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Outbreak of Multidrug-Resistant *Enterococcus faecium* with Transferable *vanB* Class Vancomycin Resistance

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Enterococcus faecium **strains resistant to ampicillin, high levels of gentamicin, and vancomycin but susceptible to teicoplanin (*vanB* class vancomycin resistance) were recovered from 37 patients during an outbreak involving a 250-bed university-affiliated hospital. Three isolates with vancomycin MICs ranging from 8 to 256 μg/ml all hybridized with a *vanB* probe. Restriction endonuclease analysis of chromosomal and plasmid DNA suggested that all isolates tested were derived from a single clone. Vancomycin resistance was shown to be transferable. Risk factors for acquiring the epidemic strain included proximity to another case patient (*P*, 0.0005) and exposure to a nurse who cared for another case patient (*P*, 0.007). Contamination of the environment by the epidemic strain occurred significantly more often when case patients had diarrhea (*P*, 0.001). Placing patients in private rooms and requiring the use of gowns as well as gloves by personnel controlled the outbreak. These findings suggest that multidrug-resistant *E. faecium* strains with transferable *vanB* class vancomycin resistance will emerge as important nosocomial pathogens. Because extensive environmental contamination may occur when affected patients develop diarrhea, barrier precautions, including the use of both gowns and gloves, should be implemented as soon as these pathogens are encountered.**

Until recently, most enterococci responsible for nosocomial infections were susceptible to ampicillin and vancomycin, which are considered the drugs of choice for treating serious enterococcal infections. However, strains of enterococci resistant to ampicillin (3, 14, 24, 26, 29, 31) or vancomycin (15, 20, 34, 39) have been reported with increasing frequency. Vancomycin-resistant enterococci have been categorized as VanA, VanB, or VanC (35). *Enterococcus faecalis* and *Enterococcus faecium* strains with *vanA* resistance characteristically have high-level, inducible resistance to both vancomycin and teicoplanin (20). Recent studies have demonstrated that the *vanA* gene cluster is carried on a transposon (2). Prototype *vanB* strains possess moderate to high-level resistance to vancomycin but are susceptible to teicoplanin (43). Rare *vanB* type strains that are resistant to teicoplanin have been reported (17). *vanB* resistance has been felt to be chromosomally mediated (8, 33). *vanC* resistance is associated with low-level vancomycin resistance and has been observed in *Enterococcus gallinarum* and *Enterococcus casseliflavus* (40).

Outbreaks of *vanA* class vancomycin-resistant *E. faecalis* or *E. faecium* have been reported in London, New York, and Philadelphia (4, 11, 16, 18, 21, 32, 39). In 1992, we noted a sudden increase in the number of patients with infections caused by enterococci resistant to ampicillin, gentamicin, and vancomycin. Isolates were also resistant to penicillin, ampicillin-sulbactam, mezlocillin, piperacillin, and imipenem. Because the organisms were resistant to all licensed antibiotics normally used for treatment of serious enterococcal infections, we instituted an investigation and implemented special infection control measures. Our investigation revealed that the outbreak was due to *E. faecium* with transferable *vanB* class vancomycin resistance. We found that the presence of diarrhea among affected patients resulted in significantly greater contamination of environmental surfaces by the epidemic strain. Such contaminated surfaces may have served as a reservoir for the epidemic strain.

MATERIALS AND METHODS

Microbial identification and susceptibility tests. Isolates recovered from clinical specimens were identified as enterococci by using established methods (10). 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 using standard criteria (27). Enterococcal isolates with vancomycin zones of inhibition of ≤14 mm in diameter were categorized as resistant (36, 37). Vancomycin and teicoplanin MICs were determined for isolates from 14 patients by using standard agar dilution methods (28). High-level aminoglycoside resistance was determined by inoculating 10^5 organisms onto Mueller-Hinton agar containing gentamicin (500 and 2,000 μg/ml) and 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 DNA. Contour-clamped homogeneous electric field electrophoresis of *Apa*I and *Sma*I restriction endonuclease digests of genomic DNA was performed on 10 outbreak isolates and several epidemiologically unrelated isolates from other hospitals by using methods described previously (7, 25). Plasmid analysis was performed on 31 vancomycin-resistant isolates (15 clinical and 16 environmental isolates) and on 6 vancomycin-susceptible

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isolates by using the technique of Anderson and McKay (1). Purified plasmid DNA from 17 isolates with the same plasmid profile was digested with *Eco*RI restriction endonuclease (New England Biolabs, Beverly, Mass.) as described in the manufacturer's recommendations, and agarose electrophoresis was performed by using standard methods (22).

Resistance transfer experiments were performed by filter mating as described previously (24). A vancomycin-resistant *E. faecium* strain was mated with rifampin- and nalidixic acid-resistant plasmid-free strains of *E. faecalis* and *E. faecium* by using 0.45-μm-pore-size nitrocellulose membrane filters (Nalge Company, Rochester, N.Y.). The mating mixture was plated on Mueller-Hinton agar containing 10 μg of vancomycin per ml, 50 μg of rifampin per ml, and 50 μg of nalidixic acid per ml. Transconjugants were restreaked on the counterselective medium and then further characterized by susceptibility testing and plasmid analysis.

Epidemiologic investigation. (i) Case patient definition. A case patient was defined as any patient from whom vancomycin-resistant enterococci were recovered. The hospital record of each case patient was reviewed by an experienced infection control coordinator, and standardized criteria were used for defining nosocomial infections (12). A case of vancomycin-resistant enterococcus infection was defined as hospital acquired if the patient's first positive culture for vancomycin-resistant enterococci occurred 72 h or more after admission. Two patients discharged from the hospital during the outbreak period were positive for vancomycin-resistant enterococci at the time of readmission. These two infections were also considered to be nosocomial in origin.

(ii) Chart review. Charts of case patients were reviewed, and the following information was recorded: age, sex, service, ward location at the time of the first culture positive for vancomycin-resistant enterococcus, all previous ward locations, body sites from which enterococci were recovered, preceding surgical procedures, and preceding antibiotic therapy.

(iii) Case-control study. To determine risk factors associated with acquisition of vancomycin-resistant enterococci in the intensive care unit, where the outbreak was centered, patients present in the unit during the period January through June 1992 served as potential case or control patients. Sixteen patients who were present in the intensive care unit at the time of their first culture positive for vancomycin-resistant enterococcus (12 patients) or who were in the unit during the month before their first positive culture (4 patients) were defined as case patients. Each case patient was matched with a control patient of similar age (±5 years), sex, and length of stay in the intensive care unit. The following data were obtained from the charts of case patients and matched controls: age, sex, service, length of stay in the unit before acquiring vancomycin-resistant enterococci (total intensive care unit stay for matched controls), and previous exposure to antibiotics, antacids or H_2 blockers, orogastric or nasogastric tubes, enteral feedings, endoscopy, rectal tube, or rectal thermister probe. Proximity to a known case patient was expressed by a proximity score as follows: a score of 1.0 if in a bed adjacent to a known case patient, a score of 10 if 10 beds removed from a known case patient, and a score of 11 if no known case patient was in the unit prior to the individual's first positive culture.

Intensive care unit nursing assignment records were used to determine how many times (number of nursing shifts) prior to acquiring vancomycin-resistant enterococcus the case or control patients had been cared for by a nurse who was assigned on the same shift to a known case patient. For case patients, this analysis included all days in the unit prior to the case patient's first positive culture; for controls, the analysis was performed for all days in the unit.

Patient and environmental culture surveys. On two occasions in April and once in July 1992, perirectal swab cultures were obtained from all patients present in the intensive care unit and screened for the presence of vancomycin-resistant enterococci. By using premoistened swabs, environmental cultures were obtained on 10 occasions from patient gowns, bed linens and side rails, overbed tables, intravenous pump buttons, stethoscopes, door handles, floors, and other items in the rooms of four patients who were currently colonized or infected with vancomycin-resistant enterococci. To assess the adequacy of routine housekeeping practices, similar cultures were obtained in three rooms that had been cleaned with a quaternary ammonium compound following the discharge of affected patients. Swabs were plated directly onto neomycin blood agar and inoculated into tryptic soy broth, which was incubated for 24 h at 35°C, and then plated onto neomycin blood agar.

Statistical analyses. Dichotomous variables for unpaired groups were compared by using Mantel-Haenzael chi-square tests; for paired groups, McNemar's test was used (44). Continuous variables for paired groups were compared by using the signed rank test (44).

RESULTS

Characteristics of vancomycin-resistant enterococci. Vancomycin-resistant enterococci were recovered from 37 patients in the period June 1991 through December 1992. All enterococci resistant to penicillin, ampicillin, and vancomycin were identified as *E. faecium*. All were resistant to 500 μg of gentamicin per ml. None produced β-lactamase. Vancomycin MICs ranged from 8 to 256 μg/ml, but all teicoplanin MICs were ≤2 μg/ml.

Whole-plasmid analysis performed on 15 clinical isolates and 16 environmental isolates of vancomycin-resistant *E. faecium* revealed that all isolates contained a common 60-kb plasmid. Electrophoresis of *Eco*RI restriction endonuclease digests of plasmid DNA from 11 clinical isolates and 6 environmental isolates revealed that all possessed identical restriction fragment patterns. Vancomycin-susceptible *E. faecalis* and *E. faecium* isolates had different whole-plasmid profiles and restriction fragment patterns.

Contour-clamped homogeneous electric field electrophoresis of restriction endonuclease digests of genomic DNA performed on 10 of the 15 clinical isolates shown to have the 60-kb plasmid revealed that all yielded similar restriction fragment patterns, with only one or two band shifts occurring with several isolates (Fig. 1). On the basis of previous studies (7, 23), such a high degree of similarity among *E. faecium* strains suggests that all isolates were derived from the same clone (epidemic strain). Restriction fragment patterns of epidemiologically unrelated glycopeptide-resistant isolates from other hospitals (Fig. 1) and vancomycin-susceptible *E. faecalis* and *E. faecium* isolates differed from the epidemic strain by numerous band shifts.

Filter mating experiments revealed that transfer of the 60-kb plasmid from a patient isolate to recipient strains of *E. faecium* and *E. faecalis* occurred at a frequency of 1.0×10^{-7} and was associated with transfer of vancomycin resistance to recipient strains.

Three isolates with vancomycin MICs ranging from 8 to 256 μg/ml were forwarded to Patrice Courvalin and colleagues, who demonstrated that all three isolates hybridized with a *vanB* probe (6a, 9, 30).

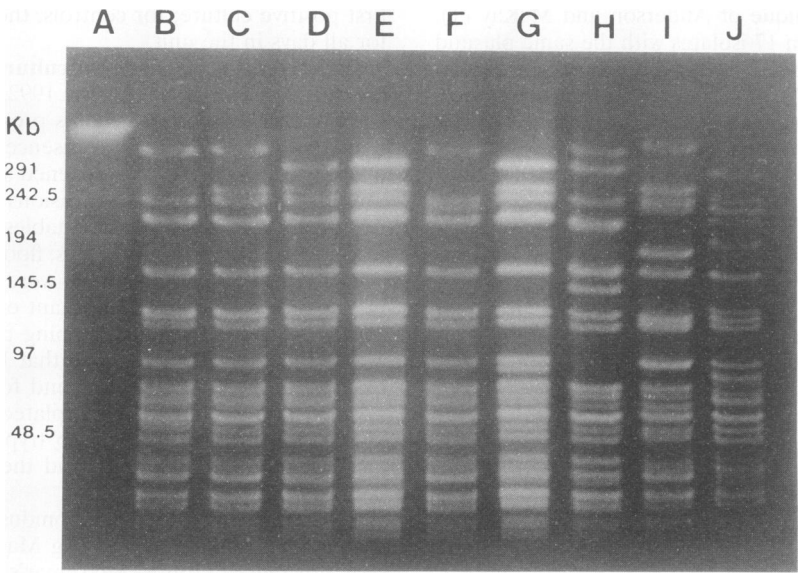

FIG. 1. Contour-clamped homogeneous electric field electrophoresis of *Sma*I digests of chromosomal DNA from clinical isolates of vancomycin-resistant *E. faecium*. Lanes: A, bacteriophage lambda ladder molecular mass standard; B to G, outbreak isolates recovered from patients at Miriam Hospital; H to J, epidemiologically unrelated isolates from geographically separate hospitals.

Case patients with epidemic strains. All 37 patients from whom the epidemic strain was isolated acquired the organism in the hospital. The patients ranged in age from 27 to 96 years old and had been hospitalized for 3 to 92 days (median, 18 days) before their first positive culture for vancomycin-resistant enterococci. The average length of stay for unaffected patients in 1992 was 6.9 days. Fifteen patients developed infections due to the epidemic strain: six patients had bacteremia, six had urinary tract infections, two had wound infections, and one had pneumonia with concomitant bacteremia.

Epidemiologic analysis. The index case patient was found to have vancomycin-resistant enterococcus in June 1991, 3 months after being transferred from a hospital in Boston. Six additional cases of vancomycin-resistant enterococcus infection or colonization occurred during the following 5 months, and then the number of cases increased significantly (Fig. 2).

Case patients were on the following clinical services: medicine (38%), surgery (32%), cardiovascular (14%), vascular (8%), and other services (8%). Cases of vancomycin-resistant enterococcus occurred on 8 of the 10 hospital wards. There was temporal and geographic clustering of cases in the intensive care unit and to a lesser extent on two general medical wards. At the time of their first positive culture for vancomycin-resistant enterococcus, 35% of the case patients were located in the intensive care unit, 19% were on ward 4B, 16% were on ward 3W, and the remaining case patients were located on other wards. Eight of the 24 case patients located on wards other than the intensive care unit at the time of their first positive culture had previous exposure to the intensive care unit. Because the outbreak was centered in the intensive care unit, the remaining investigation focused on case patients associated with the unit.

The first case in the intensive care unit occurred in October 1991. No further cases occurred in the unit until January 1992, when a patient developed bacteremia due to the epidemic strain. During the next 5.5 months, the epidemic strain was recovered from 15 additional patients exposed to the unit (Fig. 3). In addition, one patient (Fig. 3, case 6), who was cared for by intensive care unit physicians in the subacute care ward, acquired the epidemic strain while awaiting transfer to the unit.

Diarrhea (i.e., four or more loose stools per day, episodes of watery stools for more than 12 h, or presence of a rectal tube) occurred in nine intensive care unit-associated case patients shortly before (one patient) or after (eight patients) their first positive culture for vancomycin-resistant enterococci. Two patients developed *Clostridium difficile*-associated diarrhea after acquiring vancomycin-resistant enterococci. Another patient had *C. difficile*-associated diarrhea 2 weeks before she was found to have vancomycin-resistant enterococcus.

Case-control study. Of the 16 case patients who had previous exposure to the intensive care unit between 1 January and 30 June, appropriate control patients matched by age, sex, and

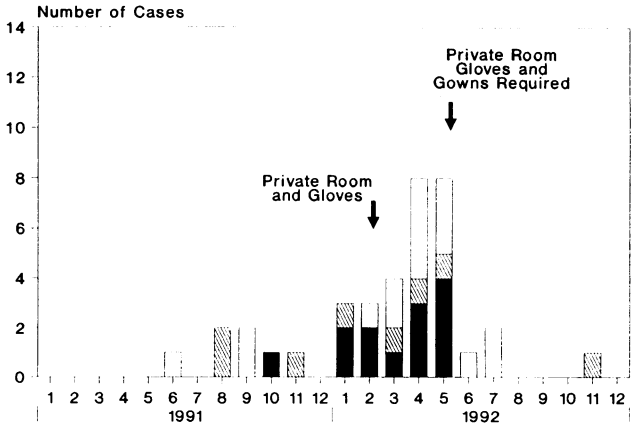

FIG. 2. Vancomycin-resistant *E. faecium* cases, by date of first positive culture for the epidemic strain (from January 1991 to December 1992). Symbols: ■, case patients in the intensive care unit at the time of the first positive culture for the epidemic strain; ▨, other case patients with previous exposure to the intensive care unit; □, case patients never in the intensive care unit.

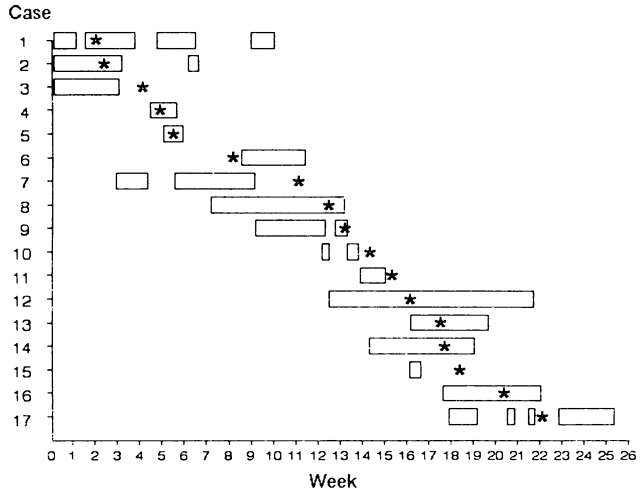

FIG. 3. Dates of stay in the intensive care unit for patients classified as intensive care unit-associated case patients, by week, from January through June 1992. An asterisk indicates the date of first positive culture for the epidemic strain. Case patient 6 was cared for by intensive care unit physicians in the subacute care unit while awaiting transfer to the intensive care unit.

length of stay in the unit were available for 12 of the case patients. Comparison of 12 intensive care unit-associated case patients with matched control patients revealed that there were no significant differences between the two groups with respect to median age (76 and 80 years, respectively), admission APACHE score (10.5 and 11.5, respectively), and length of previous intensive care unit stay (18.5 and 18 days, respectively). Case patients had received vancomycin more frequently than controls (odds ratio, 6.0; 95% confidence interval, 0.78 to 51.8), but the difference was not statistically significant. Proximity scores for case patients (median, 1.5) and controls (median, 8) revealed that case patients were more likely than controls to have been in a bed near a known case patient (*P*, 0.0005). In addition, exposure to a nurse who cared on the same shift for another known case patient occurred more frequently with case patients (median, two exposures) than with controls (median, zero exposures; *P*, 0.007).

Prevalence surveys and environmental cultures. Point prevalence culture surveys conducted on three occasions in the intensive care unit identified three patients with vancomycin-resistant enterococci that had not been detected by routine clinical cultures. Twenty-six (28%) of 92 environmental cultures performed in rooms of four affected patients yielded the epidemic strain. The epidemic strain was recovered much more frequently from environmental surfaces in rooms of patients with diarrhea (18 of 39 cultures [46%]) than in the rooms of affected patients without diarrhea (8 of 53 [15%]; odds ratio, 4.8; 95% confidence interval, 1.6 to 14.5; *P*, 0.001). When affected patients had no diarrhea, nearly all environmental isolates were obtained from patient gowns, bed linens, or bed side rails. In contrast, when affected patients had diarrhea, the epidemic strain was also isolated from intravenous pumps, electrocardiogram monitors, overbed tables, floors, and a blood pressure cuff, pulse-oximeter coupling, stethoscope, and bathroom door.

Environmental cultures performed after affected patients had been discharged from their rooms and the rooms had been cleaned were negative on all but one occasion. A tourniquet left in a patient's room yielded the epidemic strain when cultured 4 days after the affected patient had been discharged from the room.

Control measures. Initially, control measures included placing all patients colonized or infected with vancomycin-resistant enterococci in a private room and requiring the use of gloves by all health care workers entering the patient's room. However, these measures were ineffective, and additional cases of infection or colonization continued to occur (Fig. 2). When we established that many environmental surfaces were contaminated by the epidemic strain, personnel were required to wear gowns as well as gloves whenever entering an affected patient's room. Shortly after implementing the use of both gowns and gloves, the outbreak terminated (Fig. 2).

DISCUSSION

To date, all reported outbreaks of colonization or infection due to vancomycin-resistant *E. faecalis* or *E. faecium* wherein the class of resistance had been established have been due to *vanA* strains (16, 18, 21, 32, 39). One of the unique features of the outbreak we investigated was that it was due to multidrug-resistant *E. faecium* with transferable *vanB* class resistance to vancomycin. Early reports describing *vanB* class resistance suggested that the resistance is chromosomally determined (8, 33). Of interest, our findings confirmed two recent reports that the determinant responsible for *vanB* class vancomycin resistance in some strains is self-transferable to other enterococci (13, 30). The fact that transfer of vancomycin resistance may occur at the time of plasmid transfer (as we demonstrated) or without acquisition of plasmid DNA suggests that transfer of vancomycin resistance in *vanB* strains may occur via conjugative transposons (13). This fact and the demonstration that rapid spread of the organism may occur among hospitalized patients suggest that enterococci with *vanB* class vancomycin resistance may emerge rapidly as nosocomial pathogens.

Initially, the wide range of vancomycin MICs observed suggested that the outbreak was due to several different strains. However, the results of restriction endonuclease analysis of chromosomal and plasmid DNA suggest that the outbreak was due to nosocomial transmission of closely related strains derived from a single clone. Our findings are consistent with the recent report by Quintiliani et al. (30) demonstrating that widely ranging levels of vancomycin resistance may occur among enterococci with *vanB* class resistance. The fact that some *vanB* strains manifest relatively low levels of resistance (vancomycin MICs ranging from 8 to 32 μg/ml) is of concern because such strains may not be correctly identified as vancomycin resistant by automated susceptibility testing systems (38, 42). As a result, the prevalence of *vanB* strains may be underestimated in hospitals that use automated susceptibility testing methods (38). Fortunately, such strains are detected accurately by agar screening plates or by disk diffusion tests if revised zone size criteria are utilized (36, 42).

Risk factors that have been associated with acquisition of vancomycin-resistant enterococci include preceding vancomycin therapy, prolonged hospitalization, and proximity to a patient with a known infection (3–5, 16, 18, 32, 45). In the present outbreak, preceding vancomycin therapy was more common among case patients than controls, but the difference did not achieve statistical significance. We found that prolonged hospitalization, proximity to a known case patient, and exposure to a nurse who was assigned on the same shift to another known case patient were associated with acquiring the epidemic strain. The fact that patients were more likely to acquire the epidemic strain if they were cared for by a nurse who was assigned on the same shift to another known case

patient suggests that the organism was transmitted from patient to patient by nursing personnel.

On the basis of the assumption that the organism was being transmitted on the hands of personnel, patients were placed in private rooms and all individuals entering an affected patient's room were required to wear gloves. When this approach failed, we became concerned that environmental contamination may be occurring and might contribute to transmission. Subsequent environmental cultures revealed that there was considerable contamination of inanimate objects by the epidemic strain when affected patients had diarrhea, a situation not unlike that seen with *C. difficile*-associated diarrhea patients (19). The ability of enterococci to remain viable in the environment, as demonstrated by ourselves and others (6, 21, 31, 41, 46), suggests that contaminated environmental surfaces may serve as a reservoir for resistant enterococci. The presence or absence of diarrhea among affected patients may explain why strains of resistant enterococci recovered from patients have been found on environmental surfaces in some outbreaks (18, 21, 31, 41, 45) but not in others (5, 6, 16, 32, 39, 46). Perhaps the environmental contamination that occurred during the present outbreak explains why the use of both gowns and gloves controlled the outbreak, whereas gloves alone did not. Alternatively, perhaps gloves were not being changed appropriately between patients, and requiring gowns as well as gloves led to better compliance with glove use. Further studies are needed to establish the importance of the environmental contamination in the transmission of enterococci.

After the outbreak was controlled, two case patients were readmitted, and surveillance cultures revealed that one patient was still colonized with the epidemic strain 12 months after acquiring the organism. As a result, whenever known case patients are readmitted, special precautions are implemented and cultures are performed to determine whether the individual is still colonized. No further outbreaks have occurred, but continued surveillance and control measures are warranted since multidrug-resistant *E. faecium* appears to be emerging as a significant nosocomial pathogen.

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