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# Determining the Genetic Structure of the Natural Population of *Staphylococcus aureus*: a Comparison of Multilocus Sequence Typing with Pulsed-Field Gel Electrophoresis, Randomly Amplified Polymorphic DNA Analysis, and Phage Typing

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**We used a sample of *Staphylococcus aureus* strains that are carried by humans and that are representative of the natural population of *S. aureus* strains in order to assess the value of multilocus sequence typing (MLST), pulsed-field gel electrophoresis, randomly amplified polymorphic DNA analysis, and phage typing as epidemiological tools. Only MLST was able to define clonal complexes unambiguously. All DNA-based typing approaches achieved a high degree of agreement, implying phylogenetic concordance, but predicted epidemiological associations with variable accuracy.**

*Staphylococcus aureus* is one of the most important pathogens in clinical settings. It is also one of the leading causes of nosocomial infections and the dissemination of multiple-drug-resistant strains, mainly methicillin-resistant *S. aureus* (MRSA), and the recent emergence of a vancomycin-resistant MRSA is of concern to hospitals worldwide. Naturally, an understanding of the dynamics of spread and an identification of transmissions or outbreaks are of interest not only to public health epidemiologists but also to clinical microbiologists and indeed clinicians involved in patient management on a daily basis. Most of the current epidemiological typing schemes compare fragment patterns generated by restriction or amplification of chromosomal DNA or by differences in lysis patterns based on susceptibilities to lytic bacteriophages. All of these approaches are able to distinguish between unrelated strains (albeit with different precision), but the probability with which two indistinguishable patterns predict an epidemiological relationship, i.e., transmission, cannot be determined without knowledge of the underlying genetic structure of the naturally occurring population. In order to describe the genetic structure of bacterial populations and the abundance of certain clones in a given environment, typing tools such as multilocus sequence typing (MLST) provide a comprehensive genetic framework (1, 6). We used MLST and three other conventional epidemiological typing approaches to describe the genetic population structure of strains that were systematically collected from nonhospitalized individuals in the community. This way we were able to objectively assess the value of typing techniques deployed for epidemiological purposes.

## MATERIALS AND METHODS

Strains of *S. aureus* carried nasally were isolated from a representative sample of nonhospitalized elderly individuals (≥65 years old) living in the Greater Nottingham Health District (Nottinghamshire, United Kingdom) (3). A random

TABLE 1. Comparison of typing results for 117 *S. aureus* strains carried by individuals in the community[a]

| Method | Typeability (%) | No. of types | Discriminatory ability (%) ($CI_{95}$)[b] | Overall correlation between methods | | | | Group violation (%) |
|---|---|---|---|---|---|---|---|---|
| | | | | MLST | PFGE | RAPD | Phage typing | |
| MLST | 100 | 46 | 95.7 (94.4–97.1) | 1.00 | | | | |
| PFGE | 100 | 57 | 97.6 (96.8–98.5) | 0.67 | 1.00 | | | 21.6 |
| RAPD | 100 | 16 | 85.7 (82.9–88.6) | 0.53 | 0.27 | 1.00 | | 49.4 |
| Phage typing | 76.1 | 23 | 80.9 (75.5–86.3) | 0.11 | 0.04 | 0.12 | 1.00 | 86.9 |

[a] Strains were not associated with an outbreak of disease.
[b] $CI_{95}$, 95% confidence interval.

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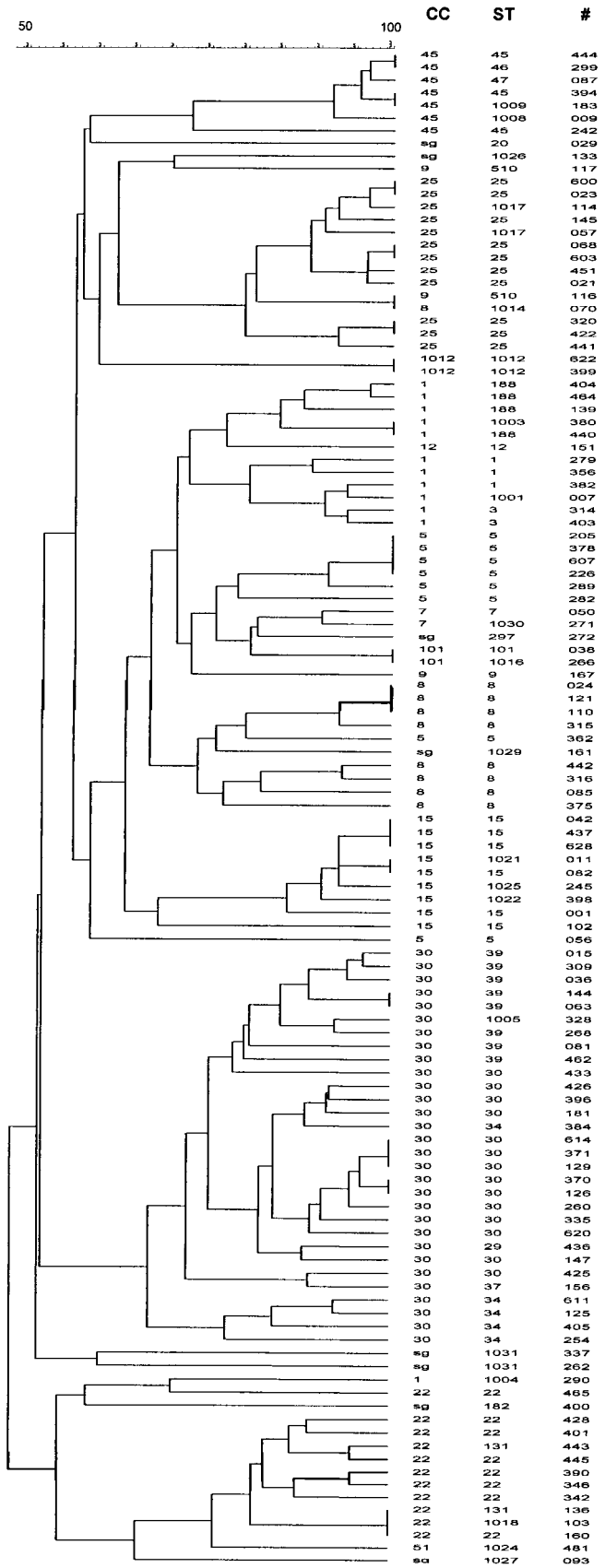


FIG. 1. UPGMA dendrogram illustrating similarity (based on Dice coefficients) of macrorestriction profiles (*Sma*I) (PFGE) of 117 isolates of *S. aureus* carried by individuals in the community. The scale at the top shows percent similarity. Abbreviations: CC, clonal cluster as defined by MLST and BURST analyses; ST, sequence type (MLST); #, isolate number; sg, singleton not grouped in a clonal cluster.

sample (117 of 257 of this strain collection) was subjected to four different typing techniques that included MLST, pulsed-field gel electrophoresis (PFGE) (*Sma*I macrorestriction analysis), randomly amplified DNA analysis (RAPD), and phage typing. All typing methods were performed by standardized published protocols (1, 9, 11), and genotypes were classified according to conventional criteria (1, 4, 7, 10). A standardized protocol for PFGE can be found at the Public Health Laboratory Service website (http://www.phls.org.uk/inter/harmony/New%20PFGE%20protocol.pdf). Typing results were stored, processed, and analyzed using the statistical suite of BioNumerics software (Applied-Maths, Ghent, Belgium). An index of diversity, defined as the probability with which two isolates chosen at random will be of a different type, was used to measure the frequency with which organisms of a particular type occur in the population of strains carried and serves at the same time as an unbiased estimate of the discriminatory ability of the relevant typing method (5, 8). Confidence intervals for discriminatory indices were calculated as previously described (2).

## RESULTS

**MLST.** MLST identified 46 different allelic profiles or sequence types (STs) among 117 strains carried by individuals in the general community. Sixteen STs were represented by more than one isolate. The genetic diversity as determined by MLST was 95.7% (95% confidence interval, 94.4 to 97.1%). We used Based Upon Related Sequence Types (BURST) analysis as a comprehensive method to classify different STs that demonstrate a recent phylogenetic relationship as described at the Multi Locus Sequence Typing website (www.mlst.net/BURST/BURSTREADME.htm). The classification was based on the data for 807 isolates of *S. aureus* for which MLST types were available by October 2001. Of the 117 total isolates, 105 could be assigned to a clonal complex, and the sample comprised all of the 13 clonal complexes identified so far. However, three clonal complexes were represented only by single isolates in our collection, and genotypes that did not correspond to any clonal complex were defined as singletons.

**PFGE.** *Sma*I macrorestriction analysis identified 57 PFGE types when three and more band differences indicated a different type. The ability of this method to discriminate strains carried by individuals in the community was 97.6% (96.8 to 98.5%) and was better but not significantly different from MLST (Table 1). Importantly, there was a high degree of concordance between the results by PFGE and MLST. Most isolates (98 of 105) assigned to clonal complexes by MLST and BURST analyses also clustered in the PFGE dendrogram after unweighted paired group mean analysis (UPGMA) clustering using band-based similarity values (Fig. 1), whereby PFGE patterns differed by a maximum of five bands within clonal complexes.

**RAPD.** DNA fingerprints generated with a single arbitrary primer identified 16 different RAPD types, resulting in a discriminatory capacity of 85.7% (82.9 to 88.6%). Thus, the ability to discriminate different strains by RAPD when using only one primer remained significantly restricted compared to those of PFGE and MLST. Still, there was a fair degree of agreement

between the pairwise similarity values generated by MLST and RAPD, as indicated by the high overall correlation value.

**Phage typing.** Of the 117 isolates, 28 were not typeable by the standard method with 23 phages of the Basic International Set at routine test dilution (typeability of 76.1%). The remaining isolates were grouped into 23 phage types corresponding to a discriminatory ability of 80.5% (75.1 to 85.8%). Notably, the overall correlation with the other typing techniques was low, indicating a poor concordance between phage typing and DNA-based approaches.

**Group violation.** Comparing all pairwise within group similarity values with between group similarity values allows an objective assessment of the robustness of the grouping of typing techniques. Within group similarity values equal to or less than those of between group values violate the group assignment. We calculated the percentage group violation as the proportion of pairwise similarity values that did not allow an unequivocal assignment to the clonal complexes defined by MLST and BURST analyses. We were thus able to determine to what extent results of typing techniques other than MLST are able to predict clonal complexes. PFGE allowed the best prediction, with 78.4% of all similarity values not in conflict with the classification; results for RAPD were fair (50.6%), whereas only 13.1% of phage typing data allowed an unambiguous prediction of the clonal complex (Table 1).

## DISCUSSION

We chose a computer-based algorithm that defines clonal complexes as groups in which each isolate is identical to at least one other isolate at five or more of the seven loci identified by MLST. This approach arrives at a rational classification by applying simple phylogenetic rules. Conversely, band-based typing techniques, such as macrorestriction analysis or RAPD, face difficulties when unequivocal types need to be assigned, as gradual changes in the positions of otherwise anonymous bands make a designation arbitrary. The genetic population structure of strains carried by individuals showed a predominance of a few clonal complexes. Moreover, the genetic diversity did not differ significantly from a sample of hospital- and community-acquired disease isolates recently collected in Oxfordshire (N. P. Day, personal communication). There is thus no evidence from MLST contradicting the assumption that isolates from cases of invasive disease represent a random sample of strains from the reservoir of *S. aureus* strains carried nasally in humans. By addressing the frequencies of naturally occurring strains carried by humans, we were also able to determine the positive predictive value with which each typing method can identify an epidemiological association (such as person-to-person transmission) when a pair of indistinguishable strains is isolated from two distinct carriers, and one would consider the lower confidence value of the discriminatory ability as a conservative estimate to this effect. We regard this as extremely valuable information when interpreting typing results for clinical practitioners or an increasingly informed public. Clearly, MLST and PFGE are the most powerful techniques for high-precision epidemiological typing when episodes of person-to-person transmission should be ascertained. RAPD and phage typing, both rapid and able to process large amounts of strains, seem, however, unsuitable for this purpose. Most importantly, MLST provides an adequate tool for producing genetic profiles for a vast number of isolates especially in nonepidemic circumstances, i.e., for national reference services or when comparing large international strain collections.

We found that DNA-based typing schemes come to very similar conclusions and combining different techniques will not increase the discrimination of strains of *S. aureus* to a large extent. Moreover, it appears that PFGE patterns could theoretically predict clonal complexes, pointing to the presence of a largely congruent phylogenetic signal in different regions of the staphylococcal genome. Future work should identify the most informative nucleotide sequences that could form the basis of a versatile strain designation of *S. aureus*.

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