JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1991, p. 2752–2757 Vol. 29, No. 12
0095-1137/91/122752-06$02.00/0
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DNA Fingerprinting of *Enterococcus faecium* by Pulsed-Field Gel Electrophoresis May Be a Useful Epidemiologic Tool

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Pulsed-field gel electrophoresis was used to compare 34 isolates of *Enterococcus faecium* from six different geographic locations. This procedure generated an average of 13 discernible fragment bands per isolate (range, 10 to 19 fragment bands) of 34 to 485 kb. The resulting restriction endonuclease digestion patterns were quite heterogeneous and were able to differentiate 27 of 34 isolates from each other, as defined by one or more mismatched fragment bands. Five patterns were shared by two or more isolates, and each set of isolates with matching patterns (shared pattern) originated in the same medical center, suggesting a common epidemiologic background, including highly penicillin resistant isolates in Richmond and Philadelphia. We conclude that pulsed-field gel electrophoresis of DNA digested with low-frequency-cleavage restriction enzymes offers a relatively simple method of comparing *E. faecium* for the purpose of epidemiologic study.

Since its discovery as a human pathogen around the turn of the century, members of the genus *Enterococcus* have distinguished themselves from other gram-positive cocci by multiple antibiotic resistances, distinct biochemical properties, and controversial virulence potential in certain mixed infections (18). The role of enterococci in urinary tract infections (17) and infective endocarditis (16) is well established, and it appears that infections with these organisms are on the rise in the nosocomial setting (15, 17). While the majority of clinical enterococcal isolates are *Enterococcus faecalis*, a significant percentage (10 to 15%) are *Enterococcus faecium*, a species which is generally more inherently resistant to antibiotics, including penicillin, ampicillin, some aminoglycosides, and imipenem (18). The clinical implications of infection with an organism which may be more difficult to treat is compounded by the apparent nosocomial transmission of this pathogen. Patients have been reported to contract bacteremia and meningitis by this mode of transmission (7, 18, 29). In addition, recent reports describe the recovery of more highly penicillin- and ampicillin-resistant clinical isolates in certain centers (5, 7, 25, 26). Thus, further characterization of *E. faecium* is an important objective. Investigations into the source and mechanism of spread of enterococci, however, have been hindered by the lack of reliable typing schemes that are able to identify particular strains beyond the species level. Methodologies that have been used include antibiotic resistance patterns, biochemical reactions (10), bacteriocin typing (13), phage typing (6), total plasmid content (31), and serologic typing (27). Most of these techniques are not sufficiently sensitive to distinguish different strains and can require considerable amounts of time, material, and expertise to perform. In addition, with some of these techniques, factors such as plasmid loss and various in vivo and/or in vitro conditions, which can change the phenotypic characteristics of certain organisms (21), could render variable results. We and other investigators have successfully used pulsed-field gel electrophoresis (PFGE) or electrophoretic devices with similar principles to make comparative chromosomal DNA analyses of *E. faecalis* (19, 20), *Escherichia coli* (3), *Pseudomonas aeruginosa* (2), *Staphylococcus aureus* (11), and other organisms (1, 2). The advantage of PFGE is its ability to separate large DNA fragments (i.e., from 10 kb to 1.5 Mb) such as those generated by low-frequency-cleavage restriction endonuclease digestion (RED) of whole chromosomes. This technique yields RED patterns which consist of relatively few, generally well separated fragment bands and which are much less ambiguous than the patterns generated by conventional electrophoresis (23). The purpose of this study was to determine whether PFGE could be used for the comparison of isolates of *E. faecium* and to determine the characteristics of the resulting RED patterns among isolates from the same and from distant geographic areas.

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MATERIALS AND METHODS

Bacterial strains. Thirty-four isolates of *E. faecium* from diverse geographic locations were used for this analysis; 11 other isolates sent to us were subsequently identified as species other than *E. faecium* (see below) and were therefore not used. The distribution of isolates was as follows: five isolates collected by the Centers for Disease Control (Atlanta, Ga.), origin unknown, kindly provided by Richard R. Facklam; four isolates from New England Deaconess Hospital (Boston, Mass.), kindly provided by George M. Eliopoulos; four isolates from University of Wisconsin Hospitals and Clinics (Madison, Wis.), kindly provided by Carol A. Spiegel; six isolates from The Medical College of Pennsylvania (Philadelphia, Pa.), kindly provided by Caroline C. Johnson; seven isolates from Rancho Los Amigos Medical Center (Downey, Calif.), kindly provided by Francisco L. Sapico; and eight isolates from Medical College of Virginia (Richmond), kindly provided by Harry P. Dalton. Strains were requested from the latter three individuals because of prior reports of highly penicillin- and ampicillin-resistant *E. faecium* in their hospitals (5, 7, 26).

The control strains used for identification were as follows: (i) *E. faecium* GE-1, kindly provided by George M. Eliopoulos; (ii) *Enterococcus gallinarum* SS-1228, *Enterococcus*

solitarius SS-1277, and *Enterococcus mundtii* SS-1232, kindly provided by Richard R. Facklam; and (iii) *E. mundtii* ATCC 43186, kindly provided by Kathryn L. Ruoff. *E. coli* MG1655 was used as a control for molecular size determination (4).

Strain identification. The identification of all isolates was performed by a combination of methods. Our primary identification scheme was one based on conventional biochemical reactions as described by Facklam (8) and Facklam and Carey (9) and as modified by Facklam and Collins (10); we also used the commercial API Rapid Strep System (Analytab Products, Plainview, N.Y.) for identification of isolates. The biochemical tests that were performed, in addition to the API tests, were the fermentation of mannitol, sorbitol (Fisher, Fair Lawn, N.J.), sucrose (J. T. Baker, Phillipsburg, N.J.), raffinose (Difco, Detroit, Mich.), lactose (BBL, Cockeysville, Md.), and sorbose (Sigma, St. Louis, Mo.); ability to grow in 6.5% NaCl (J. T. Baker); tolerance to 0.04% tellurite, pyruvate utilization, and deamination of arginine (Sigma); Gram stain and group D serologic testing (Difco) by the autoclave extraction and capillary precipitin method (9); and pigment production on tryptic soy agar and motility determination on motility media (BBL). Also tested were catalase production (EM Science, Gibbstown, N.J.) and β-lactamase production by the nitrocefin reaction (Glaxo, Research Triangle Park, N.C.). Todd-Hewitt broth was used for the carbohydrate fermentation media (Difco). The Voges-Proskauer tests; pyrrolidonylarylamidase activity; hydrolysis of esculin and sodium hippurate; and the fermentation of trehalose, inulin, L-arabinose, and ribose were directly interpreted from the API strip.

Antibiotic disk susceptibility (BBL) testing was performed on tryptic soy agar (Difco) by using ampicillin (10 μg), chloramphenicol (30 μg), erythromycin (15 μg), tetracycline (30 μg), and vancomycin (30 μg) disks. Susceptible, intermediate, and resistant isolates were defined by disk zone size according to the Zone Size Interpretative Chart included in the manufacturer's instructions. High-level gentamicin resistance was determined by the ability to grow on brain heart infusion agar containing 2000 μg of gentamicin (Schering, Bloomfield, N.J.) per ml. The MICs of penicillin G (Marsam, Cherry Hill, N.J.) were also determined by the agar dilution method (30). Isolates for which the MICs were 256 μg/ml or above were considered to be highly resistant to penicillin G.

DNA analysis. Genomic DNA was prepared from all *E. faecium* isolates by previously described methods (19, 28). Briefly, an overnight culture grown in 5 ml of Todd-Hewitt or brain heart infusion broth (Difco) was centrifuged, and the packed cells were suspended in 5 ml of PIV solution (1 M NaCl, 10 mM Tris-HCl [pH 7.6]). A total of 750 μl of this suspension was mixed with 250 μl of 3.2% agarose (Incert Agarose; FMC, Rockland, Maine) and pipetted into small rectangular molds (final agarose concentration, 0.8%). The cells suspended in the agarose plugs were then lysed overnight at 37°C by using a solution containing 6 mM Tris-HCl (pH 7.6), 1 M NaCl, 100 mM EDTA (pH 7.5), 0.5% Brij 58, 0.2% deoxycholate, 0.5% sodium lauroyl sarcosine (Sarkosyl), 20 μg of RNase (DNase free) per ml, and 1 mg of lysozyme per ml. Next, the lysis solution was replaced with a solution containing proteinase K (50 μg/ml), 1% Sarkosyl, and 0.5 M EDTA (pH 9 to 9.5); and the plugs were further incubated overnight at 50°C with slow shaking. The plugs were then washed three times (30 min each time at 37°C) with TE solution (10 mM Tris-HCl [pH 7.5], 1 mM EDTA) and stored in fresh TE solution at 4°C until and after their use.

All *E. faecium* DNAs were digested with the restriction enzyme *Sma*I (recognition sequence, CCCGGG), and the DNA of the size control *E. coli* MG1655 was digested with *Not*I (recognition sequence, GCGGCCGC). Lambda concatemers (New England BioLabs, Beverly, Mass.) were also used as size controls in some gels. The enzymes were obtained from Boehringer-Mannheim (Indianapolis, Ind.) or New England BioLabs. The digestions were performed by placing a 4- to 5-mm slice of each plug in 225 μl of 1× restriction buffer solution, and approximately 20 to 40 U of the respective enzyme was added. Incubations were as follows: (i) *Sma*I, 25°C for 4 to 20 h; (ii) *Not*I, 37°C for 12 to 20 h. After incubation, the plugs were washed and equilibrated with TE solution for 1 h at 37°C. The TE solution was subsequently discarded, and the plugs were then melted at 55 to 60°C and pipetted into the wells of a 1.2% Seaplaque GTG agarose gel (FMC) (in 0.5× TBE buffer [0.089 M Tris-HCl, 0.089 M boric acid, 0.0025 M EDTA). Electrophoresis was performed with the Contoured-Clamped Homogeneous Electric Field apparatus (CHEF-DRII; Bio-Rad) by using ramped pulse times beginning with 5 s and ending with 35 s at 200 V for 32 to 40 h. For selected isolates, DNA was also run with a fixed pulse time of 5 s in order to discern the lower-molecular-weight fragments. The gels were then stained with ethidium bromide for 30 min and destained in distilled water for 12 to 20 h before they were photographed with UV radiation.

RESULTS

Chromosomal patterns of isolates. A unique pattern was arbitrarily defined in this study as any RED pattern generated with a ramped pulse time of 5 to 35 s which varied from another pattern by one or more clearly visible fragment bands, or simply, any nonidentical RED pattern (ramped at 5 to 35 s) constituted a pattern of its own. This notwithstanding, our experience suggests that RED patterns with differences in only a few fragment bands would be expected to represent closely related isolates of a single strain (19, 20). By using a ramped pulse time of 5 to 35 s, the bands visualized best were those corresponding to the higher-molecular-weight fragments (range, 200 to 500 kb). By decreasing the pulse time to a 5-s fixed interval, the lower-molecular-weight fragments became more visible (range, 30 to 190 kb) at the expense of those with higher molecular weights, which segregated poorly or not at all.

In the present study, all but 1 of the 34 isolates analyzed displayed evaluable RED patterns by PFGE on the first attempt. The remaining isolate was evaluable after a new plug was made. The range of clearly visible fragment bands on each gel when a ramped pulse time of 5 to 35 s (many isolates were repeated on different gels) was used was 10 to 19 fragment bands, with an average of 13 to 14 fragment bands visible per isolate. In all, 27 distinct RED patterns were seen, 5 of which were shared by two or three members, which segregated the organisms by geographic locale. The RED patterns which were shared (designated with the suffix SP) contained organisms isolated in the same medical center and, in one case, from the same patient (Fig. 1A, lanes b, c, and d, pattern MCV-SP1, and Table 1). Shared RED patterns were seen in isolates from Richmond, Va. (MCV-SP1 and MCV-SP2); Philadelphia, Pa. (MCP-SP1); Downey, Calif. (RLA-SP1); and Boston, Mass. (NEDH-SP1) (data not shown, except for the isolate from Richmond in Fig. 1A).

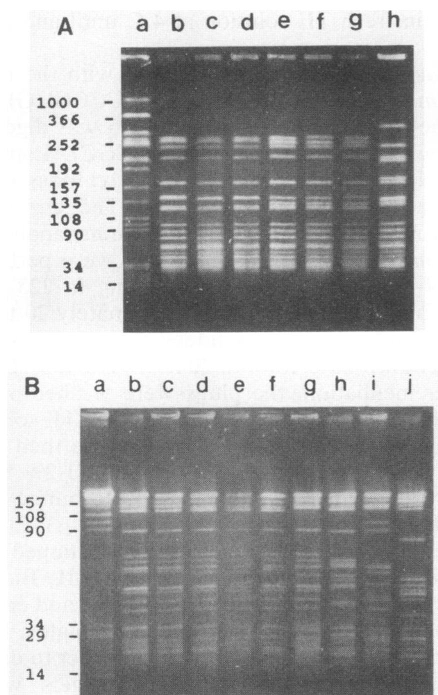

FIG. 1. (A) Agarose gel showing *Sma*I digestion patterns of *E. faecium* from Richmond, Va., by using PFGE with ramped pulse times of 5 to 35 s to resolve the higher-molecular-weight fragment bands (see panel B). Lanes b, c, and d, isolates with pattern MCV-SP1; lanes e, f, and g, isolates with pattern MCV-SP2; lane h, an isolate with pattern MCV-3; lane a, *E. coli* MG1655 (see Fig. 2A). (B) Agarose gel showing *Sma*I digestion patterns of *E. faecium* by PFGE by using a fixed pulse time of 5 s to resolve the lower-molecular-weight fragment bands (see panel A). Two members of MCV-SP1 (lanes b and c) are also shown in panel A, lanes b and d. Two members of MCV-SP2 (lanes d and f) are also shown in lanes f and e of panel A. Lane e is the third member of MCV-SP2. Note the one to two fragment band differences in the lower-molecular-weight range among members with the same shared pattern. Lane g, an isolate from Philadelphia, Pa. (MCP-2) (also shown in lane h of Fig. 2A); lane h, an isolate from Madison, Wis. (WIS-1) (also shown in lane d of Fig. 2A); lane i, an isolate from Downey, Calif. (RLA-2) (also shown in lane b, Fig. 2A, and lane a, Fig. 2B); lane j, an isolate from the Centers for Disease Control (CDC-2) (also shown in lane f, Fig. 2A); lane a, *E. coli* MG1655 (see Fig. 2A). The numbers on the left are molecular sizes, in kilobases. SP denotes a shared pattern.

Most isolates from Richmond, Philadelphia, and Downey differed from others in the same locale by only a few fragment bands (in Fig. 1A, similarities between isolates MCV-SP1 and MCV-SP2 from Richmond are shown). All except one of the isolates from these three areas had high-level resistance to penicillin G (see below).

In addition to similarities within a given geographic area, the isolate with pattern MCP-2 from Philadelphia and one of the isolates with pattern MCV-SP2 from Richmond had many similarities in both their higher- and their lower-molecular-weight fragment bands (Fig. 2A, lanes h and i, and Fig. 1B, lanes g and d, respectively). These isolates were both highly resistant to penicillin, shared the same API profile number, and had identical antibiograms and biochemical reactions.

We also showed that for those isolates which had identical higher-molecular-weight RED patterns and for which the lower-molecular-weight fragments were analyzed, the latter were also identical or differed by only one to two fragment bands. This is illustrated in Fig. 1A and B, in which both the higher- and the lower-molecular-weight fragment bands, respectively, are shown for two members of MCV-SP1 (Fig. 1A, lanes b and d, and Fig. 1B, lanes b and c) and two members of MCV-SP2 (Fig. 1A, lanes f and e, and Figure 1B, lanes d and f).

Phenotypic characteristics of isolates. Of the 45 isolates that we received, we identified 34 of them as *E. faecium* using a biochemical scheme. Two isolates were identified as *E. faecium* by API Rapid Strep but as *E. gallinarum* by conventional tests and were not included in the analysis. The remaining nine isolates were identified as enterococcal species other than *E. faecium* by both conventional biochemical reactions and the API Rapid Strep, and in general, no discernible bands were generated or the patterns were difficult to interpret (Fig. 2B, lane f). These isolates were not included in the results reported here.

Of 34 isolates, 21 displayed high-level resistance to penicillin G. All isolates from Downey and Philadelphia, locations previously known to have such isolates (5, 25, 26), displayed this phenotype. In addition, isolates that were highly resistant to ampicillin have been reported in Richmond (7), and all but one of the isolates from that location demonstrated high-level resistance to penicillin G. Conversely, only one isolate from Madison was highly resistant to penicillin G; all other isolates (from Madison, the Centers for Disease Control, and Boston) either had low-level resistance or were susceptible to penicillin G (MIC for 50% of

TABLE 1. RED patterns of isolates examined in this study

Isolate origin (designation)	No. of isolates	No. of distinct RED patterns (pattern name)[a]	RED patterns containing more than one isolate (no. of isolates)[b]
Centers for Disease Control (CDC)	5	5 (CDC 1-5)	0
Medical College of Pennsylvania (MCP)	6	5[b] (MCP-SP1 and MCP 2-5)	MCP-SP1 (2)
Medical College of Virginia (MCV)	8	4[b] (MCV-SP1-2 and MCV 3-4)	MCV-SP1 (3)[c], MCV-SP2 (3)
Wisconsin Hospitals and Clinics (WIS)	4	4 (WIS1-4)	0
New England Deaconess Hospital (NEDH)	4	3 (NEDH-SP1 and NEDH 2-3)	NEDH-SP1 (2)
Rancho Los Amigos Medical Center (RLA)	7	6[b] (RLA-SP1 and RLA 2-6)	RLA-SP1 (2)
Total	34	27	5 (12)

[a] A distinct pattern is defined as any RED pattern generated with a ramped pulse time of 5 to 35 s which varies from another by one or more fragment bands (i.e., any nonidentical RED pattern). Organisms with matching patterns (i.e., RED shared pattern) are designated with the suffix SP.
[b] Similarities were seen in the RED patterns of some isolates from the same locale, e.g., patterns MCV-SP1 and MCV-SP2. These similarities may indicate that some organisms from the same area were clonally derived (see text).
[c] All three isolates with the MCV-SP1 pattern were obtained from the same patient.

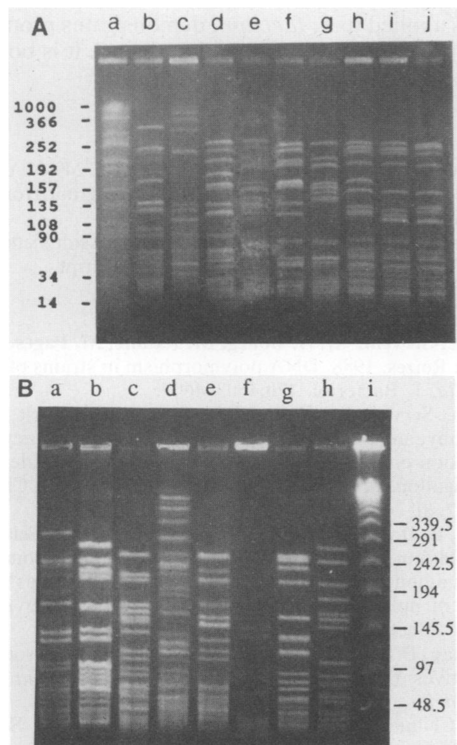

FIG. 2. (A) Agarose gel showing *Sma*I digestion patterns of *E. faecium* (ramped pulse time, 5 to 35 s) from Downey, Calif. (lane b; RLA-2); Centers for Disease Control (lane c; CDC-1); Madison, Wis. (lane d; WIS-1); Boston, Mass. (lane e; NEDH-2); Centers for Disease Control (lanes f [CDC-2] and g [CDC-3]); Philadelphia, Pa. (lane h; MCP-2); and Richmond, Va. (lanes i [MCV-SP2] and j [MCV-SP1]). *E. coli* MG1655 (lane a) was digested with *Not*I. (B) Agarose gel showing *Sma*I digestion patterns of *E. faecium* (ramped pulse time, 5 to 35 s) from Downey, Calif. (lane a [RLA-2; also shown in lane b in panel A and lane i, Fig. 1B] and lane b [RLA-3]); Centers for Disease Control (lanes c [CDC-4], d [CDC-5], and e [CDC-6]); Richmond, Va. (lane g; MCV-SP1 [also shown in lane j, Fig. 2A]); and Madison, Wis. (lane h; WIS-2). Lane f, an *E. gallinarum* isolate from Madison, Wis.; note the difference in the quality of the pattern compared with the pattern of *E. faecium*. Lane i, lambda concatemers. The numbers on the left and right are molecular sizes, in kilobases. SP denotes a shared pattern.

isolates tested, 16 μg/ml). No isolate in this study produced β-lactamase.

API profile numbers, biochemical reactions, and antibiotic susceptibility patterns could not be relied upon to predict the relatedness of isolates. For example, for organisms with shared RED patterns, only MCP-SP1 members had identical API profile numbers, adjunct biochemical reactions, and antibiotic susceptibilities (including high-level penicillin and high-level gentamicin resistance), although the only difference found among members of MCV-SP2 was the susceptibility of one member to tetracycline and chloramphenicol. NEDH-SP1 members had minor differences in their API profile numbers and their susceptibilities to chloramphenicol; RLA-SP1 members differed in their API profile numbers, reaction to sorbose, and susceptibilities to chloramphenicol; MCV-SP1 members differed in their API profile numbers and antibiotic susceptibilities.

Identical API profile numbers were seen in a number of isolates with different RED patterns by PFGE, regardless of their site of origin. For example, the API profile for the organisms who were members of MCV-SP2 and MCP-SP1, two additional Philadelphia isolates, and one of the organisms with pattern RLA-SP1 was the same (i.e., 7357711).

DISCUSSION

The usefulness of any typing scheme is predicated on the particular scheme's discriminatory power, which, in turn, depends on the characteristics being measured. Previous methods of typing enterococci (6, 10, 12, 13, 27, 31), except plasmid typing (14, 31), have relied on the phenotypic properties of the bacterium, the expression of which can be affected by many factors (as reviewed in reference 21 for *Pseudomonas aeruginosa*). Likewise, the loss, acquisition, and transfer of plasmids and transposons can create difficulties when plasmid patterns are used, since they may not be able to distinguish among epidemiologically related isolates or may result in patterns that are difficult to interpret, or the plasmids may be absent altogether (11, 22, 24). Combining several typing schemes might facilitate epidemiologic investigation (13, 14), but the additional methods and material needed renders this strategy less desirable and frequently more time-consuming and costly. Coudron et al. (7) suggested that profile numbers (seven digits) of epidemic strains tested by the API 20S system (Analytab Products) could differentiate these from other unrelated environmental and reference strains. We found that certain profile numbers (using the seven-digit code of the API Rapid Strep system) were generated by strains from distant geographic areas and with different RED patterns determined by PFGE. Furthermore, not all members with a particular shared RED pattern had the same profile number, a phenomenom that is known to occur even with repeat testing of a single strain. On the basis of this, we do not think that API profile numbers are a reliable way of comparing different *E. faecium* isolates, but it may prove somewhat useful in outbreaks if the profile number is not a common one. Conventional biochemical reactions were also not sufficiently discriminatory, nor were antibiograms.

The need for a simple and reliable method of comparing bacterial strains is therefore evident. For enterococci in particular, the need is becoming more urgent as these bacteria become increasingly implicated in serious illness (15, 17, 18) and evidence mounts for their epidemic spread (7, 14). In addition, a reliable comparison tool would be useful in delineating issues such as endogenous versus exogenous origins of strains, vectors for epidemic spread, and whether new infectious episodes with enterococci are due to persistence or reinfection.

By digesting the bacterial chromosome with restriction enzymes which cleave the DNA in only a few sites, a pattern consisting of a reasonable number (e.g., 10 to 25) of high-molecular-weight DNA fragments is generated, and these fragments can be easily compared with those obtained from the DNAs of other isolates. The resolution of these large fragments can be achieved with the use of PFGE, which, by nature of its changing electrical field, is able to separate DNA fragments that are orders of magnitude larger than conventional unidirectional gel electrophoresis can (28). In contrast to the patterns generated by PFGE, patterns generated by conventional gel electrophoresis (and restriction enzymes with a large number of recognition sites within the genome) usually show numerous and poorly visible fragment bands (19, 23).

In this study, a working assumption was that the chromosome is a relatively stable property of a microorganism. That

the RED patterns generated by PFGE can be quite stable, at least within a short period of time in evolutionary terms, is supported by the reproducibility of our results with bacteria prepared up to 1 year apart (data not shown) and by results of other studies which have shown consistently stable patterns for the same organism even after many in vitro passages (3, 11), after recovery from different sites from the same patient (3) or longitudinal recovery from chronically infected patients (11), and as seen with certain β-lactamase-producing strains of *E. faecalis* (20).

RED patterns generated by RED of *E. faecium* genomic DNA demonstrated a considerable amount of polymorphism among isolates from distinct geographic sites and even among a number of isolates within a single locale. In addition, some isolates which had identical (i.e., shared) RED patterns were seen; and each of these shared patterns was unique to a particular geographic location and, in one instance, was unique to a particular patient. The similarities between the RED patterns of some other isolates within the same locale (some differed by only two fragment bands) suggest recent evolutionary divergence from a common isolate and could suggest the possibility of epidemiologic relatedness. A more detailed epidemiologic study will be required to verify this hypothesis. The data presented here, however, support this notion, since RED patterns closely resembling each other were nearly always seen in the same medical center. It was interesting that isolates with apparently similar RED patterns within each locality (Richmond, Philadelphia, and Downey) tended also to have higher levels of resistance to penicillin. High-level penicillin and ampicillin resistance has been increasingly reported and was, in fact, reported by investigators from three locations from which some of the isolates in this study were obtained (5, 7, 25, 26). Indeed, we requested isolates from these investigators for this reason. It is likely, then, that PFGE correctly identified related isolates which were selected for their common resistance properties or epidemiologic background. In the only instance in which the RED pattern of an isolate from one site closely resembled those of isolates from a different site (Philadelphia and Richmond), all phenotypic properties studied were identical, including high-level resistance to penicillin G and API profile number. The significance of this finding is not clear, but it is interesting to speculate on the possible clonal spread of these organisms. Clonal spread has been demonstrated for β-lactamase-producing *E. faecalis* isolates from five diverse geographic areas (i.e., Delaware, Texas, Pennsylvania, Florida, and Virginia) with the use of PFGE and low-frequency-cleavage endonuclease digestion (20).

While this study was not meant to include epidemiologic observations beyond the origin of isolation of individual organisms (these data were not available for the isolates from the Centers for Disease Control), it is clear that this technique generates a number of very diverse RED patterns and appears to be sufficiently discriminatory to be of value for the comparison of *E. faecium* isolates. These observations can be exploited in the investigation of outbreaks and to further characterize the role of *E. faecium* as normal flora and human pathogen, its mode of spread, and the virulence of particular strains. A potential weakness of this or any technique would be the presence of prolonged and widespread dissemination of a given strain, making epidemiologic comparison of isolates less useful. For this reason, implementation of this technique for detailed epidemiologic studies of *E. faecium* or other organisms should include investigation of background strains as well. We conclude that PFGE, as applied to *E. faecium*, demonstrates promise as an epidemiologic and investigational tool since it is both simple to perform and reproducible.

ACKNOWLEDGMENTS

This work was supported in part by grant AI-27249-01A1 from the National Institutes of Health and in part by a grant from Lederle Laboratories, American Cyanamid Company.

We thank George Weinstock for helpful discussions and Deborah Marsales for assistance in preparing the manuscript.

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