

## Epidemiologic Analysis and Genotypic Characterization of a Nosocomial Outbreak of Vancomycin-Resistant Enterococci

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We are reporting on a nosocomial outbreak of 213 cases of vancomycin-resistant enterococcus infection involving 2,812 enterococcal isolates from patients over a period of 36 months. In 1990, the *Enterococcus faecium* vancomycin susceptibility rate was found to be 85.7% (36 of 42 cases), and an incidence of 10.9% (42 of 383) was noted. The 1991 data showed *E. faecium* with a vancomycin susceptibility rate of 61.8% (110 of 178) and an incidence of 26.0% (178 of 684). Subsequently, in 1992, the incidence of *E. faecium* increased to 34.0% (599 of 1,745), with a decreased vancomycin susceptibility rate of 25.8% (155 of 599). The *E. faecalis* vancomycin susceptibility rate remained near 97% (1,768 of 1,823) over the 36-month period. Of 115 vancomycin-resistant enterococcus (VRE) clinical isolates identified by the MicroScan MIC Combo-6 panels (Baxter Healthcare, Sacramento, Calif.), the agar dilution method indicated the resistance rate to be 92.3% (106 of 115) (high level), 3.5% (4 of 115) midlevel, and 3.5% (4 of 115) (low level). Genotypic characterization of 32 different VRE isolates by field-inversion gel electrophoresis demonstrated 19 dissimilar restriction endonuclease patterns, with 9 patterns associated with VRE quinolone resistance. Statistical analysis of case-control data for 32 patients with VRE infections indicated a positive association with intrabdominal surgical procedures (odds ratio, 24.12), multidrug therapy (odds ratio, 37.80), preexposure to vancomycin (odds ratio, 20.21), and death (odds ratio, 17.50).

*Enterococcus* species have been recognized as human pathogens since their discovery at the turn of the century and have been commonly characterized as multiple-drug-resistant gram-positive cocci (5, 16, 18). It has been shown that the inappropriate use of antibiotics can potentially lead to the emergence of resistant strains of bacteria (3, 11, 26, 33, 34). However, the role of enterococci as a virulent group of organisms in mixed infection is still difficult to establish by conventional epidemiology techniques (4). Advances in molecular probe technology potentiate their use as powerful tools for solving complex problems in epidemiology (6, 15, 24). One basic problem is that enterococci are frequently isolated as part of the normal human flora and from the surrounding environment (5). Prior to 1986, vancomycin-resistant enterococci (VRE) were considered a clinical rarity. Since 1988, reports of VRE have been limited to a small number of outbreaks (12, 29). However, within the last 6 years, enterococci have emerged as the second most common cause of nosocomial infections (16, 18).

In 1991, we investigated an increase in the frequency of VRE among patients throughout the hospital. A case-control study was carried out, and probable risk factors associated with VRE infections were analyzed by a 2-by-2 table odds ratio analysis. Field-inversion gel electrophoresis (FIGE) was used to genotype the VRE isolates and to assist in establishing the etiology of the VRE infections.

### MATERIALS AND METHODS

**Setting.** Cabrini Medical Center is an acute-care teaching hospital that serves a diverse patient population, including

frequently hospitalized individuals such as AIDS patients and nursing home residents.

**Bacteriologic methods.** Catalase-negative, esculin-positive, pyrrolidonyl naphthylamide-positive, gram-positive cocci in short chains were recovered from a variety of clinical sources. The methods of isolation and vancomycin (30 µg/ml) disk diffusion were performed according to previously described procedures (5, 22).

The gram-positive MIC Combo-6 panels (Baxter Healthcare, Sacramento, Calif.) were prepared according to the manufacturer's directions. The panels were inoculated by using the MicroScan Prompt system (8, 9). The panels were incubated for 16 to 18 h at 35°C in ambient air and then read with the MicroScan AutoScan-4 instrument.

The MIC agar dilution method was used as the "gold standard" for confirming the accuracy of the MicroScan MIC Combo-6 freeze-dried panel to detect VRE. The culture identifications by the MicroScan panels were confirmed by a routine battery of conventional biochemical tests, which included those for bile esculin, pyrrolidonyl naphthylamide, 6.5% sodium chloride, glycerol, arabinose, sorbitol, pyruvate, tellurite, and mannitol. For yellow-pigmented isolates, a motility test was included in the tests. The agar dilution assays to confirm vancomycin resistance and the culture identifications by conventional biochemical tests were performed at the Division of Microbiology, New York City Department of Health.

**Statistical analysis.** Case-control methods were used to analyze the relationship between VRE infections and risk factors. Our study group included 32 patients with infections of vancomycin-resistant *E. faecium* and/or vancomycin-resistant *E. faecalis* isolated over a 3-month period (August to October) in 1991. Our control group included 35 patients with vancomycin-susceptible enterococci isolates collected

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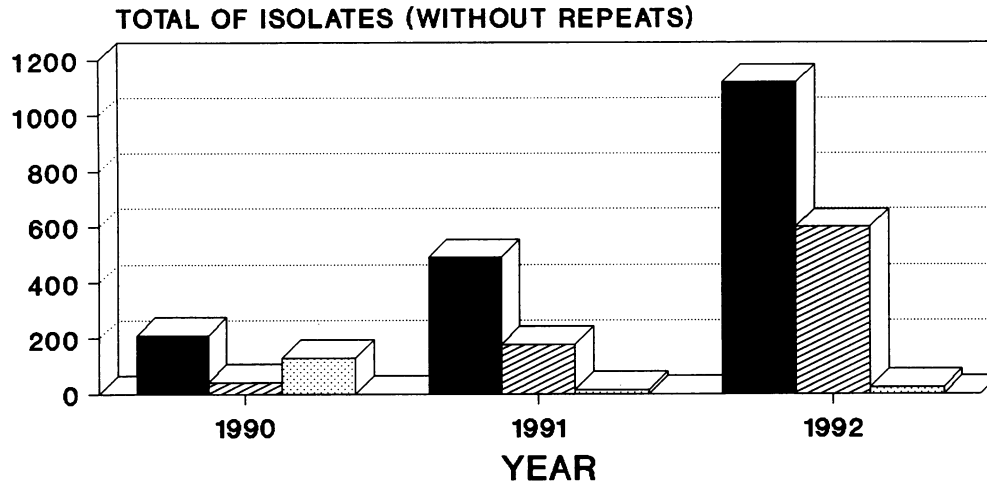


FIG. 1. The enterococcal trend report for 1990 to 1992 shows the incidence of 2,812 isolates, excluding repeats, over the course of 36 months. *Enterococcus* species include vancomycin-susceptible *E. avium*, *E. durans*, and enterococci not otherwise specified. *E. faecalis* showed an incidence of 64.8% (1,823 of 2,812) for the last 3 years, but *E. faecium* was the predominant VRE isolate. ■, *E. faecalis*; ▨, *E. faecium*; ▤, *Enterococcus* spp.

during the same period as the study group. If during the same hospitalization, cultures of more than one specimen from the same source in a patient contained VRE, only the first specimen was included in the study. Epi Info, version 5, computer software was used to calculate odds ratio and confidence limits (5).

**Restriction endonuclease analysis (REA).** For chromosomal DNA, a 10-ml sample of log-phase bacterial cells was centrifuged and washed in 10 ml of TE (Tris-EDTA) buffer. The cells were resuspended in 2 ml of EC buffer (6 mM Tris hydrochloride [pH 7.6], 1 mM NaCl, 100 mM disodium EDTA [pH 7.5], 0.5% Brij, 0.2% sodium deoxycholate, 0.5% sodium lauroylsarcosine) (Sigma Chemical Co., St. Louis, Mo.) of which 1 ml was mixed with 1 ml of 1% InCert Agarose (FMC, Rockland, Maine) prepared in EC buffer. To this suspension, 100  $\mu$ l of lysozyme (Sigma) was added, and 100  $\mu$ l of this mixture was cast into a mold (Pharmacia LKB Biotechnology, Inc., Piscataway, N.J.). The inserts were allowed to solidify and then were incubated at 35°C in 20 ml of EC buffer overnight with gentle shaking. After incubation, the inserts were stored in TE buffer at 55°C overnight. The inserts were stored in TE buffer at 4°C until used. For restriction endonuclease digestion, the inserts were removed from storage, equilibrated in 225  $\mu$ l of sterile water–25  $\mu$ l of React 4 buffer (GIBCO BRL, Gaithersburg, Md.), and incubated overnight at 4°C. Inserts were then supplemented with 30 U of *Sma*I and incubated at 30°C for 1 h. Following digestion, the inserts were placed in TE buffer for 15 min at 4°C. Before the inserts were loaded into the gel, they were equilibrated in 50% TBE (Tris-Borate-EDTA) buffer for 10 min at 4°C. Thin slices of the inserts were loaded into each of two gels for separating chromosomal DNA by FIGE. Two programs were run to separate DNA fragments with sizes of 48.5 to 485 kb and below 48.5 kb (28).

## RESULTS

In 1991, an investigation of the vancomycin susceptibility of enterococcal isolates had shown an overall increase in the incidence of enterococci (Fig. 1). In 1990, the overall enterococcal susceptibility rate of vancomycin was 97.9% (375 of 383 cases). The 1991 report showed that the incidence of *E.*

*faecium* increased to 26.0% (174 of 684) among other enterococci, and the reported vancomycin susceptibility rate was 61.8% (110 of 178). Subsequently, in 1992, the incidence of *E. faecium* increased to 34.0% (599 of 1,745), with a decreased vancomycin susceptibility rate of 25.8% (155 of 599).

During the course of the outbreak, 115 enterococcal isolates that were identified and for which susceptibilities to vancomycin were determined with the MicroScan system were forwarded to the Division of Microbiology, New York City Department of Health. All 115 isolates were verified as having been correctly identified. The results of the agar dilution testing of the VRE isolates were as follows: 92.2% (106 of 115 isolates) showed high-level [HL] vancomycin (>500  $\mu$ g/ml) resistance, 4.3% (5 of 115) showed midlevel vancomycin (>100  $\mu$ g/ml) resistance, and 3.4% (4 of 115) low-level vancomycin (>50  $\mu$ g/ml) resistance. *E. faecium* represented 94.7% (109 of 115) of the VRE isolates tested by agar dilution (Table 1).

The streptomycin and gentamicin synergy test results are limited to enterococci isolated from blood cultures. The MicroScan system screens isolates at a concentration of 2,000  $\mu$ g/ml. The Department of Health microbiology laboratory performs the streptomycin and gentamicin synergy test screens at a concentration of 500  $\mu$ g/ml. Agreement of the synergy results for 21 VREF blood isolates sent to the Department of Health was 90.5% (19 of 21) and 85.7% (18 of 21) for streptomycin and gentamicin, respectively. The MicroScan streptomycin and gentamicin synergy test results are presented as percentages of susceptibility (Table 1).

In the study group, 96% (30 of 32) of the patients were found to have had prior exposure to vancomycin or broad-spectrum antibiotics (Table 2). In comparison, only 47% (16 of 35) of the patients in the control group had been exposed to antibiotics. The calculated odds ratio was 16.31 when any use of antibiotics was considered as a risk factor associated with VRE infection. The odds ratio increased to 37.80 when only multi-antibiotic therapy was considered as a risk factor associated with VRE infection. There was a positive association with preexposure to vancomycin as a risk factor to acquiring a VRE infection on the basis of the odds ratio of 20.21.

TABLE 1. Susceptibility of VRE

Year evaluated and organism	No. of strains tested	% Susceptibility to MIC <sub>90</sub> (mg/ml) of <sup>a</sup> :					
		AMP (≤8)	IMP (≤8)	CIP (≤2)	TE (≤8)	VA (≤4)	STREP/GEN (2,000)
<b>1990</b>							
<i>E. faecalis</i>	211	97	92	75	30	99	NT
<i>E. faecium</i>	42	29	57	43	51	86	NT
<i>Enterococcus</i> sp.	130	43	86	71	29	100	NT
Total	383	71	86	70	33	97	
<b>1991</b>							
<i>E. faecalis</i>	491	90	88	61	30	97	57/52
<i>E. faecium</i>	178	3	39	49	51	62	25/38
<i>Enterococcus</i> sp.	15	70	85	70	70	100	NT
Total	684	67	73	58	36	88	
<b>1992</b>							
<i>E. faecalis</i>	1,121	95	95	59	49	97	64/67
<i>E. faecium</i>	599	5	69	49	79	25	63/54
<i>Enterococcus</i> sp.	25	95	100	59	100	100	NT
Total	1,745	36	80	52	81	80	

<sup>a</sup> MIC<sub>90</sub>, MIC for 90% of strains tested for cumulative urinary and blood dosage levels. AMP, ampicillin; IMP, imipenem; CIP, ciprofloxacin; TE, tetracycline; VA, vancomycin. STREP/GEN, streptomycin-gentamicin synergy. NT, not tested. The synergy screen percentage based on blood isolates only.

<sup>b</sup> *Enterococcus* species include vancomycin-susceptible strains of *E. durans*, *E. avium*, and *Enterococcus* species not otherwise specified.

Urine was the predominant source for the enterococcal isolates in both the study group (62%) and the control group (100%). There were 41 *E. faecalis* and 16 *E. faecium* isolates from blood in 1991 and 36 *E. faecalis* and 31 *E. faecium*

isolates from blood in 1992. The distribution of other sources for enterococcal isolates in the case-control study is described in Table 2.

Of the study group, 34% (11 of 32 patients) had abdominal surgery, and 25% (8 of 32) had lower gastrointestinal endoscopy, whereas only 6% of the control group (2 of 35) had abdominal surgery. The calculated odds ratio of 24.12 indicates a positive association between intrabdominal surgeries and acquiring a VRE infection (Table 2).

Data for patients from whom VRE had been isolated in several services were investigated, and we found no association between the service and VRE infection on the basis of the odds ratio (1.02). The average length of stay for the study group was 52.9 days compared with 28.6 days for the control group. The time between the date of admission and isolation of the VRE was 25.16 days for the study group compared with 13.06 days for the control group. The mortality rate for the study group was 50% (16 of 32 patients) compared with 5.7% (2 of 35) for the control group.

FIGE was performed on the restriction endonuclease digests for VRE isolates from 32 patients. The resulting REA patterns are presented in Fig. 2. These FIGE results demonstrated 15 different restriction endonuclease patterns for *E. faecium* and 4 different patterns for *E. faecalis* (Table 3). There were possible REA profiles associated with specific antibiotic patterns. The *E. faecium* isolate showing a Bb fragment pattern was found to be associated with quinolone susceptibility and the *E. faecium* isolate showing an Ic fragment pattern was found to be associated with quinolone resistance by FIGE. The overall VRE quinolone susceptibility rate of *E. faecium* was 46% (12 of 26) while that of *E. faecalis* was 60% (3 of 5).

## DISCUSSION

Recent reviews have described the emergence of enterococci and identified this group as one of the leading causes of

TABLE 2. Clinical features of case patients and controls

Variable	No. of patients		Odds Ratio <sup>a</sup>
	Case (n = 32)	Control (n = 35)	
Average age (years)	61.2	66.2	
Nosocomial acquisition of infection	32	24	
Hospital service			
Surgical	12	12	1.00
Medical	20	23	1.02
Invasive procedure			
Abdominal surgery	11	2	
Colonoscopy & Sigmoidoscopy	8	0	
Foley catheter	15	7	
Central venous catheter	6	0	
Avg length of hospital stay (days)	52.9	28.6	
Mean no. of days before VRE isolation <sup>b</sup>	25.2	13.1	
Antibiotic regimen			
Antibiotic therapy	30	16	16.31
Preexposure to vancomycin	12	1	20.21
Multidrug therapy	27	5	37.8
Bacterial infection			
Infection/colonization	22/32	16/35	
Bacteremia	7	0	
Urinary tract infection	18/32	16/35	
Wound infection	4	0	

<sup>a</sup> Risk factors associated with VRE.

<sup>b</sup> Number of days of hospitalization before isolation of enterococci.

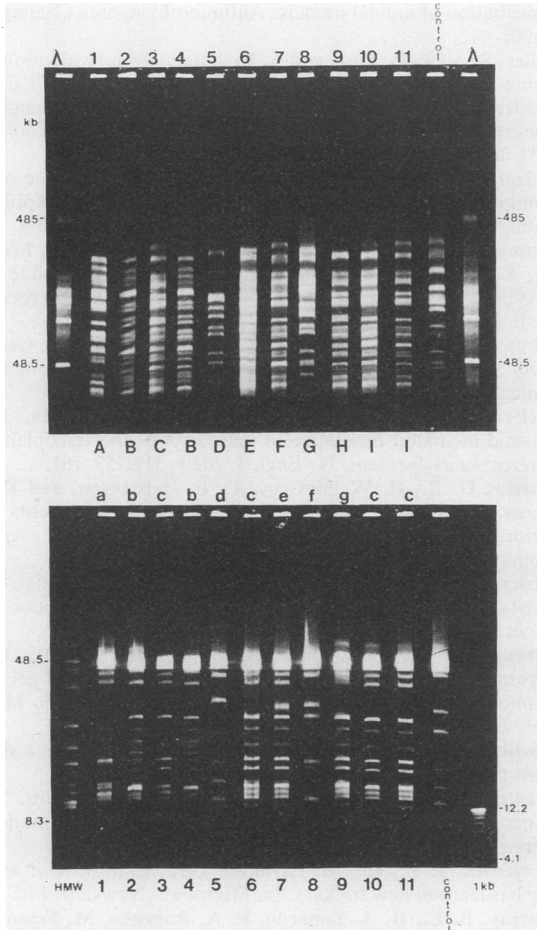


FIG. 2. REA patterns for *E. faecium* isolates from 11 patients demonstrated DNA fragments with sizes from 48.5 to 485 kb (top panel) and 4.1 to 48.5 kb (bottom panel). The DNA fragments resulting from *Sma*I digestion were Aa, Bb, Cc, Bb, Dd, Ec, Fe, Gf, Hg, Ic, and Ic (lanes 1 to 11, respectively, as labeled between the panels). Lanes labeled λ, size markers; HMW, high molecular weight.

nosocomial infections (1, 3, 14, 17, 18). Within the genus, *E. faecalis*, followed by *E. faecium* and *E. durans*, is responsible for most human disease (5). HL-aminoglycoside-resistant *E. faecalis* has been recognized in the United States for nearly a decade (14). More recently, several intrahospital

nosocomial outbreaks have been attributed to HL-aminoglycoside-resistant *E. faecalis* and ampicillin-resistant *E. faecium* (1, 19, 33, 34).

Conventional epidemiological techniques are dependent on the presence of phenotypic bacterial properties and lack the ability to detect genotypic changes due to plasmids or transposons (15, 25). The disadvantage of using plasmid content as an epidemiological marker is the inherent problem in the loss, acquisition, or transfer of plasmids (3, 34). In this study, our approach was to characterize the chromosomal DNA of the bacterium by REA (4, 15).

Recent studies have clearly documented that antibiotic-resistant strains of enterococci can be spread exogenously (patient to patient) in the hospital (1, 19, 29, 33). In this study, the REA patterns generated by FIGE demonstrated the capability, with this technique, of distinguishing between epidemiologically related isolates of both *E. faecium* and *E. faecalis* found in a nosocomial hospital outbreak.

The interesting features of this study are the dissimilarity of the genotypes and their widespread distribution in the hospital but with transient clusters of similar genotypes. There is the possibility that two or more nosocomial outbreaks had occurred on the basis of the dissimilarity in the four REA patterns for *E. faecalis*, with no strain predominating, and the 15 different patterns for *E. faecium*, with two predominant strains. In addition, there is the possibility that the two *E. faecium* strains (with an Ic and a Bb pattern) were superinfections and may have served as a focus for the exogenous transfer of VRE. The *E. faecium* isolates with a strain Bb pattern were vancomycin-resistant quinolone-susceptible strains, and *E. faecium* isolates with an Ic pattern were quinolone-resistant VRE strains. These isolates should be further evaluated for plasmids associated with quinolone resistance and experimental triple combination synergism (6, 21).

The statistical analysis of VRE case-control data using odds ratio analysis as an index of chance occurrence for risk factors indicates a positive association with preexposure to vancomycin (20.21), multi-antibiotic therapy (37.8), invasive procedures (i.e., sigmoidoscopy and colonoscopy) (24.12), and length of stay, with a mean difference of 24.3 days. All the features found in this study are consistent with previously reported outbreaks of methicillin-resistant *Staphylococcus aureus* (26), gentamicin-resistant *E. faecalis* (14), and ampicillin-resistant enterococci (1), which suggest that VRE with HL resistance are an endemic problem.

We found that patients with VRE infections showed no significant differences in terms of age, gender, and hospital distribution compared with patients with vancomycin-susceptible enterococci. In addition, having a human immunodeficiency virus-positive seroconversion in itself was not a risk factor for acquiring an enterococcal infection on the basis of comparison to our control group. More important considerations for VRE risk factors, are the extent of the patient's immunocompromised condition and the patient's immunosuppressive drug regimens (13, 30).

Urine was the most common source for enterococcal isolates in both the study and control groups. Enterococci have been implicated in over 15% of all nosocomial urinary tract infections (16). However, we found vancomycin-resistant *E. faecium* in various other sources, which included blood, wound, and sputum in which it was most often associated with infection. The most serious infections in our study group involved patients who had abdominal surgery (11 of 32), Foley catheters (15 of 32), or central venous

TABLE 3. REA of VRE from the study group

<i>E. faecium</i> (n = 26) <sup>a</sup>		<i>E. faecalis</i> (n = 5)	
CIP resistant (n)	CIP susceptible (n)	CIP resistant (n)	CIP susceptible (n)
Ic (6)	Aa (1)	Gf (2)	Dd (1)
Jc (2)	Bb (5)		Lf (1)
Cc (1)	Kk (1)		Pi (1)
Ec (1)	Nb (2)		
Fe (1)	Oh (1)		
Mg (1)	Bj (1)		
Qc (1)	Pj (1)		
Hc (1)			

<sup>a</sup> n, number of isolates; CIP, ciprofloxacin.

catheters (6 of 32). The mortality rate for these patients was 50% (16 of 32).

A quantitative drug usage evaluation performed independently in our hospital from 1989 to 1991 revealed a 106% increase in vancomycin usage. The qualitative drug usage evaluation results indicated that 85% (34/40) of the patients were prescribed vancomycin for one of the approved indications, but only 40% (16 of 40) were given the appropriate dosage. Interestingly, in the evaluation, no cases of subinhibitory dosages were encountered. Thus, treatment with subtherapeutic doses of vancomycin and inappropriate usage were not proven to be contributing factors in promoting vancomycin resistance.

The sensitivity of automated microbiology systems to detect HL vancomycin and aminoglycoside resistance has been described as inadequate for routine use of the systems (7, 27, 31). However, we have found the MicroScan automated microbiology and data management system to be a reliable system for the identification of enterococci and the detection of VRE.

Enterococci can be a normal component in the normal flora of the human gut, but they have the potential to cause serious infections. Patients receiving large enough doses of antibiotics could be predisposed to colonization by enterococci, leaving them at risk for developing an enterococcal infection (30). Colonization in our study group (10 of 32 patients) versus the control group (19 of 35) was calculated to an odds ratio of 2.61. Within this group of patients with colonized enterococci, those receiving recent vancomycin therapy before or at the time of colonization were placed at a higher risk for developing a serious VRE infection. This finding appears to be consistent with other data from our study showing that multi-antibiotic therapy (odds ratio, 37.8) increases the risk of VRE infection. Thus, it appears that the widespread use of glycopeptides needs to be reevaluated in an effort to reduce the risk of VRE infections (10, 20). Prescribers should be encouraged to seek consultation with an infectious disease specialist and microbiologist when considering a glycopeptide in the treatment of *Clostridium difficile*-associated diarrheal disease, methicillin-resistant *S. aureus* infections, and uncomplicated urinary tract infections.

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