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Random Amplified Polymorphic DNA Assay Is Less Discriminant than Pulsed-Field Gel Electrophoresis for Typing Strains of Methicillin-Resistant *Staphylococcus aureus*

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Twenty-six strains of methicillin-resistant *Staphylococcus aureus* with different pulsed-field gel electrophoresis fingerprints were tested by random amplified polymorphic DNA assay with three primers, resulting in 15 to 20 different random amplified polymorphic DNA fingerprints. By summing the results for the three primers, the number of different fingerprints increased to 25, but two strains could not be differentiated. We conclude that pulsed-field gel electrophoresis remains the best method of typing methicillin-resistant *S. aureus* strains.

Nosocomial infections caused by strains of methicillin-resistant *Staphylococcus aureus* (MRSA) are disquieting (4, 5), and control of such infections requires strain typing to identify the source of contamination. However, serotyping (23), lysotyping (3), capsular typing (29), and numerical analysis of electrophoresis protein patterns (8, 22) can be performed only in reference laboratories, because they require specific reagents or sophisticated equipment. On the other hand, the technology for total or plasmid DNA typing (11, 15, 16, 18, 20, 34) is gradually becoming available in most laboratories and can be used to type any microbial species. The ideal technique should be cost-effective and easy to perform. Its results should preserve a balance between increased discriminating power and applicability. In this respect, pulsed-field gel electrophoresis (PFGE) has successfully been used to separate large total DNA restriction fragments and generate what are termed pulsotype profiles (1, 7, 17, 25–27, 31). For MRSA, the best results were apparently obtained when the DNA was restricted with the *Sma*I enzyme (7, 17, 25, 31). This technique was more discriminant than ribotyping (25) with a universal ribosomal probe (9, 13, 14, 30).

Recently, a simple fast technique called the random amplified polymorphic DNA (RAPD) assay was proposed for genetic analysis (33). In this technique, single short primers with arbitrary nucleotide sequences are used in a polymerase chain reaction to amplify genomic DNA. The profiles obtained after electrophoretic separation of the amplification products can be used to fingerprint strains of various prokaryotic and eukaryotic species (6, 21, 33).

In the study described here, we compared the effectiveness of this RAPD assay with that of PFGE.

We used MRSA clinical isolates, numbered 1 to 26, which were previously shown to have different PFGE fingerprints (25). Strains were grown overnight at 37°C on Mueller-Hinton agar (BioMérieux, Charbonnières-les-Bains, France) and lysed as described previously (2), except that the last incubation with sodium dodecyl sulfate lasted for 1 h and the lysate was extracted, first with 1 volume of phenol-chloroform-isoamyl alcohol (25:24:1) and then with 1 volume of chloroform-isoamyl alcohol (24:1). Total bacterial DNAs were purified by ethanol precipitation and dissolved in a buffer containing 10 mM Tris-HCl (pH 7.6) and 0.1 mM EDTA. DNA concentrations were estimated on agarose gels.

The RAPD assay was performed as described previously (33), except that DNA was amplified in 50 μl of a modified solution containing 25 ng of template DNA, 100 nM primer, 2.5 U of AmpliTaq DNA polymerase (Perkin-Elmer/Cetus, Norwalk, Conn.), the four deoxynucleoside triphosphates (200 μM each; Boehringer Mannheim, Meylan, France), 4 mM $MgCl_2$, 15 mM Tris-HCl (pH 8.3), 40 mM KCl, and 100 μg of gelatin (Sigma Chemical Co., St. Louis, Mo.) per ml. The mixtures were overlaid with 60 μl of mineral oil and were subjected to 44 cycles of amplification (94°C for 1 min, 36°C for 1 min, and 72°C for 2 min for each cycle) in a thermocycler (Gene Ataq Controller; Pharmacia, Saint-Quentin-en-Yvelines, France) to which we added one cycle in which the incubation at 72°C lasted for 5 min and the first denaturation at 94°C lasted for 2 min. The primers (Organic Chemistry Laboratory, Institut Pasteur, Paris, France) used were AP2 (5'TCATGATGCA-3'; GC content, 40%), AP3 (5'-TCACGATGCA-3'; GC content, 50%), and AP5 (5'-TCACGCTGCG-3'; GC content, 60%). The products of the RAPD assay were separated by 0.8% agarose gel electrophoresis and were visualized after staining with ethidium bromide. Preliminary experiments showed good reproducibility of the profiles obtained by this technique (data not shown).

The number of bands obtained with each strain and the number of bands that each strain shared with other strains were both counted. The percentage of similarity between two strains was then estimated by using the coefficient of Dice (10). Dendrograms were constructed from the percentage similarity values by the unweighted pair group method with arithmetic averages (19, 28) and an adapted software kindly provided by M. Strulens (Hospital Erasme, Brussells, Belgium). A dendrogram was first plotted by using the percent similarities calculated from the previously published results for PFGE (25), and the dendrogram was then compared with the dendrograms obtained by using the results of the RAPD assay performed with primer AP2, AP3, or AP5. Lastly, we constructed a dendrogram using the combined

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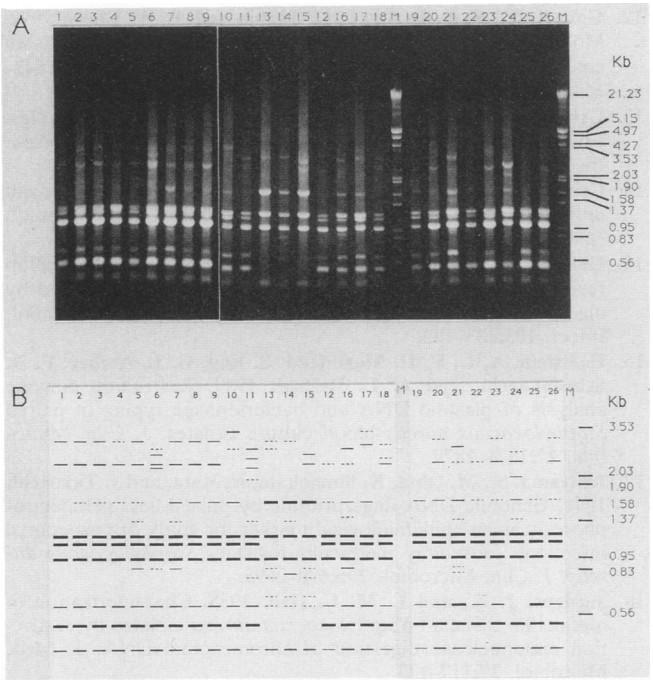

FIG. 1. Results of RAPD assay with primer AP2 on 26 MRSA strains. With PFGE, each strain had a different profile of *Sma*I restriction fragments. (A) Ethidium bromide-stained agarose gel. (B) Schematic representation of fragments of less than 3.53 kb. Strains are identified by numbers ranging from 1 to 26. Lanes M, size standards obtained by digesting bacteriophage λ with *Eco*RI and *Bam*HI.

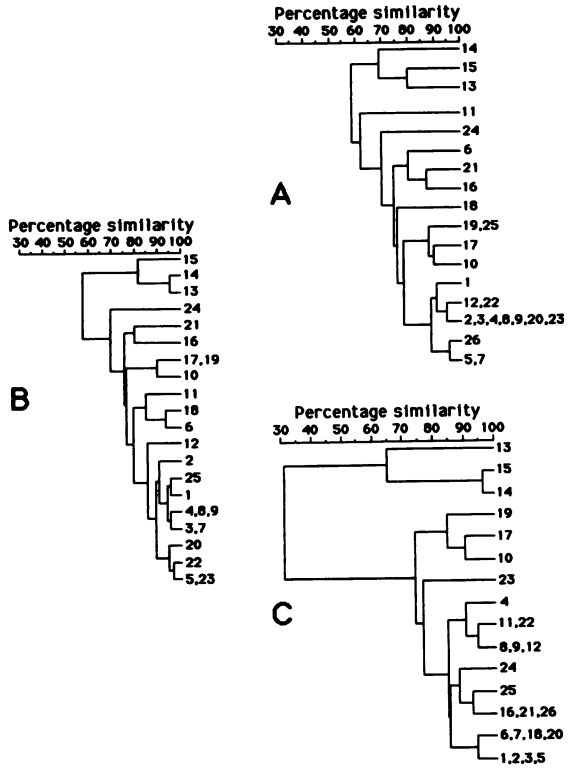

FIG. 2. Dendrograms showing estimates of the percent similarities among 26 MRSA strains on the basis of the individual results of the RAPD assay with primer AP2 (A), AP3 (B), or AP5 (C). Strains are identified by numbers ranging from 1 to 26.

results obtained with the three primers. This combination was obtained by simply summing the number of bands (total and common) obtained in the three RAPD assays.

Figure 1A shows, as an example, the RAPD fingerprints obtained with primer AP2. The number of amplified fragments ranged from 7 (e.g., strains 19 and 25) to 12 (e.g., strains 11 and 21) (Fig. 1B). Major bands were shared by all strains except strains 13, 14, and 15, which were clearly different. However, 17 different RAPD fingerprints could be observed when minor, less intense bands were taken into account. Twenty and 15 different RAPD fingerprints were obtained when primers AP3 and AP5, respectively, were used (data not shown).

Dendrograms constructed on the basis of these results showed that several strains could not be distinguished from each other (Fig. 2). In addition, the strains that clustered together sometimes differed, depending on which primer was used.

When calculations were made by using the summed results for the three primers, the number of different fingerprints increased to 25 (Fig. 3A). Only strains 8 and 9, which are indicated in a box in Fig. 3A, were clustered. However, when we compared these results with those obtained by PFGE of *Sma*I restriction fragments (Fig. 3B), we found that the average percent similarities between the strains were higher by the RAPD assay than by PFGE.

Because PFGE, the reference method used to type MRSA strains which are of a clonal nature (7, 22, 24), is time-consuming and difficult to perform, we evaluated the effectiveness of the RAPD assay, which has been reported to be both discriminant and easy to perform (6, 21, 33). Using a collection of 26 isolated clinical strains with different PFGE fingerprints (25), we found, first, that RAPD was less discriminant, whatever primer was used, since several strains could not be distinguished. Even when the results obtained with the three different primers were used, two strains were

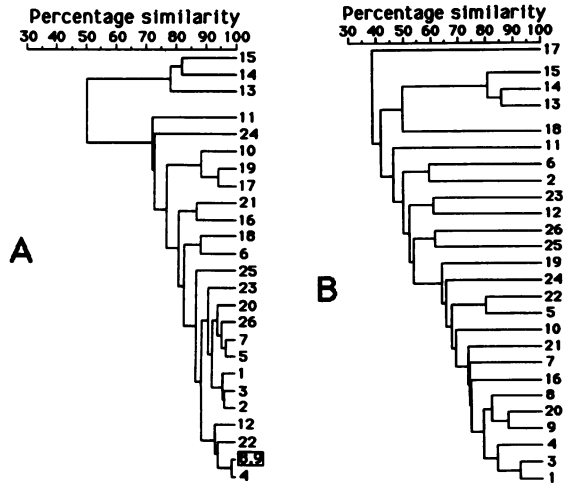

FIG. 3. Dendrograms showing estimates of the percent similarities among 26 MRSA strains by using the summed results of the RAPD assays with the three primers AP2, AP3, and AP5 (A) and previously published results (25) for PFGE of *Sma*I restriction fragments (B). Strains are identified by numbers ranging from 1 to 26.

clustered. Second, the average percent similarity between strains was higher by the RAPD assay than by PFGE. Third, although the RAPD assay is easy to perform with a single primer, it becomes more cumbersome when the number of primers increases. Lastly, the intensities of the discriminating bands obtained with the RAPD assay were often weak, making the analysis of the different profiles difficult.

The arbitrarily primed polymerase chain reaction, a technique very similar to the RAPD assays was previously found to be useful for discriminating between species of staphylococci but not for differentiating between strains within a given species (32).

Recently, another method of MRSA strain typing was proposed; the method uses amplification of the 3′ end of the coagulase gene and subsequent restriction enzyme digestion of the polymerase chain reaction product(s) (12). However, its results have not yet been compared with those of PFGE.

In conclusion, the RAPD assay is a technique in which minimum changes in primer sequences or amplification conditions can result in dramatic changes in the profiles that are obtained (25). Therefore, other results might have been obtained under conditions different from those that we used. However, our results suggest that PFGE of *Sma*I restriction fragments remains the best method of typing MRSA strains.

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