

Typing of *Staphylococcus aureus* by PCR for DNA Sequences Flanked by Transposon Tn916 Target Region and Ribosomal Binding Site

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The continuous intra- and interhospital spread of multiresistant *Staphylococcus aureus* demands a rapid molecular typing system. This study describes the fingerprinting of *S. aureus* by PCR amplification of DNA sequences flanked by the target site for transposon Tn916 and the ribosomal binding site and neighboring nucleotides (target 916–Shine-Dalgarno PCR [tar 916-shida PCR]). Both starting points for PCR are known to be randomly distributed on the *S. aureus* chromosome. By use of *Sma*I-macrorestriction patterns as the reference method it was shown that this PCR genotyping discriminates among strains of the major clonal groups of the species *S. aureus* (strains with phage patterns 29,+ , 94,96, and 95 as well as group II and group III patterns) and identifies the six epidemic methicillin-resistant *S. aureus* strains prevalent in German hospitals. All of the investigated strains including methicillin-sensitive *S. aureus* were typeable. Tar 916-shida patterns are stable during the dissemination of epidemic methicillin-resistant *S. aureus* among different hospitals.

During the last 5 years genomic fingerprinting of *Staphylococcus aureus* became a powerful tool for epidemiological typing (6); especially, macrorestriction patterns were found to be discriminative (for an overview, see references 11, 14, and 18) and stable (20). This method, however, is rather time-consuming and labor-intensive. The use of PCR for genetic typing of medically important bacteria was meanwhile described in a large number of reports, especially for arbitrarily amplifying variable regions of the bacterial genomic DNA (AP-PCR) (for a review, see references 15 and 16). For *S. aureus* the individual use of three different primers resulted in sufficient strain discrimination by AP-PCR (16). Besides AP-PCR, PCR for detection of length polymorphisms of repetitive DNA sequences such as those of the coagulase gene was also described (13); this PCR, however, does not reach the resolution of macrorestriction patterns (13). The same was observed by us for PCR patterns of rRNA gene spacers (6). A sufficient degree of strain dissemination should be obtained by use of a sequence from the target site of a nonspecific transposon (because of location at different sites of the staphylococcal chromosome) and of a sequence present in nearly every reading frame as primers. For this we have chosen a sequence of the target site of multilocus transposon Tn916 (5) as one primer and the Shine-Dalgarno sequence and most frequent neighboring nucleotides (according to reference 10) as the other primer. This article reports the application of these primers for PCR fingerprinting of *S. aureus* strains of different epidemiological origins which also have been typed by *Sma*I macrorestriction patterns.

We have checked this method for its capacity for discrimination among strains belonging to the major clonal groups of the species *S. aureus* such as those exhibiting phage pattern 29,+ and producing toxic shock syndrome toxin 1 (6, 9), strains with phage pattern 94,96 (3), and strains with phage pattern 95 (12) and also for typing methicillin-resistant *S. aureus* (MRSA) disseminated over several federal German counties (17, 19).

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MATERIALS AND METHODS

Bacterial strains. *S. aureus* strains were chosen on the basis of the frequency distribution of phage patterns and related *Sma*I macrorestriction patterns. They were isolated in unrelated cases of infections in outpatients and in hospitals (strains 1 to 19); MRSA strains (strains 25 to 30) were isolated in outbreaks of nosocomial infections.

Strains 1 to 25 had the following phage patterns: strains 1 to 5, pattern 29,+; strains 6 to 10, pattern 94,96; strains 11 to 14, pattern 55,71; strains 15 to 19, pattern 95; strains 20 to 24, group III pattern. All these strains are methicillin-sensitive *S. aureus* (MSSA). Strains 10, 15, and 19 were completely sensitive to antibiotics.

MRSA strains 25 to 30 were isolated in epidemics in the following regions: 25, northern Germany; 26, southern Germany; 27, Hannover area; 28, southeastern Germany; 29, Vienna; 30, Berlin.

S. aureus strains from infections in surgical units were isolated from postoperative wound infections after clean surgery in a surgical unit of a district hospital.

Phage typing and *Sma*I macrorestriction patterns. Bacteriophage typing was performed by the method described in reference 1; for *Sma*I macrorestriction patterns the protocol described previously was used (18).

DNA isolation. Ten colonies from an overnight culture on nutrient agar plates were suspended in 150 μ l of TGE buffer (25 mM Tris-HCl, 50 mM glucose, 10 mM Na₂-EDTA); 75 μ l of lysostaphin (100 μ g/ml in TGE) was added, and the sample was incubated at 37°C for 1 h; 1 ml of a 4 M guanidine isothiocyanate solution (freshly prepared) was added; and after a brief vortexing 10 μ l of a Celite suspension was added. The Celite suspension was prepared as described elsewhere (2) by use of Celite 545 from Serva. The sample was vortexed for 15 s and afterwards was incubated at 4°C for 3 h. After centrifugation for 5 min in a microcentrifuge (\sim 13,000 \times g) the pellet was subjected to the following washing procedure: once with 1 ml of TGE, twice with 1 ml of 25 mM Tris-HCl (pH 7.2), twice with 1 ml of 70% ethanol, and once with 1 ml of acetone. The pellet was resuspended in 20 μ l of 10 mM Tris-HCl with 1 mM EDTA (pH 8.0) and incubated at 56°C for 1 h. After centrifugation at 4°C at 36,000 \times g the supernatant was stored at -20°C . One microliter of the supernatant was used as template for PCR (\sim 10 ng of DNA).

DNA amplification. The PCR mixture consisted of approximately 20 ng of template DNA, 100 pmol of each primer, 200 μ M each deoxyribonucleotide, 2 to 5 U of the polymerase (Replitherm [Biozym, Markoldendorf, Germany] and *Taq* polymerase [Appligene]), and an appropriate buffer. The cycling scheme consisted of 94°C for 2 min, 25°C for 1 min, and 72°C for 1 min; 94°C for 30 s, 25°C for 30 s, and 72°C for 30 s 29 times; and 94°C for 30 s, 25°C for 30 s, and 72°C for 4 min. Primers used were AGAGAGCTATTTTA for the target site of Tn916 (3) and AAAGGAGGAATTA for the Shine-Dalgarno sequence and neighboring nucleotides (8). PCR products were detected by electrophoresis in 1.4% agarose gels and subsequent ethidium bromide staining.

Documentation of PCR and similarity analysis of amplicon patterns. Agarose gels were documented and image processed by the MWG-Biotech restriction fragment length polymorphism Scan system. Cluster analysis started from patterns of molecular masses of PCR products for each of the investigated strains which were stored in the data bank system. This database is implemented in

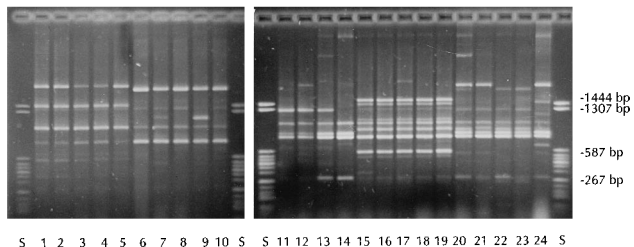


FIG. 1. PCR for 24 strains belonging to major clonal groups of *S. aureus*. S, molecular mass standard.

MS-Access version 2.0. The cluster analysis is based on an optimizing similarity algorithm (4).

RESULTS

Discrimination of strains belonging to the major clonal groups of *S. aureus*. As shown in Fig. 1, each group of the investigated strains exhibits a characteristic pattern of PCR products. Whereas strains with phage pattern 29,+ and strains with phage pattern 95 exhibit rather uniform target 916-Shine-Dalgarno (tar 916-shida) patterns, strains exhibiting phage pattern 55,71, strains with phage pattern 94,96 and strains reacting with group III phages can be further differentiated within each group. A corresponding dendrogram of similarity based on amplimers with common molecular masses is presented in Fig. 2.

Discrimination among epidemic virulent MRSA strains. In Germany MRSA strains from the majority of outbreaks of nosocomial infections can be differentiated into six clonal groups which exhibit characteristic *Sma*I macrorestriction pat-

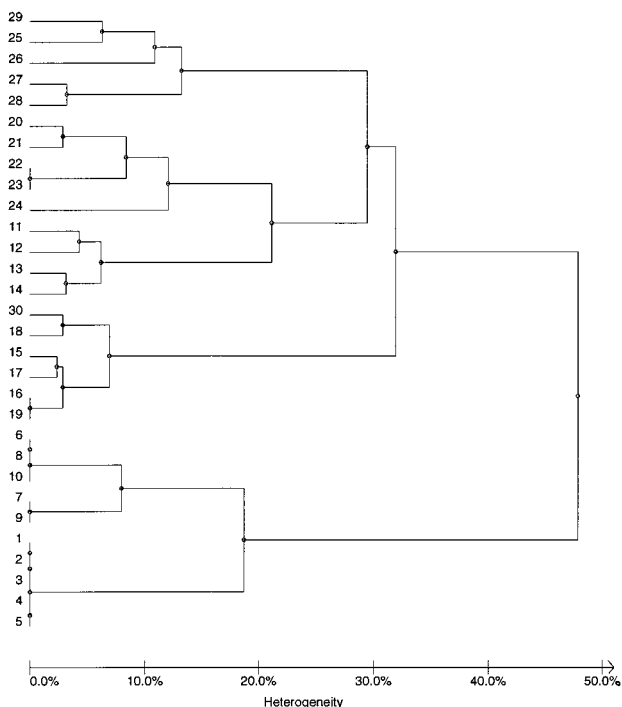


FIG. 2. Similarity dendrogram of amplimer patterns obtained by PCR for the *Tn*916 target site and ribosomal binding site for 24 strains belonging to major clonal groups of *S. aureus* and representative epidemic MRSA strains (strains 25 to 30).

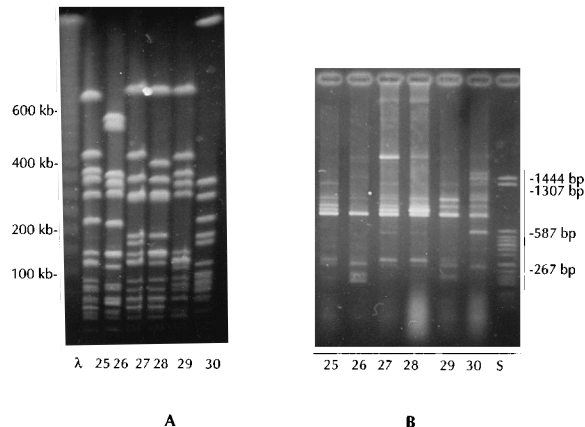


FIG. 3. *Sma*I macrorestriction patterns (A) and PCR patterns (B) for epidemic MRSA (see Materials and Methods). λ, genome of phage λ as a molecular mass standard; S, molecular mass standard.

terns (Fig. 3A). Each of these strains is also characterized by a specific PCR pattern (Fig. 3B).

Typing of MRSA strains belonging to definite clonal groups from infections in different hospitals. Stability of molecular fingerprints along with interhospital dissemination of multiresistant *S. aureus* is an important prerequisite for use in epidemiology. *Sma*I macrorestriction patterns and tar 916-shida patterns were identical for six isolates of the northern German epidemic MRSA, each from an outbreak of infections in a different hospital. The same was found for nine isolates of the southern German epidemic MRSA from nine hospitals and also for nine isolates of the Berlin epidemic MRSA from nine Berlin hospitals (data not shown in detail).

Typing of MSSA from infections in a surgical unit. Within a period of 3 weeks eight *S. aureus* isolates were obtained from postoperative wound infections in eight patients and sent for typing. Isolates 1, 3, 4, 5, 7, and 8 were resistant only to penicillin; isolates 2 and 6 were sensitive to antibiotics.

Isolates 3, 5, and 6 have similar *Sma*I macrorestriction patterns which are identical for isolates 3 and 5; tar 916-shida patterns of these isolates are also similar and identical for

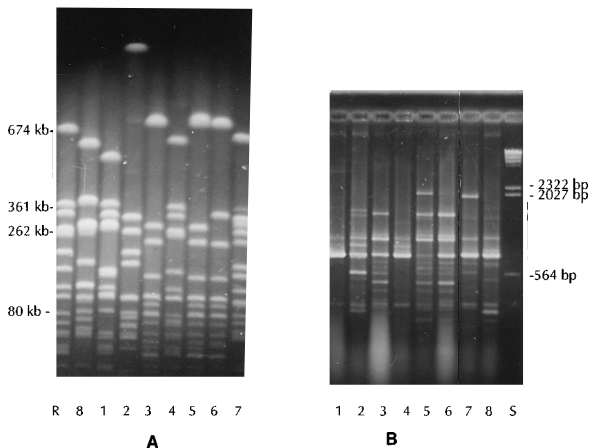


FIG. 4. *Sma*I macrorestriction patterns (A) and PCR patterns (B) for *S. aureus* strains isolated from wound infections in a surgical ward. R, *Sma*I digest of *S. aureus* 8325 as a standard; S, molecular mass standard.

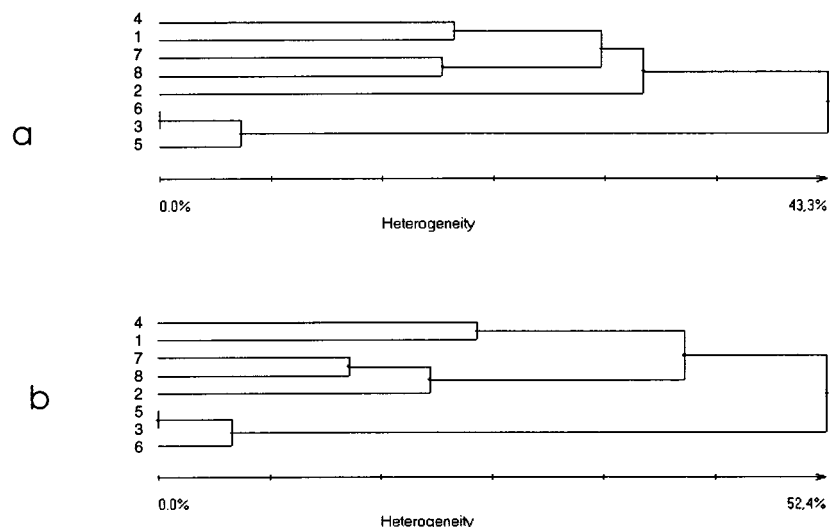


FIG. 5. Similarity dendrograms of *Sma*I macrorestriction patterns (a) and of amplicon patterns of tar 916-shida PCR (b) for eight MSSA strains from wound infections in a surgical ward. The corresponding gels are shown in Fig. 4.

isolates 3 and 6. Isolates 1, 2, 4, 7, and 8 are obviously different (Fig. 4).

Figure 5a shows a dendrogram of similarity based on common molecular masses of *Sma*I fragments; Fig. 5b shows a dendrogram based on common molecular masses of tar 916-shida amplicons. Both dendrograms result in the same clusters of isolates. Within the cluster of isolates 3, 5, and 6 the difference between the two typing methods in grouping isolate 3 with isolate 5 or with isolate 6 is clearly demonstrated.

Reproducibility of PCR patterns. PCR patterns are reproducible by use of at least three different preparations of genomic DNA (checked for strains 1 to 30) and independent from two different kinds of polymerases (Replitherm polymerase and *Taq* polymerase) used in these experiments (data not shown in detail).

DISCUSSION

The aim of this study was to establish molecular typing of *S. aureus* by means of a PCR-detected length polymorphism of DNA sequences flanked by a multilocus transposon target region and the ribosome binding site. The use of primers specific for the target site of Tn916 and for the *S. aureus* Shine-Dalgarno sequence and neighboring nucleotides (tar 916-shida PCR) resulted in reproducible amplicon patterns of sufficient discriminatory power. This is especially important for the discrimination of MRSA.

The amplicon patterns obtained by tar 916-shida PCR were stable for different MRSA clones among different hospitals.

MSSA can also be typed by tar 916-shida PCR, since sequences complementary to both of the primers are obviously common on the *S. aureus* chromosome and independent from antibiotic resistance determinants. The comparative typing of eight MSSA isolates by *Sma*I macrorestriction patterns and by tar 916-shida PCR resulted in the same clustering by dendrograms of similarity. Differences in grouping between the two techniques for isolates 3, 5, and 6 (Fig. 5b) might be due to a few independent genetic events. Theoretically, a chromosomal rearrangement in isolate 5 might have generated an additional configuration of the Tn916 target region and ribosomal binding site permitting PCR of the 2-kb DNA segment in between with no detectable influence of the distribution of *Sma*I cleav-

age sites. The difference in *Sma*I fragments of isolates 5 and 6 might be due to an insertion of 54 kb into the 281-kb fragment present in isolates 3 and 5 which leads to a 335-kb fragment in isolate 6.

This example demonstrates that dendrograms of similarity should be interpreted in the context of their methodological backgrounds and that differences within a cluster of probably related strains might be in the range of natural variability.

The use of a repetitive sequence from *Mycoplasma pneumoniae* which very likely has a random chromosomal distribution in *S. aureus* also was highly discriminative (7). Among methods applied to molecular typing of *S. aureus* until now, those screening the whole chromosome for polymorphisms are obviously superior to those examining definite regions. This was already described for the comparison between *Sma*I macrorestriction patterns and ribotyping (9) and was also the case in PCR typing by means of rRNA gene spacer polymorphism (6) and coagulase gene variation (13).

As already found by use of other typing systems based on genomic polymorphisms (*Sma*I macrorestriction patterns [15] and rRNA gene spacer patterns [6]), amplicon patterns of tar 916-shida PCR are also characteristic for clonal groups of *S. aureus* which are also defined by multilocus enzyme analysis (3, 9, 12). This is especially evident for the MRSA strain which also exhibits *Sma*I macrorestriction and rRNA gene spacer patterns similar to those of strains exhibiting phage pattern 95 (Berlin epidemic MRSA) and might be a derivative of this clonal group (6, 19). Other epidemic MRSA strains (strains 25 and 27 to 29) share PCR patterns with phage group III strains (especially for the three amplicons of 2,000, 880, and 820 bp) which correspond to phage and macrorestriction patterns (6).

Together with a simple method of template DNA extraction, tar 916-shida-PCR seems to be very useful for epidemiological typing of *S. aureus* in routine surveillance.

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