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Evaluation and Characterization of Multiresistant *Enterococcus faecium* from 12 U.S. Medical Centers

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Forty-two *Enterococcus faecium* isolates resistant to ampicillin, penicillin, gentamicin, streptomycin, vancomycin, and teicoplanin (VanA phenotype) from 12 U.S. medical centers were analyzed by pulsed-field gel electrophoresis of chromosomal DNA. The isolates were tested for susceptibility to 12 alternative drugs. The results indicated both intrahospital and interhospital diversity among multiresistant *vanA* enterococcal isolates. Furthermore, the finding of isolates with identical pulsed-field gel electrophoresis patterns in different centers strongly suggests some interhospital clonal transmission.

Enterococci are now the second most frequently reported cause of surgical wound infection and nosocomial urinary tract infection and the third most frequently reported cause of bacteremia (15). Along with this rapid rise in incidence, resistance of enterococci to traditional antimicrobial agents, including the glycopeptides (4, 11), is increasing, and outbreaks caused by these resistant organisms have been recently reported with increased frequency (2, 5). Despite the publication of several studies on this topic, understanding of the dissemination of multiresistant enterococci (MRE) and their epidemiology is incomplete. Both intrahospital and interhospital transmissions of strains resistant to one or two of the antimicrobial agents used for enterococcal infections have been reported. However, most studies evaluate only one hospital or a few medical centers in a limited geographic area. The evaluation of a large group of MRE selected from consecutively collected isolates from representative areas of the country can provide a more valuable analysis of the epidemiology and spread of this organism in U.S. hospitals.

In the present study, we evaluated a set of 42 enterococci which were selected from 1,936 clinical isolates because of their demonstrated clinically relevant resistance to vancomycin, teicoplanin, penicillin G, ampicillin, gentamicin, and streptomycin. The principal objective of the study was to evaluate the clonal variability and dissemination of MRE among U.S. hospitals. We also evaluated the correlation between phenotypic and genotypic methods of characterizing glycopeptide-resistant isolates and analyzed the ability of different systems to detect in vitro antimicrobial resistance to these agents. In addition, the isolates were tested for susceptibility to 12 alternative antimicrobial agents.

The 42 MRE isolates evaluated were selected from a previous study (10) in which the prevalence of resistance to ampicillin, penicillin, gentamicin, streptomycin, teicoplanin, and vancomycin among 1,936 clinical isolates was assessed. The isolates of the earlier study were consecutively collected and processed in the last quarter of 1992 from bloodstream or other non-urinary tract infections in 97 U.S. medical centers (approximately 20 isolates from each center). The participant centers were distributed among 46 states and the District of Columbia. In the previous study (10), 42 isolates (2.2% of all strains) were resistant to all six antimicrobial agents tested. These 42 MRE isolates were sent to the University of Iowa for validation and were further analyzed in the present study. The isolates were collected from 12 medical centers (Table 1). Six centers were located in New York (26 isolates), two were located in New Jersey (7 isolates), and one each was located in Virginia (5 isolates), Connecticut (2 isolates), New Hampshire (1 isolate), and Illinois (1 isolate). The isolates were identified to the species level with the API 20S system (bioMérieux Vitek, Inc., Hazelwood, Mo.), with the Vitek gram-positive identification cards with the industrial mode software 8.1 (bioMérieux Vitek, Inc.), and by a modified version (3) of the conventional method proposed by Facklam and Collins (8).

The MRE isolates were tested against vancomycin and teicoplanin by broth microdilution, E test, and disk diffusion methods and against ampicillin, penicillin, and gentamicin by both the E test (9) and disk diffusion techniques (13). Streptomycin was tested only by the E test with the high-range (0.125- to 2,048-μg/ml) strip. Susceptibility was determined by the criteria described by the National Committee for Clinical Laboratory Standards (12, 13). The isolates were categorized into phenotypes of glycopeptide resistance on the basis of the MICs of vancomycin and teicoplanin (1). All 42 isolates were further tested against chloramphenicol, doxycycline, novobiocin, rifampin, imipenem, spectinomycin, trospectomycin, ciprofloxacin, clinafloxacin, sparfloxacin, erythromycin, and trimethoprim-sulfamethoxazole by either the E test (9) or National Committee for Clinical Laboratory Standards methods (13).

The isolates were analyzed for the presence of *vanA*, *vanB*, and *vanC* resistance genes by PCR and hybridization as described in a previous study (6). Pulsed-field gel electrophoresis (PFGE) of chromosomal DNA was performed as described by Pfaller et al. (14). Restriction digestion of chromosomal DNA was performed with *Sma*I (New England Biolabs, Inc.). The resultant restriction fragments were resolved in a 1% agarose gel with a CHEF-DR II system (Bio-Rad Laboratories, Richmond, Calif.). The pulse time ramped from 5 to 30 s over 23 h at 13°C and 6 V/cm. PFGE patterns were considered identical if they shared every band, similar if they differed from

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TABLE 1. PFGE patterns of 42 MRE from 12 medical centers

Isolate no.	Center no. (state)	PFGE pattern[a]
1	9 (NY)	1
2	11 (NY)	2
3	11 (NY)	3
4	12 (NY)	4a
5	12 (NY)	4b
6	12 (NY)	6a
7	12 (NY)	7
8	12 (NY)	6b
9	12 (NY)	4c
10	12 (NY)	10
11	12 (NY)	11
12	23 (VA)	6b
13	23 (VA)	6b
14	23 (VA)	6b
15	23 (VA)	6b
16	23 (VA)	6b
17	25 (CT)	17
18	25 (CT)	17
19	30 (NH)	19
20	33 (NY)	6b
21	33 (NY)	6b
22	33 (NY)	6b
23	33 (NY)	6b
24	38 (NY)	24
25	38 (NY)	25
26	38 (NY)	26a
27	38 (NY)	26b
28	38 (NY)	26b
29	38 (NY)	26b
30	38 (NY)	26b
31	38 (NY)	31
32	38 (NY)	26c
33	80 (NY)	33
34	80 (NY)	34
35	83 (NJ)	35
36	83 (NJ)	36a
37	83 (NJ)	36b
38	83 (NJ)	36b
39	83 (NJ)	36c
40	83 (NJ)	36b
41	94 (NJ)	41
42	99 (IL)	26b

[a] The major patterns are represented by Arabic numerals, and the subtypes are represented by lowercase letters.

one another by only one or two clearly visible bands, and different if they differed by three or more bands.

Analysis of the *Sma*I restriction digests of genomic DNA by PFGE resulted in 19 major patterns (Table 1 and Fig. 1), and only 2 of those were demonstrated in more than one medical center. PFGE pattern 6b was demonstrated in two hospitals in New York City and in a third hospital located in Virginia, and PFGE pattern 26b was demonstrated in two medical centers, one located in New York City and the other in Chicago. In addition, more than one pattern were demonstrated in five of

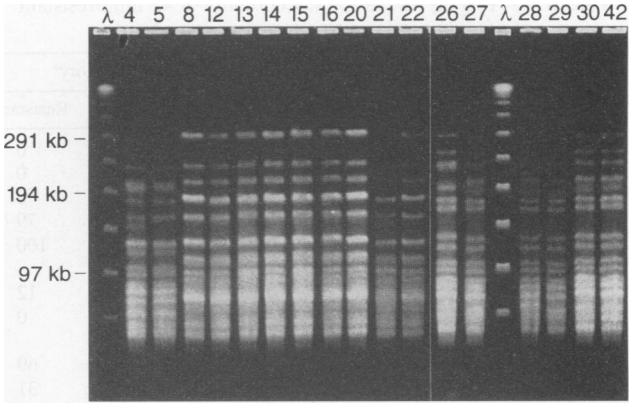

FIG. 1. PFGE patterns of *E. faecium* isolates after digestion of genomic DNA with *Sma*I and electrophoresis with switch time ramping from 5 to 30 s. The lane numbers match the isolate numbers (Table 1). Isolates 4, 5, and 8 are from center 12 (New York); isolates 12 to 16 are from center 23 (Virginia); isolates 20 to 22 are from center 33 (New York); isolates 26 to 30 are from center 38 (New York); and isolate 42 is from center 99 (Illinois). Lanes λ, lambda ladder molecular mass standards. Molecular masses are shown on the left.

eight medical centers that referred more than one MRE isolate. Five PFGE patterns were verified among eight isolates sent by medical center 12, and four PFGE patterns were demonstrated among nine isolates from medical center 38. Both hospitals are located in New York City (Table 1).

PFGE has been successfully used by many investigators and appears to be the technique of choice for epidemiologic evaluations of enterococci (2, 5, 7). The isolates were also evaluated by restriction endonuclease analysis of plasmid DNA (data not shown). Our results agreed with those of other studies in showing discrepant results between the two molecular methods. The discrepancy is probably due to plasmid instability (7). In addition, the results of restriction endonuclease analysis of plasmid DNA were very difficult to interpret because of the large number of bands and the presence of faint bands. In summary, the PFGE pattern appears to be more stable and easier to interpret than the restriction endonuclease analysis of plasmid DNA pattern.

Our results confirm the impressions of other investigators, showing a great genomic variability among multiresistant *Enterococcus faecium* isolates collected in different hospitals or even in the same hospital. This finding can be explained by horizontal transmission of resistance genes via either plasmids or transposons (1). However, mutation and subsequent selection of resistant strains caused by the extensive use of certain antimicrobial agents in some institutions may be occurring in genomically distinct strains. In addition, the discovery of isolates with identical PFGE patterns in different medical centers strongly suggests interhospital transmission.

The agreement among the applied susceptibility testing methods was very high, i.e., no major or very major errors were identified. Minor errors occurred only when teicoplanin was tested. The disk diffusion results disagreed (false intermediate) with the results (resistant) of both other methods for only three isolates. Several studies have reported the inability of some susceptibility testing methods, especially automated systems and disk diffusion, to detect resistance to β-lactams, aminoglycosides, and glycopeptides (9, 16). On the other hand, our results indicate that the E test appears to be an acceptable alternative for testing enterococci against the antimicrobial

TABLE 2. Testing results for susceptibility of 42 multiresistant *E. faecium* isolates to alternative compounds

Antimicrobial agent	% of strains in category[a]		
	Susceptible	Intermediate	Resistant
Chloramphenicol	100	0	0
Doxycycline	93	7	0
Novobiocin	100	0	0
Rifampin	21	0	79
Imipenem	0	0	100
Aminocyclitols			
Spectinomycin	10	78	12
Trospectomycin	100	0	0
Fluoroquinolones			
Ciprofloxacin	2	29	69
Clinafloxacin	64	5	31
Sparfloxacin	55	10	35
Erythromycin	0	0	100
Trimethoprim-sulfamethoxazole	2	0	98

[a] Breakpoint criteria used to define the susceptible and resistant categories, respectively, were as follows: novobiocin, ≤1 and ≥4 μg/ml; spectinomycin, ≥18 and ≤14 mm; trospectomycin, ≤16 and ≥64 μg/ml; clinafloxacin, ≤1 and ≥4 μg/ml; and sparfloxacin, ≤1 and ≥4 μg/ml. Results for all other drugs were interpreted by National Committee for Clinical Laboratory Standards criteria (12, 13).

agents most frequently used to treat infections caused by these species.

When analyzed by hybridization and PCR gene amplification, all isolates (all were VanA) produced the expected 1-kb product with the *vanA* primers and also hybridized with the *vanA* probe. All isolates produced negative results with both *vanB* and *vanC* primers. In contrast with other investigators, we did not identify any discrepancy between phenotype and genotype in our MRE strain collection.

The results of tests for susceptibility to alternative drugs are summarized in Table 2. The highest levels of in vitro inhibition were for chloramphenicol (100%), novobiocin (100%), trospectomycin (100%), and doxycycline (93%). However, further evaluation of the antibiotics tested showed that none demonstrated bactericidal activity either alone or in combination (data not shown). A great variation in the in vitro activity of the quinolones against enterococci was also noted. Although ciprofloxacin showed poor activity, 64% of the isolates were susceptible to the investigational fluoroquinolone clinafloxacin (MIC ≤ 1 μg/ml). In addition, susceptibility to the fluoroquinolones varied among isolates with identical PFGE patterns. This discrepancy was noticed for all three tested compounds. Since there is no proven effective alternative therapy for MRE infections, treatment options are limited to unproven combinations of older drugs or experimental compounds (11). This problem underscores the need to investigate new combinations and to develop new antimicrobial agents for these multiresistant strains.

In summary, our results show a great genomic variety among the most drug-resistant *E. faecium* isolates in numerous U.S. hospitals. PFGE appeared to be an acceptable and preferred method for epidemiologic typing of this species. In addition, the glycopeptide resistance phenotype VanA showed a good correlation with the resistance genotype (*vanA*), and the E test appeared to be a reasonable susceptibility testing method for detecting and precisely quantitating resistance to the antimicrobial agents most frequently used to treat enterococci infections as well as some alternative drugs used for MRE chemotherapy.

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REFERENCES

1. **Arthur, M., and P. Courvalin.** 1993. Genetics and mechanisms of glycopeptide resistance in enterococci. Antimicrob. Agents Chemother. **37**:1563–1571.
2. **Boyle, J. F., S. A. Soumakis, A. Rendo, J. A. Herrington, D. G. Gianarkis, B. E. Thurberg, and B. G. Painter.** 1993. Epidemiologic analysis and genotypic characterization of a nosocomial outbreak of vancomycin-resistant enterococci. J. Clin. Microbiol. **31**:1280–1285.
3. **Buschelman, B. J., M. J. Bale, and R. N. Jones.** 1993. Species identification and determination of high-level resistance among enterococci. Comparison study of sterile body fluid isolates, 1985–1991. Diagn. Microbiol. Infect. Dis. **16**:119–122.
4. **Centers for Disease Control.** 1993. Nosocomial enterococci resistant to vancomycin—United States, 1989–1993. Morbid. Mortal. Weekly Rep. **42**:597–599.
5. **Chow, J. W., A. Kuritza, D. M. Shlaes, M. Green, D. F. Sahm, and M. J. Zervos.** 1993. Clonal spread of vancomycin-resistant *Enterococcus faecium* between patients in three hospitals in two states. J. Clin. Microbiol. **31**:1609–1611.
6. **Clark, N. C., R. C. Cooksey, B. C. Hill, J. M. Swenson, and F. C. Tenover.** 1993. Characterization of glycopeptide-resistant enterococci from U.S. hospitals. Antimicrob. Agents Chemother. **37**: 2311–2317.
7. **Donabedian, S. M., J. W. Chow, J. M. Boyce, R. E. McCabe, S. M. Markowitz, P. E. Coudron, A. Kuritza, C. L. Pierson, and M. J. Zervos.** 1992. Molecular typing of ampicillin-resistant, non-β-lactamase-producing *Enterococcus faecium* isolates from diverse geographic areas. J. Clin. Microbiol. **30**:2757–2761.
8. **Facklam, R. R., and M. D. Collins.** 1989. Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. J. Clin. Microbiol. **27**:731–734.
9. **Huang, M. B., C. N. Baker, S. Banerjee, and F. C. Tenover.** 1992. Accuracy of the E test for determining antimicrobial susceptibilities of staphylococci, enterococci, *Campylobacter jejuni*, and gram-negative bacteria resistant to antimicrobial agents. J. Clin. Microbiol. **30**:3243–3248.
10. **Jones, R. N., M. E. Erwin, and the Enterococcus Study Group.** 1993. Emerging multiply resistant enterococci (MRE) among clinical isolates: prevalence data from 97 medical centers, abstr. 1052. Program Abstr. 33rd Intersci. Conf. Antimicrob. Agents Chemother.
11. **Moellering, R. C., Jr.** 1992. Emergence of *Enterococcus* as a significant pathogen. Clin. Infect. Dis. **14**:1173–1178.
12. **National Committee for Clinical Laboratory Standards.** 1993. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
13. **National Committee for Clinical Laboratory Standards.** 1993. Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A5. National Committee for Clinical Laboratory Standards, Villanova, Pa.
14. **Pfaller, M. A., R. J. Hollis, and H. S. Sader.** 1994. Chromosomal restriction fragment analysis by pulsed-field gel electrophoresis, Suppl. 1, p. 10.5.c.1–10.5.c.12. *In* H. D. Isenberg (ed.), Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
15. **Schaberg, D. R., D. H. Culver, and R. P. Gaynes.** 1991. Major trends in the microbial etiology of nosocomial infection. Am. J. Med. **91**(Suppl. 3B):72S–75S.
16. **Tenover, F. C., J. Tokars, J. Swenson, S. Paul, K. Spitalny, and W. Jarvis.** 1993. Ability of clinical laboratories to detect antimicrobial agent-resistant enterococci. J. Clin. Microbiol. **31**:1695–1699.