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DNA Fingerprinting by Pulsed-Field Gel Electrophoresis Is More Effective than Ribotyping in Distinguishing among Methicillin-Resistant *Staphylococcus aureus* Isolates

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Pulsed-field gel electrophoresis (PFGE) after *Sma*I restriction of DNA from 239 methicillin-resistant *Staphylococcus aureus* isolates (from 142 patients) produced 26 different fingerprints. The deduced chromosome sizes ranged from 2,200 to 3,100 kb (±100 kb). A total of 81 isolates taken from 65 patients were then typed by PFGE and ribotyping with *Cla*I, *Eco*RI, and *Hin*dIII. Ribotypes were less discriminating than PFGE. Ribotyping did not discriminate isolates from a given PFGE fingerprint into different subsets. PFGE may be a more effective epidemiological tool than ribotyping for the typing of methicillin-resistant *S. aureus* strains.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is still one of the most frequent agents of nosocomial infections. Although MRSA strains have not been shown to be more virulent than other strains, the multiple antimicrobial resistance of these isolates makes them responsible for high mortality rates in compromised patients (13). In hospital units, these strains are spread by patients, staff, and environmental factors (5). Strains need to be distinguished from each other for epidemiological purposes. Convenient markers have already been proposed, and the methods include lysotyping (4), serotyping (15), capsular typing (20), numerical analysis of electrophoresis protein patterns (6), restriction endonuclease digestion of chromosomal DNA (11), and restriction analysis of plasmid DNA (10, 12). Two features are generally expected from these systems: stability and polymorphism.

The aim of this work was to evaluate the polymorphism provided by two chromosome-based epidemiological marker systems, pulsed-field gel electrophoresis (PFGE) (2, 8, 14, 17, 19) and ribotyping (7, 9, 22), which both offer the advantages of chromosomal stability and applicability to all bacterial species. A collection of 239 MRSA strains isolated over 2 years was first classified according to the PFGE patterns of the strains. Of these isolates, 81 were further analyzed by ribotyping in an attempt to compare the polymorphisms of the two markers, to establish a possible link between the two markers, and to distinguish strains exhibiting the same PFGE fingerprint.

MATERIALS AND METHODS

Bacterial strains and identification. *S. aureus* organisms were identified as gram-positive cocci that were facultative anaerobes producing free coagulase (tested by the tube coagulase test with reconstituted citrate rabbit plasma [Biomérieux, Marcy l'Etoile, France]), and acetoin and that were deficient for β-galactosidase production. Testing for methicillin resistance was performed by inoculating Mueller-Hinton broth (Oxoid)–5% NaCl agar plates according to the recommendations of Barry and Thornsberry (3). Resistance to methicillin was recorded when the diameter of the zone of inhibition was less than 20 mm for 5-μg oxacillin disks, as defined by the French Committee for Antibiogram of the French Society for Microbiology (1). MRSA isolates were kept frozen in brain heart infusion medium containing 10% horse serum and 10% glycerol.

Over 2 years, 239 MRSA isolates were collected from 141 patients in different care units of two hospitals (in Strasbourg and Villeneuve-Saint-Georges, France). The sampling procedure, the number and distribution of isolates, and the number of patients are indicated in Table 1. A group (group IV) of 32 isolates collected in Villeneuve-Saint-Georges Hospital was considered a control group, since that hospital and Strasbourg University Hospital are remote and do not share the same patients. No outbreak of MRSA was observed during the study; thus, there were no epidemiologically related isolates, only multiple isolates collected from single patients. The number of multiple isolates per patient is recorded in Table 2.

DNA preparations for PFGE analysis. The isolates were grown overnight at 37°C in 100 ml of 2× YT medium (18) to a density of 0.4 (measured as A_{600}). One milliliter of 0.5 M EDTA (pH 8.0) was added to the culture, and 25 ml of the bacterial suspension was centrifuged at 5,000 × *g* for 10 min at 4°C, washed, and resuspended in 1 ml of 10 mM Tris-HCl–5 mM EDTA–1 M NaCl (pH 8.0). The bacterial suspension was then mixed with 1.5 ml of deionized low-melting-point agarose (Appligène, Strasbourg, France) maintained at 50°C. The mixture of cells and agarose was poured into the slots of a plastic mold (25 by 3 by 3 mm) and cooled. The agarose plugs were then transferred into sterile tubes containing 1 ml of lysis buffer (6 mM Tris-HCl, 100 mM EDTA, 1 M NaCl, 0.5% Brij 58 [wt/vol], 0.2% sodium deoxycholate [wt/vol], 0.5% lauroyl sarcosine [wt/vol] [pH 7.6]) supplemented with 50 μg of lysostaphin (Sigma) per ml and 500 μg of lysozyme (Sigma) per ml. Lysis of the bacterial envelopes was performed overnight at 0°C. Lysis buffer was then replaced with 1 ml of proteolysis buffer [0.25 M EDTA, 20 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 1% lauroyl sarcosine (wt/vol) (pH 9.0), 250 μg of proteinase K (Sigma) per ml]. Digestion was performed for 24 h at 60°C with a change of proteolysis buffer after 12 h. Cell debris and proteinase K activity were eliminated by three washes in 5 ml of 10 mM Tris-HCl–0.1 mM EDTA (pH 8.0) (TE) buffer–1 mM phenylmethylsulfonyl fluoride (pH 8.0) for 10 min at 4°C. Before DNA

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TABLE 1. Distribution of the 239 MRSA isolates

Origin of isolates[a]	Period of collection	Type (no.) of hospital care unit	Source of sampling (no. of patients)	No. of isolates
I. Strasbourg	1988	All types	Infected patients (33)	35
II. Strasbourg	1989 (August)	Intensive care (4)	Infected patients (61)	100
III. Strasbourg	1989 (October)	Intensive care (1)	Weekly systematic sampling (15)[b]	72
IV. Villeneuve-Saint-Georges	1989	All types	Infected patients (32)	32

[a] Isolates identified by group number and hospital of origin.
[b] Sampling from hands, anterior nares, and tracheal aspirations (17).

hydrolysis, the agarose plugs were equilibrated in TE buffer (three washes for 10 min at 4°C). Plugs could be stored at 4°C for 12 months as long as the TE buffer was changed every month.

DNA fingerprinting by transverse alternating PFGE was carried out after *Sma*I restriction of bacterial DNA. For restriction analysis, plugs (5 by 3 by 3 mm) were equilibrated for 30 min in 300 μl of hydrolysis buffer and then digested in 60 μl of the corresponding buffer with 20 U of restriction endonuclease. All enzymes, including *Apa*I, *Nar*I, *Sma*I (all purchased from Boehringer GmbH, Mannheim, Germany), and *Eag*I (Biolabs Laboratories, Beverly, Mass.) were used according to the manufacturers' recommendations. Electrophoretic runs were performed at 13°C with a Beckman Geneline transverse alternating field electrophoresis (TAFE) system at 150 mA in 0.6× TAFE running buffer (20× TAFE is 200 mM Tris, 0.5 mM free-acid EDTA, and 87 mM acetic acid [pH 8.2]). For the separation of *Sma*I and *Eag*I DNA fragments, each run began with 4-s pulses for 1 h, followed by 8-s pulses for 10 h, 20-s pulses for 3 h, and 8-s pulses for 4 h. For the separation of *Apa*I and *Nar*I DNA fragments, the runs were performed at 120 mA in 0.8× TAFE for 1 h with 4-s pulses and then for 8 h with 6-s pulses and 12 h with 10-s pulses. Gels were stained for 30 min with ethidium bromide (5 μg/ml), washed further in water, and photographed under UV light.

Designation of different PFGE patterns. We studied the within- and between-day reproducibility of the 26 PFGE fingerprints (see Fig. 1) by scanning negative photographs with an LKB 2222-020 UltroScan laser densitometer (LKB-Pharmacia, Uppsala, Sweden). Absorbance was recorded at 8-μm intervals along the gel, yielding 1,250 values per 50 mm of gel. The absorbance range was set from 0.2 to 1.2 absorbance units (full scale). A spot beam was used to scan each lane three times. The peak with the highest molecular weight was taken as a reference origin to avoid the inaccuracy resulting from an imprecise location of the migration origin. The resulting values were recorded by using LKB Gelscan XL laser densitometer software (LKB-Pharmacia). Under these conditions, peaks with more than 80 kb were recorded and both their average distances and their standard deviations were estimated. The standard deviation values ranged from 2 to 5%. We considered PFGE patterns different (i) when they had the same number of DNA fragments but when the size of at least one band varied by more than 1 standard deviation (5%) (this allowed the distinction of the different PFGE patterns visible to the naked eye), (ii) when they exhibited a different number of DNA fragments, or (iii) when the sum of the sizes of the differing bands in the first PFGE pattern did not correspond to that of the differing DNA fragments in the second PFGE pattern.

TABLE 2. Distribution of patients with single or multiple MRSA isolates

Isolate group	No. of patients with the following no. of isolates:		
	1	2	≥3
I	31	2	0
II	36	15	9
III	2	0	13
IV	32	0	0

Ribotyping. Ribotyping was performed as described previously (9). The intact DNA that was previously included in agarose for PFGE analysis was restricted for 4 h at 37°C by *Cla*I or *Eco*RI or *Hin*dIII restriction endonuclease, which was used according to the manufacturer's recommendations (Boehringer GmbH). Restriction fragments were separated overnight by conventional electrophoresis (2 V/cm) on a 0.8% agarose gel in 0.5× TEB (10× TEB is 0.89 M Tris, 0.89 M boric acid, and 25 mM $EDTANa_2$ [pH 8.3]) and transferred onto an Immobilon P membrane (Millipore) with 0.5 M NaOH by the method of Southern (21). Prehybridization was performed with 6× SSPE (1× SSPE is 0.18 M NaCl, 10 mM $NaPO_4$, and 1 mM EDTA [pH 7.7]) (18), 5× Denhardt's solution (18), and 0.1% (wt/vol) sodium dodecyl sulfate (SDS) at 50°C for 2 h. *Escherichia coli* 16S and 23S rRNAs previously dephosphorylated with calf intestine alkaline phosphatase (Boehringer GmbH) were 5′ labeled with [γ-^{32}P]ATP by T4 polynucleotide kinase. The hybridization between staphylococcal DNA and *E. coli* rRNA was performed overnight at 60°C in the prehybridization solution supplemented with 2.5×10^6 dpm (50 ng) of labeled rRNA per 20 ml and per membrane. After the hybridization step, the filters were washed twice in 2× SSPE–0.1% (wt/vol) SDS at 50°C and twice in 0.1× SSPE–0.1% (wt/vol) SDS at 30°C for 10 min each wash. Hybridized bands were visualized by autoradiography at −70°C with intensifying screens by using noninterleaved films (NIF) (3M) for a maximum of 24 h.

RESULTS

PFGE fingerprints. PFGE analysis of 239 MRSA isolates with *Sma*I revealed 26 different patterns (Fig. 1). As reported previously (17), the different PFGE patterns were stable even after 40 subcultures of the corresponding isolates on blood agar plates. The standard deviation values were 2 to 5% for various DNA fragments. For example, patterns 21 and 22 were determined to be different, with three bands differing in size (410 versus 440, 350 versus 325, and 230 versus 210 kb, respectively). PFGE patterns 13 and 14, which differed only in the location of a single DNA fragment (200 and 150 kb, respectively), were considered different because the difference in length (50 kb) between the two fragments was more than 5%. PFGE fingerprints 3 and 4 (and 22 and 25) were also considered different because pattern 4

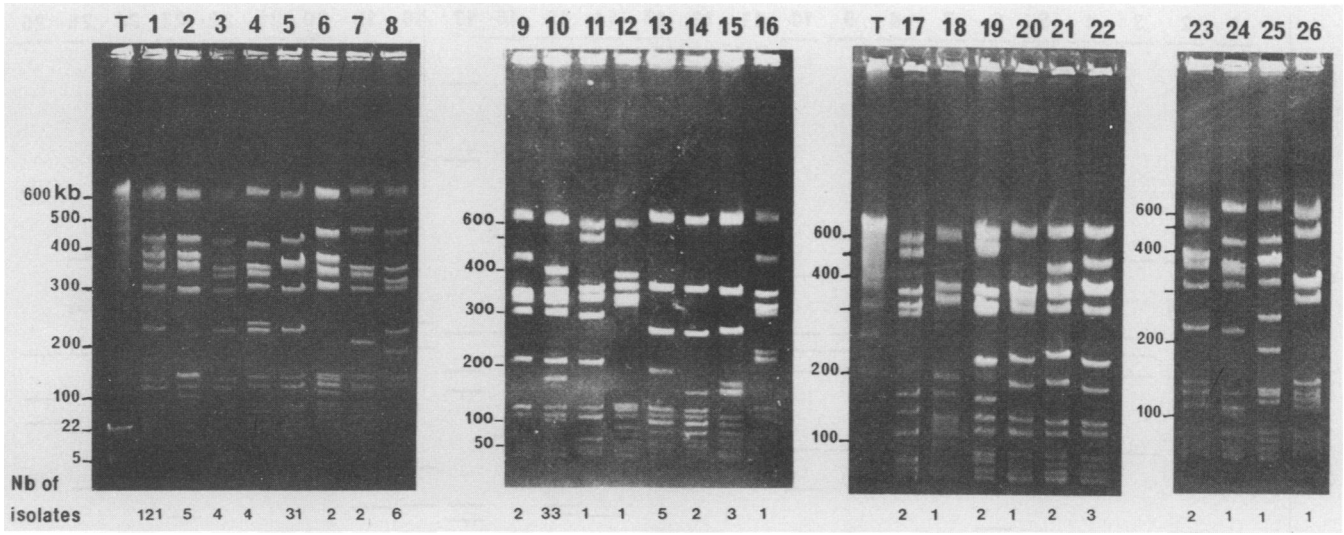

FIG. 1. DNA fingerprint analysis of MRSA isolates by transverse alternating PFGE. Chromosomal DNAs were digested with *Sma*I restriction endonuclease, and the fragments were separated by PFGE. Molecular masses in kilobases were obtained from bacteriophage lambda DNA concatemers (FMC Products) (lanes T). DNA fragments of 22 and 6 kb were from lambda DNA digested by *Eco*RI and *Hin*dIII restriction endonucleases. The different fingerprints identified from the analysis of 239 MRSA isolates are numbered (lanes 1 to 26). The number of strains analyzed for each fingerprint is listed below each pattern.

(and pattern 25) had an additional DNA fragment of 240 kb (135 kb for pattern 25) compared with PFGE pattern 3 (and pattern 22).

The multiple dialysis steps required for DNA preparation allowed greater release of small nucleic acids from the agarose plugs. This may be the reason plasmid DNA was not detected by PFGE. The fingerprints obtained were, therefore, specific for chromosomal DNA (17). PFGE patterns with large-molecular-size DNA fragments also were obtained after digestion with other restriction endonucleases (i.e., *Apa*I, *Eag*I, and *Nar*I). Four isolates representing the eight most frequent PFGE patterns (Fig. 2) were analyzed by *Eag*I restriction before and after 40 subcultures. As for *Sma*I restriction, stability of the marker was observed. There was no polymorphism shown by testing *Eag*I, *Apa*I, and *Nar*I which was greater than that of *Sma*I (Fig. 3). Moreover, *Apa*I digestions produced more than 20 DNA fragments which were not well separated after PFGE (Fig. 3); complete digestion with *Nar*I endonuclease was difficult to obtain. Figures 3 and 4 show that the strains displaying *Sma*I PFGE fingerprints 13, 14, and 15 (Fig. 3A, lanes 1 through 3, respectively) also exhibit different *Eag*I PFGE fingerprints (Fig. 3A, lanes 4 through 6, respectively). Four strains that exhibited a single *Sma*I PFGE pattern (pattern 1) (Fig. 3B, lanes 1 to 4) could not be resolved into separate *Eag*I (Fig. 3B, lanes 5 to 8), *Apa*I (Fig. 3B, lanes 9 to 12), or *Nar*I (Fig. 3B, lanes 13 to 16) patterns. Therefore, the polymorphism obtained for MRSA with the restriction endonucleases *Apa*I, *Eag*I, and *Nar*I was not greater than that obtained with *Sma*I. These results seem to indicate a homologous organization of the chromosome for strains displaying a given PFGE profile, particularly PFGE fingerprint 1. *Sma*I restriction was preferred to *Apa*I, *Eag*I, and *Nar*I restriction because the DNA fragments obtained with it ranged from 30 to 700 kb and provided the most readable fingerprints.

When multiple isolates from a single patient were analyzed, two observations were noted. First, with four patients from whom multiple MRSA isolates (8 to 16 samples) were obtained during 2-week periods, no changes in PFGE profiles (patterns 1 and 10) were detected. Second, three other patients were demonstrated to carry multiple MRSA isolates with different PFGE profiles (patterns 1, 5, and 10), with each type unique to a given body site (hand, anterior nares, or tracheal aspiration). This observation was repeatedly confirmed over 2 weeks, and the definitive replacement of one PFGE pattern by another was never observed.

As shown in Fig. 2, the number of *Sma*I fragments in each fingerprint ranged from 11 (i.e., fingerprints 12 and 17) to 14 (i.e., fingerprints 6, 13, and 14). Nevertheless, 26 fingerprints displayed the 35- and 50-kb fragments, 23 contained the 85- and 135-kb DNA fragments, 22 contained the 115-kb DNA fragment, and 15 contained the 300-kb DNA fragment. There were only 10 fingerprints exhibiting these six restriction fragments (i.e., those with lengths of 35, 50, 85, 115, 135, and 300 kb). The sizes of the DNA fragments observed for each pattern allowed an estimation of the molecular size of the staphylococcal chromosomes. The sizes ranged from 2,200 to 3,100 kb (±100 kb) according to the strain.

Considering the classification of the 239 strains into 26 PFGE fingerprints, it appeared that fingerprints 1, 5, and 10 were the most frequently encountered, with fingerprint 1 being predominant (as shown in Fig. 1), representing 51% of the isolates. Fingerprints 5 and 10 accounted for 13 and 14% of these isolates, respectively. Strains bearing fingerprint 1 were isolated at rates ranging from 18 to 80%, depending on the hospital unit.

Ribotyping. In an attempt to determine whether another chromosome-based marker system could define subsets of isolates within a single PFGE profile, we analyzed 81 MRSA isolates by *Cla*I, *Eco*RI, and *Hin*dIII ribotyping. In the first step, 26 strains representing each of the 26 different PFGE fingerprints from 26 patients were randomly selected in order to test for a possible link between the two markers (Fig. 5). These 26 strains with distinct fingerprints were distributed in only four *Cla*I, six *Eco*RI, and 4 *Hin*dIII ribotypes (Table 3), schematically represented in Fig. 6. Most of the PFGE fingerprints were classified as *Cla*I ribotype 1 (C1), *Eco*RI ribotype 1 (E1), or *Hin*dIII ribotype 1 (H1), although the

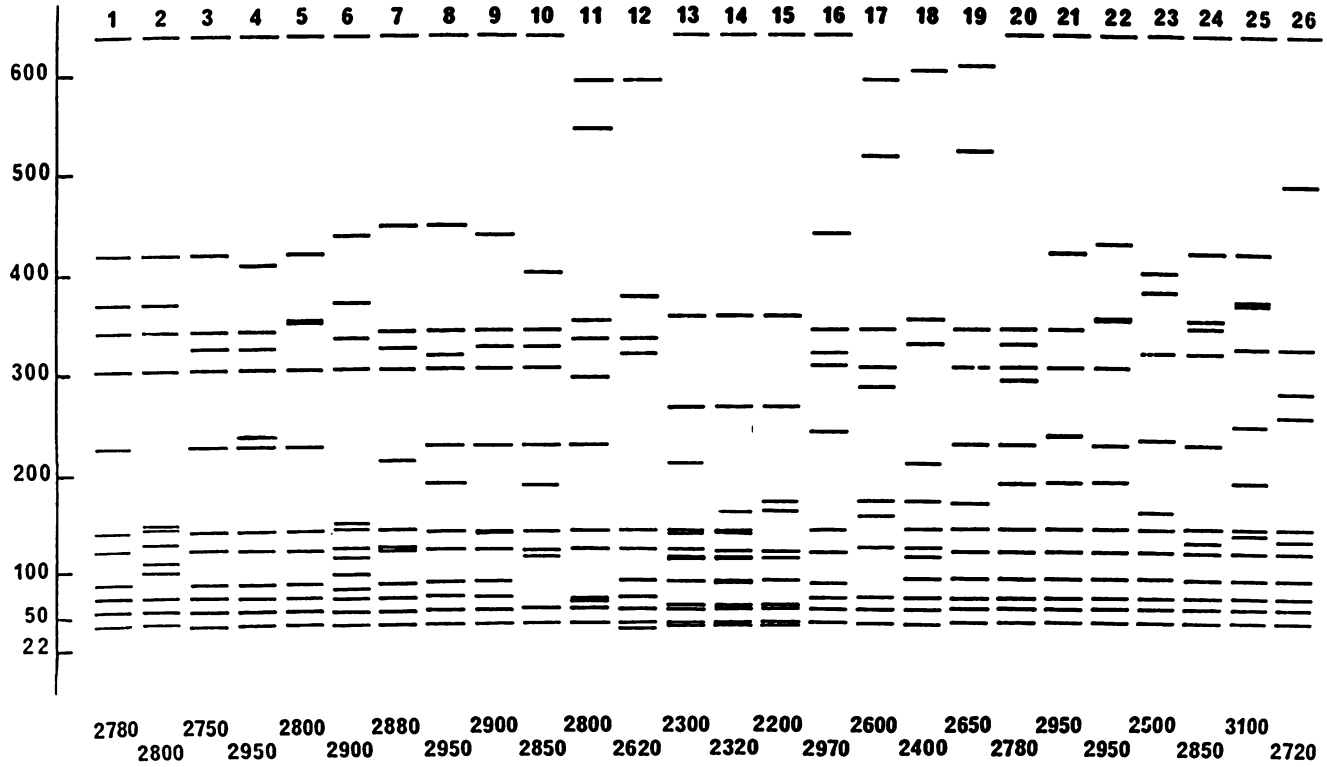

FIG. 2. Schematic representation of the 26 *Sma*I PFGE fingerprints numbered as in Fig. 1. The size (in kilobases [±100 kb]) of each chromosome according to PFGE analysis is shown at the bottom.

sizes of the corresponding chromosomes ranged from 2,400 kb (PFGE pattern 18) to 3,100 kb (PFGE pattern 25). The distribution of *Cla*I ribotypes within the PFGE patterns was identical to that of *Hin*dIII ribotypes (Table 3).

In the second step, we examined whether there were several ribotypes within a given PFGE profile. For this purpose, we analyzed 40% of each of the most frequently encountered PFGE profiles (patterns 1, 5, and 10) from the isolates collected from patients belonging to each of the four patient groups: 24 isolates of PFGE pattern 1, 9 isolates of

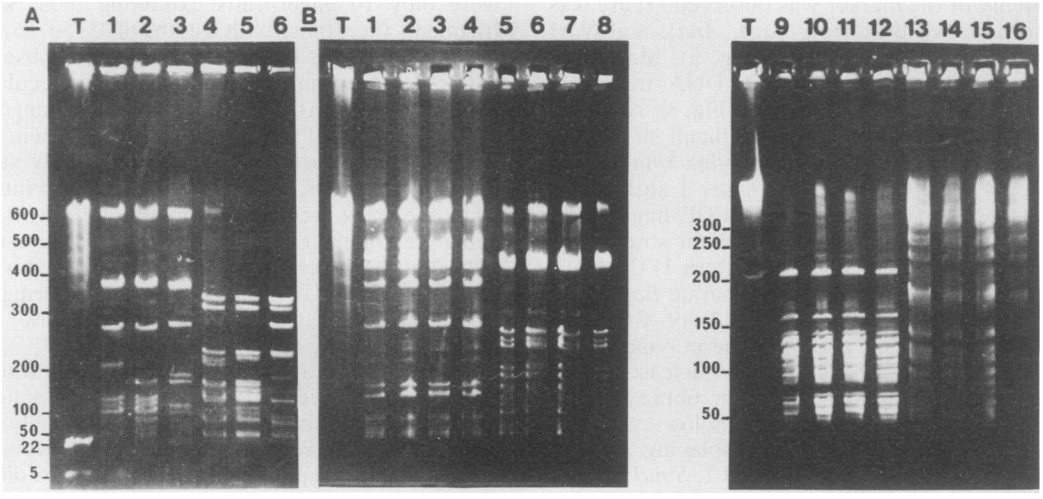

FIG. 3. PFGE after hydrolysis with restriction endonuclease *Eag*I of DNAs from MRSA isolates exhibiting *Sma*I PFGE fingerprints 13, 14, and 15 and from strains exhibiting *Sma*I PFGE pattern 1 (A) *Sma*I PFGE fingerprints 13, 14, and 15 (lanes 1 through 3, respectively) and the corresponding *Eag*I fingerprints (lanes 4 through 6, respectively). (B) Identical *Sma*I PFGE fingerprints (fingerprint 1) of four strains isolated from different hospital units (lanes 1 through 4) and the corresponding *Eag*I (lanes 5 through 8, respectively), *Apa*I (lanes 9 through 12, respectively), and *Nar*I (lanes 13 through 16, respectively) fingerprints. Molecular masses in kilobases were obtained from bacteriophage lambda DNA concatemers (lanes T). The 22- and 5-kb DNA fragments were obtained from lambda DNA digested by *Eco*RI and *Hin*dIII restriction endonucleases.

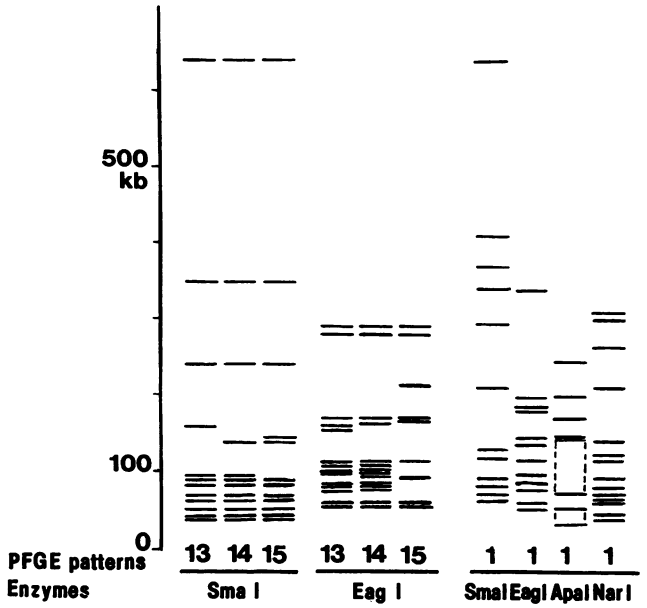

FIG. 4. Schematic representation of PFGE patterns. *Sma*I PFGE patterns 13, 14, and 15 and the corresponding PFGE patterns when the chromosomal DNAs were restricted by *Eag*I and the *Sma*I, *Eag*I, *Apa*I, and *Nar*I PFGE patterns of four epidemiologically unrelated isolates are shown. The lengths of fragments (in kilobases) are indicated. The boxed areas show the nonseparated DNA fragments.

PFGE pattern 5, and 9 isolates of PFGE pattern 10. Of these 42 strains, 3 (one each of PFGE profiles 1, 5, and 10) had been compared in the first step of this PFGE-ribotyping comparison. Despite the number of isolates tested, ribotyping could not discriminate among epidemiologically unrelated isolates belonging to the same PFGE profile. All the isolates tested exhibited *Cla*I ribotype 1 (C1), *Eco*RI ribotype 1 (E1), or *Hin*dIII ribotype 1 (H1).

Sixteen additional isolates, consisting of four isolates with PFGE pattern 1 from each of four patients, were analyzed. These isolates comprised four subsets of 4 epidemiologically related isolates. Again, ribotyping provided no further discrimination among the strains.

The results obtained by ribotyping indicated that (i) there was no link demonstrated between PFGE profiles and ribotyping, (ii) the polymorphism observed for ribotyping was not as great as that for PFGE, (iii) ribotyping was not related to the chromosome size, as determined by PFGE, and (iv) in our study, ribotypes did not discriminate within a given PFGE pattern of even epidemiologically unrelated isolates.

TABLE 3. Classification of the 26 *Sma*I PFGE fingerprints of MRSA strains by ribotype

*Sma*I PFGE fingerprint(s)	Ribotype[a]
1, 3, 4, 5, 8, 9, 10, 12, 17, 18, 20, 21, 22, 24, 25	E1
2, 7, 11, 16, 23	E2
19, 26	E3
13, 14	E4
6	E5
15	E6
1, 2, 3, 4, 5, 8, 9, 10, 12, 14, 15, 17, 18, 20, 21, 22, 24, 25	C1 or H1
7, 11, 16, 26	C2 or H2
19, 23	C3 or H3
6, 13	C4 or H4

[a] E, *Eco*RI; C, *Cla*I; H, *Hin*dIII.

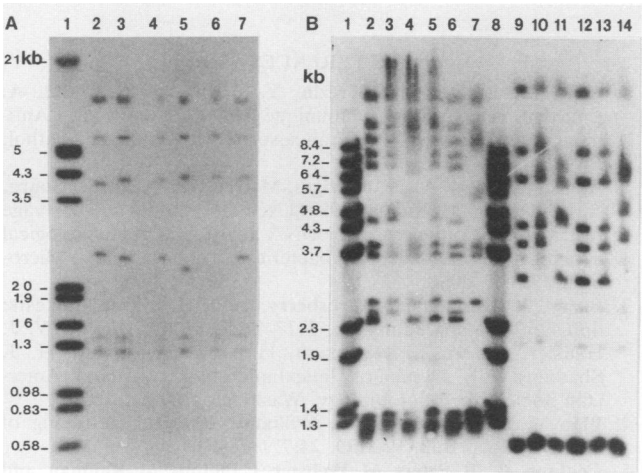

FIG. 5. Examples of the different *Cla*I, *Eco*RI, and *Hin*dIII ribotypes. (A) Epidemiologically unrelated isolates whose chromosomal DNAs had *Sma*I PFGE fingerprints 6, 8, 12, 16, 19, and 22 were analyzed for *Cla*I ribotyping (lanes 2 to 7, respectively). (B) Epidemiologically unrelated isolates whose chromosomal DNAs had *Sma*I PFGE fingerprints 10, 23, 26, 13, 22, and 6 were analyzed for *Eco*RI (lanes 2 to 7, respectively) or *Hin*dIII (lanes 9 to 14, respectively) ribotyping. The lengths of DNA fragments were obtained from 5′-labeled *Eco*RI-*Hin*dIII fragments of lambda DNA (panel A, lane 1) or from 5′-labeled *Bst*EII fragments of lambda DNA (panel B, lane 1).

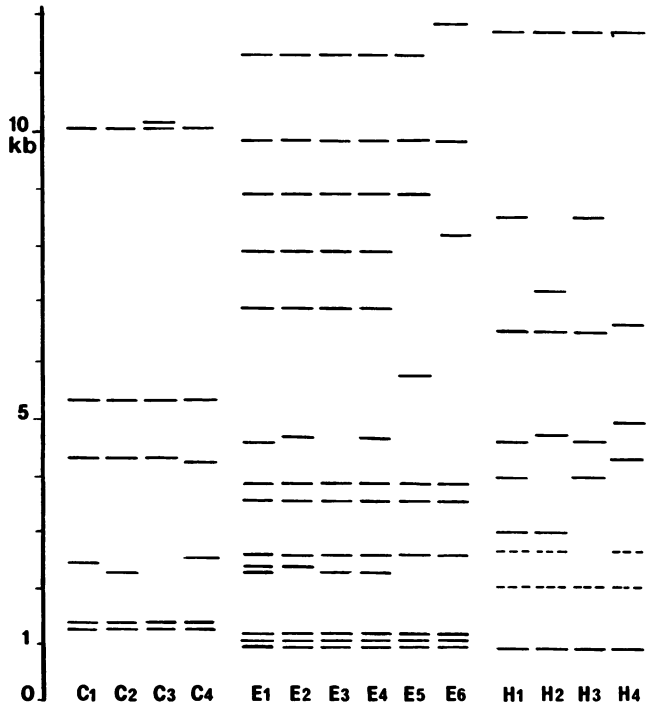

FIG. 6. Schematic patterns of the different *Cla*I (C1 through C4), *Eco*RI (E1 through E6), and *Hin*dIII (H1 through H4) ribotypes according to the length of each DNA fragment in kilobases.

DISCUSSION

PFGE fingerprints reflect the structural organization of bacterial chromosomes. The stability of the marker was demonstrated by the identification of the same PFGE patterns before and after 40 subcultures of several isolates (17). As described in Results, PFGE patterns 3 and 4 (or 22 and 25) differed only in the size of one fragment. Such a loss or acquisition of DNA fragments of 135 kb (or 240 kb) cannot be explained by a simple genetic event such as transposon or prophage excision or insertion. The only explanation of the loss of a large chromosomal segment would be a huge deletion, which should not influence the further viability of the bacteria. The most similar PFGE fingerprints (patterns 13 and 14) differed only in the length of one band representing about 50 kb. This difference could be the result of a prophage excision or insertion, but we never observed any change in PFGE patterns 13 and 14 after more than 40 subcultures of the corresponding isolates. The difference between these two isolates apparently did not result from the excision or insertion of a prophage belonging to the set of phages used for typing, since these isolates had the same lysotype (sensitivity to bacteriophages 79 and 80) (3). However, according to our definition of separate patterns and in the absence of observed in vitro variation, PFGE profiles 13 and 14 were considered different, thereby determining different strains. The comparison between PFGE profiles other than 1 and 3, 3 and 4, 1 and 5, 5 and 22, and 22 and 25 that also differed from each other by only one DNA fragment did not show any simple mechanism that would explain the transformation of the first fingerprint into the second. Similarly, in the four patients harboring MRSA isolates with two PFGE profiles at different body sites, there was no replacement of one PFGE pattern by the other over a 2-week interval. Since the analysis of strains isolated from Villeneuve-Saint-Georges Hospital (Table 1) allowed the characterization of seven more patterns than were previously identified in Strasbourg University Hospital, one could expect that the number of PFGE fingerprints for MRSA exceeds the 26 patterns reported here. However, in this study, strains displaying 3 of the 26 *Sma*I PFGE fingerprints (profiles 1, 5, and 10) were more frequently encountered than strains classified as having the other PFGE fingerprints. These three particular patterns were recovered at different frequencies in different hospital units.

PFGE profiles and ribotypes for 81 isolates were compared. In order to obtain sufficient polymorphism by ribotyping (7, 9, 21), we used several restriction endonucleases (*Cla*I, *Eco*RI, and *Hin*dIII). The numerous DNA fragments were separated by conventional electrophoresis and then hybridized with two rRNAs (i.e., 16S and 23S). Despite this methodology, ribotyping did not allow us to characterize subsets within epidemiologically unrelated isolates or related isolates with the same PFGE fingerprint. Moreover, 15 strains with different PFGE fingerprints were classified as ribotypes C1, E1, and H1 (Table 3). PFGE, therefore, appears to be more discriminating than ribotyping. Our ribotyping procedure differs from that previously described (7), since we used *E. coli* rRNA as a probe instead of the cloned 16S rRNA gene from *Bacillus subtilis*. Our rRNA gene patterns E1, E2, and E3 shared a high degree of homology with those (16) corresponding to strains STH 6444, Bri 6, and STH 1584, respectively. Again, our rRNA gene patterns H1 and H3 are very similar to those described for strains C8466 and STH 17956. The previous study (16) used biotinylated ribosomal cDNA (16S and 23S rRNAs) from *S. aureus* NCTC 10442 as probes. By comparison with ribotypes obtained by using the 16S rRNA gene from *B. subtilis*, there was a high degree of pattern similarity for DNA fragments of 1.1, 1.2, 2.6, 3.5, and 10 kb for *Eco*RI ribotypes and for DNA fragments of 1, 2, and 6.4 kb for *Hin*dIII ribotypes, as previously reported (7).

Ribotyping and PFGE typing are based on the same principle: the study of the distribution of restriction sites on chromosomal DNA. Ribotyping detects a single region of the chromosome (rRNA gene copies) that is well conserved among the bacteria. The sizes of the DNA fragments seen after hybridization with *E. coli* rRNA can vary among the isolates of a species and produce different ribotypes. This phenomenon is correlated with the variations of the restriction sites either within the rRNA genes or within the sequences surrounding this particular chromosomal region.

In contrast to ribotyping, PFGE detects the distribution of restriction sites throughout the chromosome. This distribution varies because of ancestral strain-to-strain mutational differences and because of the variations in the gene content of the staphylococcal chromosome. For example, several genes may or may not be present in the chromosome of a particular strain (e.g., the exfoliative toxin A gene, the enteroxin B and E genes, and the toxic shock syndrome toxin gene), leading to variable restriction patterns and chromosome sizes as shown by PFGE. It is clear, therefore, that PFGE profiles may offer greater discriminative power than ribotyping. This study underscores the suitability of PFGE for investigating MRSA-caused nosocomial infections.

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