additional evidence that nosocomial transmission had occurred. Although a few reports of the recovery of *E. raffinosus* from hospitalized patients have been published (3, 28, 34, 36), only one other report has provided epidemiologic evidence of the nosocomial spread of this newly described *Enterococcus* species (3).

Increasing imipenem use appears to have contributed to the emergence of genetically unrelated strains of ampicillin-resistant enterococci in our hospital. Imipenem use increased nearly fivefold during the study period, and a logistic regression analysis revealed that patients with nonepidemic strains were 16 times more likely to have received preceding imipenem therapy than patients who had infections yielding ampicillin-susceptible enterococci. The association between preceding imipenem therapy and acquisition of ampicillin-resistant enterococci was independent of preceding length of stay and could not be attributed to the clustering of affected patients by service, ward, or intensive care unit, in which imipenem might have been used frequently. Since this report is the first to describe an association between imipenem use and the emergence of ampicillin-resistant enterococci, further prospective studies designed to assess the incidence of colonization by resistant enterococci among patients receiving imipenem and appropriate controls are needed to establish the importance of imipenem as a risk factor for the nosocomial acquisition of such multiply resistant strains.

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