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Clonal Spread of Vancomycin-Resistant *Enterococcus faecium* between Patients in Three Hospitals in Two States

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The DNAs of 38 vancomycin-resistant *Enterococcus faecium* isolates from five hospitals in three states were analyzed by contour-clamped homogeneous electric field electrophoresis and plasmid analysis. There were 22 strain types. One strain type was common to patients in three hospitals in two states. These results suggest the apparent intra- and interhospital spread of vancomycin-resistant *E. faecium*.

In the past decade there has been a rapid rise in the number of serious nosocomial infections caused by enterococci resistant to multiple antibiotics. Enterococci with high-level gentamicin resistance (MIC, >2,000 μg/ml) became a major concern beginning in the 1980s, when both the intrahospital and the interhospital spread of this resistance was seen in nursing homes and acute-care hospitals (32–35). Subsequently, ampicillin-resistant enterococci, including both β-lactamase-producing and non-β-lactamase-producing strains, have been isolated in many hospitals (4, 5, 12, 20, 25, 26). Moreover, Murray and colleagues (23) found evidence for the clonal spread of a β-lactamase-producing *Enterococcus faecalis* strain among six hospitals in five states. In recent years, glycopeptide-resistant enterococci have been reported in both European and North American hospitals (2, 13, 15–19, 27, 28, 31). To help determine the basis for the spread of this resistance trait in hospitals in the midwestern United States, we analyzed the DNA contents of vancomycin-resistant *Enterococcus faecium* clinical isolates from five hospitals in three states.

Thirty-eight clinical isolates of enterococci collected from separate patients during 1990 to 1992 were studied. Isolates were from two hospitals in Chicago, Ill. (hospital A, 12 isolates; hospital B, 9 isolates) (13), two hospitals in Detroit, Mich. (hospital C, 4 isolates; hospital D, 7 isolates), and one hospital in Pittsburgh, Pa. (hospital E, 6 isolates). Isolates were from patients who were both epidemiologically related and unrelated in time and geographic location. The patients from hospitals A and B were hospitalized during approximately an 18-month period from 1990 to 1991. The patients from hospital C were hospitalized in February 1992. The patients from hospital D were hospitalized within 3 months of each other in the beginning of 1992. Patients from hospital E were hospitalized during the first 6 months of 1992. Duplicate isolates from the same patient were excluded from the study. Study isolates were also compared with control strains. Control strains were 40 vancomycin-susceptible clinical isolates of *E. faecium*. Isolates were identified to the species level by using the biochemical reactions described by Facklam and Collins (10). Antibiotic susceptibility was determined by a standard microdilution method (24). All antibiotics except vancomycin (Eli Lilly & Co., Indianapolis, Ind.) and teicoplanin (Marion Merrell Dow Inc., Cincinnati, Ohio) were obtained from Sigma Chemical Company (St. Louis, Mo.). Plasmid DNA was prepared as described previously (8, 9, 14) and was digested with *Eco*RI according to the instructions of the manufacturer (BRL Life Technologies, Inc., Gaithersburg, Md.). Genomic DNA for contour-clamped homogeneous electric field (CHEF) electrophoresis was prepared by modifying a previously described procedure (9, 22, 29) and was digested with the restriction endonucleases *Sma*I or *Apa*I. Genomic DNA was electrophoresed in a 0.8% agarose gel in 0.5× TBE buffer (45 mM Tris, 45 mM boric acid, 1 mM EDTA) by using a CHEF DRII apparatus (Bio-Rad) (initial switch time, 1 s; final switch time, 20 s; start ratio, 1; voltage, 200; run time, 21 h; temperature, 4°C) and was stained with ethidium bromide. Strains were differentiated by visual inspection of patterns on the agarose gels. Isolates were considered identical if their CHEF electrophoresis patterns were similar or the same by both *Sma*I and *Apa*I digestions on separate gels. Isolates were considered different if their CHEF electrophoresis patterns differed by more than one band. The transferability of resistance markers was determined by using cross-streak and filter mating experiments as described previously (1, 11). *E. faecium* FA2-2 (7) and JH2-2 (30) and *E. faecium* 9790RF (6) were used as enterococcal recipients.

All isolates were *E. faecium*. The MICs of vancomycin for isolates ranged from 16 to 512 μg/ml. The MICs of ampicillin, rifampin, teicoplanin, and tetracycline for 90% of isolates tested were 64, 64, 0.25, and 32 μg/ml, respectively. Vancomycin resistance was not transferable by cross-streaking or filter matings. Isolates contained zero to five plasmids. CHEF electrophoresis performed on the 38 isolates identified 22 total strain types. In hospital A, there were eight strain types among the 12 isolates; one strain type was identical between three patients, a second strain type was identical between two patients, and a third strain type was identical between two other patients. In hospital B, there were five strain types among the nine isolates; one strain type was identical between four patients and a second

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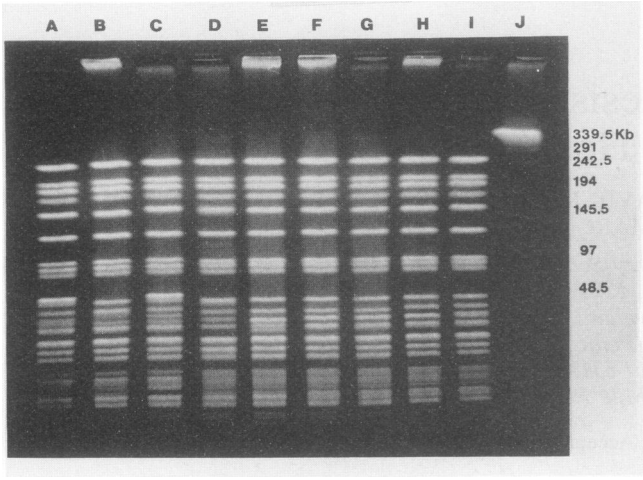

FIG. 1. CHEF electrophoresis of *Sma*I-digested chromosomal DNA from clinical isolates of *E. faecium*. Lanes A to C, isolates from hospital A; lanes D to G, isolates from hospital B; lanes H and I, isolates from hospital C; lane J, bacteriophage lambda ladder molecular mass standard. Molecular masses (in kilobases) are shown to the right.

strain type was identical between two patients. In hospital C, there were three strain types among the four isolates; only one strain type was identical between two patients. In hospital D, there were two strain types among the seven isolates; one strain was identical between six patients (the seventh isolate differed from the other six by only two chromosomal bands). In hospital E, there were four strain types among the six isolates; one strain type was identical between three patients.

CHEF electrophoresis identified a single strain type among isolates from three patients in hospital A (Illinois), four patients in hospital B (Illinois), and two patients in hospital C (Michigan) (Fig. 1). Of the nine isolates that were identical by CHEF electrophoresis, plasmid analysis revealed that six (two isolates from hospital A, two isolates from hospital B, and two isolates from hospital C) had identical patterns when plasmid DNA was digested with *Eco*RI. The remaining three isolates (one from hospital A and two from hospital B) had plasmid patterns that were very similar but not identical to those of the other six isolates. A second strain type was found to be identical by CHEF electrophoresis between a patient in hospital A (Illinois) and a patient in hospital C (Michigan); however, plasmid analysis revealed that their patterns were different. None of the control strains had patterns similar to those of the vancomycin-resistant isolates.

The results of the present study suggest that both the intrahospital and the interhospital spread of vancomycin-resistant *E. faecium* may have occurred. Especially striking is the apparent spread of one clone between hospitals in two adjacent states. Because of our strict criteria for determining the relatedness of strains, we may have missed additional isolates that were evolutionarily related. Isolates that differed by only two chromosomal bands in the CHEF electrophoresis gel could have been derived from the same ancestral clone. CHEF electrophoresis has proven useful in typing enterococci in epidemiologic studies (3, 9, 21, 23). The rapid emergence of vancomycin-resistant enterococci in many hospitals across the United States is a major concern. These strains are often resistant to ampicillin and to high levels of aminoglycoside as well, thus eliminating the possibility of effective antibiotic therapy. A knowledge of their epidemiology is essential for the control of further spread. The use of CHEF electrophoresis in typing these enterococci may prove helpful to clinicians attempting to initiate effective infection control measures to contain the spread of this organism both within each institution and between hospitals in different geographic locations.

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