Comparative Evaluation of Use of \textit{cna}, \textit{fnbA}, \textit{fnbB}, and \textit{hlf} for Genomic Fingerprinting in the Epidemiological Typing of \textit{Staphylococcus aureus}

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We used a genomic fingerprinting protocol to characterize 59 \textit{Staphylococcus aureus} strains and a single \textit{S. intermedius} isolate, all of which were previously typed by 13 different methods (F. C. Tenover et al., J. Clin. Microbiol. 32:407–415, 1994). These 60 strains were divided into three groups of 20 strains each, with each group including internal controls. Two of the three groups (groups SB and SC) included 29 strains from four relatively well-defined outbreaks. The epidemiological relationships of the strains in the third group (group SA) were unclear. Fingerprints were established by Southern blotting with \textit{Hae}III-digested genomic DNA and a probe mixture consisting of DNA fragments corresponding to the \textit{S. aureus} collagen adhesin (\textit{cna}), fibronectin-binding protein (\textit{fnbA} and \textit{fnbB}), and \(/3\)-toxin (\textit{hlf}) genes. An unambiguous fingerprint was obtained for all \textit{S. aureus} isolates. No hybridization signal was observed with \textit{S. intermedius}. Twenty-seven of the 29 related strains in the SB and SC groups were correctly identified as belonging to one of the four epidemiologically related groups. Our protocol was less successful with respect to the exclusion of unrelated strains. Specifically, only 6 of 11 unrelated strains in the SB and SC groups had a fingerprint that was distinct by comparison to the fingerprints of the outbreak strains. Nevertheless, our protocol was relatively accurate by comparison to the accuracies of the other methods and was one of only six methods that accurately identified all of the repetitive strains included as internal controls.

\textit{Staphylococcus aureus} is among the most prominent of all nosocomial pathogens. Its prevalence within the hospital often leads to nosocomial outbreaks associated with a single strain. In such cases, it is imperative that the identity of the offending strain be established such that appropriate control measures can be implemented. The importance of the accurate identification of clinical \textit{S. aureus} isolates is evidenced by the number of typing techniques developed for that purpose. Tenover et al. (6) compared 13 different typing techniques using a set of 59 relatively well-defined strains of \textit{S. aureus} and a single strain of \textit{Staphylococcus intermedius}. The specific techniques included (i) antibiogram analysis, (ii) bacteriophage typing, (iii) biotyping, (iv) restriction fragment length polymorphism (RFLP) analysis with \textit{mec}, \textit{Tn}554, \textit{agr}, and \textit{aph}(2\textsuperscript{-})-\textit{aad}(6\textsuperscript{-}) gene probes, (v) insertion sequence typing with an IS257/43I gene probe, (vi) field-inversion gel electrophoresis (FIGE) with \textit{Sma}I-digested genomic DNA, (vii) immunoblotting with EDTA extracts of \textit{S. aureus} cell surface proteins and pooled human serum, (viii) multilocus enzyme electrophoresis (MLEE), (ix) pulsed-field gel electrophoresis (PFGE) of \textit{Sma}I-digested genomic DNA, (x) PCR amplification of coagulase gene (\textit{cna}) variants followed by digestion with \textit{Alu}, (xi) plasmid profile analysis with \textit{EcoRI} or \textit{HindIII}-digested plasmid DNA, (xii) ribotyping with \textit{HindIII}-digested genomic DNA and labelled rRNA, and (xiii) ribotyping with \textit{Cla}I-digested genomic DNA and a gene probe corresponding to the \textit{Escherichia coli} \textit{rne} operon. These techniques were compared on the basis of typeability (the ability to generate an unambiguous result for all isolates), reproducibility, discriminatory power (the ability to discriminate between unrelated isolates), ease of use, and ease of interpretation of results. These parameters reflect the need for a reproducible protocol that can identify all related strains to the exclusion of all unrelated strains without exceeding the limits of technical practicality. To date, no single typing method has met that standard (6).

We recently reported a genomic fingerprinting protocol for the epidemiological differentiation of clinical isolates of \textit{S. aureus} (5). The protocol used DNA probes for the \textit{S. aureus} collagen adhesin (\textit{cna}), fibronectin-binding proteins (\textit{fnbA} and \textit{fnbB}), and \(/3\)-toxin (\textit{hlf}) genes and was used to investigate an outbreak of methicillin-resistant \textit{S. aureus} in a neonatal intensive care unit (NICU). Of 46 isolates examined, 22 were found to have identical fingerprints (5). Of these 22 strains, 21 were isolated from the affected NICU. These results suggested that a single strain was responsible for the NICU outbreak and that our protocol was useful for the epidemiological typing of clinical isolates of \textit{S. aureus}. However, because our results were based on an isolated incident and involved strains that had not been typed by any other method, the overall utility of our fingerprinting protocol relative to those of existing typing techniques remained unclear. To address that issue, we used our genomic fingerprinting protocol to characterize the 59 \textit{S. aureus} isolates previously characterized by Tenover et al. (6). By comparison to other protocols, our method had an intermediate degree of discriminatory power but had the advantages of 100% typeability and an exceptionally high degree of reproducibility.

MATERIALS AND METHODS

Bacterial strains. The 59 \textit{S. aureus} isolates examined in this study were obtained from four confirmed outbreaks and one pseudo-outbreak (6). As in the

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previous study (6), we typed these isolates in groups of 20 isolates each (designated groups SA, SB, and SC), with each group including internal controls that were either duplicate submissions of the same isolate or isolates obtained from the same patient on the same day. S. aureus ATCC 12600 was included in all three groups (strains SA-4, SB-7, and SC-3). A single strain of S. intermedius (ATCC 49052) was included in the SA group (strain SA-16) (6). The remaining 18 strains in the SA group included nine isolates (isolates SA-1, -2, -3, -5, -10, -13, -14, -17, and -19) that were obtained from two different nursing homes and that were originally thought to represent a single strain (6). However, a clear epidemiological link could not be established, these nine strains were subsequently classified as pseudo-outbreak isolates (6). Two of these pseudo-outbreak isolates (isolates SA-9 and SA-15) as internal controls. The SA group also included seven strains that were unrelated to each other or to the pseudo-outbreak strain (6).

The SB group contained isolates from two different and unrelated outbreaks as well as eight isolates (isolates SB-1, -8, -9, -13, -14, -17, and -18) with no clear epidemiological link to either outbreak (6). S. aureus ATCC 12600 (isolate SB-7) was included as an internal control. The outbreak I strains (strains SB-2, -3, -5, -10, -12, -15, -19, and -20) were obtained at the Iowa Veterans Affairs Medical Center and were originally defined as outbreak related on the basis of plasmid profile analysis (6). Six of the strains thought to be unrelated to either outbreak (strains SB-2, -8, -14, -16, -17, and -18) were isolated from the same hospital. Two of these isolates (isolates SB-2 and SB-16) were obtained from patients in the same surgical service in which outbreak I occurred but were considered unrelated because the outbreak could not be established after the defined outbreak period (6). Outbreak II was associated with a contaminated anesthetic and contained four isolates (isolates SB-2, -4, -6, and -11), three of which (isolates SB-2, -4, and -6) were obtained from the same patient (6). The outbreak II isolates were originally characterized by bacteriophage typing.

The SC group of isolates also contained strains from two relatively well-defined outbreaks as well as S. aureus ATCC 12600 (isolate SC-3). The strains from outbreak III (strains SC-1, -4, -5, -9, -11, -12, -14, -15, and -17) were isolated at the Sepulveda Veterans Affairs Medical Center in Sepulveda, Calif. (6). One of these (isolate SC-17) was duplicated (isolate SC-20) as an internal control. Additionally, two isolates (isolates SC-14 and SC-15) were obtained from different sites on the same patient on the same day (6). The outbreak III isolates were originally characterized by immunoblotting (6). The outbreak IV strains (strains SC-2, -6, -7, -10, -13, -16, -18, and -19) were also associated with a contaminated anesthetic and were originally characterized by bacteriophage typing (6). The remaining isolate (isolate SC-8) was originally grouped with the outbreak IV strains, however, the epidemiological link between SC-8 and the outbreak IV strain was not confirmed in a subsequent analysis (6).

To verify the identity of each of the 59 S. aureus strains, CiaI-digested genomic DNA was hybridized with a probe corresponding to the S. aureus accessory gene regulator (agr) (2). This blot was chosen because it was included in the previous study and yielded a hybridization signal from all S. aureus strains (6). Although the restriction profiles were not shown, a numerical description representing a total of seven different CiaI-agr hybridization profiles was reported (6). For all strains other than S. intermedius (strain SA-16), which did not yield a hybridization signal with the agr probe, the groups defined in the previous study were confirmed in our analysis (data not shown). The typing methods used in the previous study (6) are listed in Table 1.

**Fingerprinting protocol.** To isolate genomic DNA for use in our fingerprinting protocol, each strain was grown overnight in 20 ml of tryptic soy broth. Because our protocol uses probes for chromosomesomally genes that are not associated with antibiotic resistance (see below), cultures were grown with no antibiotic selection. Cells were harvested by centrifugation, washed with 10 ml of TES buffer (30 mM Tris pH 8.0, 25 mM EDTA, 50 mM NaCl), and resuspended in 5 ml of high-salt TES (TES with 2.5 M NaCl) (5). After treating the cells with 30 µg of lysostaphin (Ambicin L; Aplin and Barrett, Trowbridge, United Kingdom) per ml for 30 min at 37°C, the cells were lysed by adding 0.5 ml of 20% n-laurylsarcosine (Sarkosyl). The suspension was gently inverted, and as soon as lysis was complete, 3.69 g of guanidine hydrochloride was added and the incubation was continued for 60 min at 55°C. Genomic DNA was purified from the lysate by a cesium chloride step gradient (2.85 M CsCl over 5.9 M CsCl) as described previously (5).

To establish a fingerprint for each strain, genomic DNA was digested with HaeIII and resolved by electrophoresis in 0.8% agarose. DNA was transferred to nylon membranes and was hybridized with a probe mixture containing DNA fragments corresponding to the S. aureus collagen adhesin (cnA), fibronectin-binding protein (fnbA and fnbB), and lipotoxin (hld) genes. Probes were generated by PCR amplification of genomic DNA (cna, fnbA, and fnbB) or from a plasmid clone (hld) as described previously (5). DNA fragments were labelled by random primer extension with digoxigenin-11-dUTP (5). Hybridizations were carried out overnight (~16 h) at 65°C with 10 ng of each probe fragment per ml of hybridization buffer (10% dextran sulfate, 1% sodium dodecyl sulfate, 1 M NaCl, 100 ng of each of the two specific digoxigenin-11-dUTP-labeled DNA fragments per ml). Hybridization signals were detected with anti-digoxigenin antibodies (Fab fragments) conjugated to alkaline phosphatase (2) and

**TABLE 1. Numerical comparison of S. aureus typing techniques**

<table>
<thead>
<tr>
<th>Method*</th>
<th>No. of related strains</th>
<th>No. of unrelated strains</th>
<th>Total no. of correctly identified strains</th>
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<td>This study</td>
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* Methods other than our genomic fingerprinting protocol are fully described in the report by Tenover et al. (6).

† RFLP analysis refers to the protocol with mecA, agr, Tn554, and aph(2'')-lact(6) probes (6).

**RESULTS**

Characterization of group SA isolates. Counting the two strains that were duplicated as internal controls, the SA group included 11 strains that were originally thought to be epidemiologically related. When genomic DNA from the SA strains was digested with HaeIII and hybridized with a probe mixture containing DNA fragments corresponding to the S. aureus cna, fnhA, fnbB, and hld genes, 15 strains were found to have identical fingerprints (Fig. 1). Included among these 15 strains were eight of the nine pseudo-outbreak strains as well as both of the internal controls (strains SA-9 and SA-15). Although the results obtained by our fingerprinting protocol are difficult to

**FIG. 1. Characterization of the group SA isolates by Southern blotting of HaeIII-PstI digoxigenin-labeled DNA probes.** Lane designations correspond to the strain numbers listed in the text. Lanes 1, 2, 3, 4, 5, 10, 13, 14, 17, and 19, genomic DNA from the nine pseudo-outbreak strains, respectively. The strains represented in lanes 1 and 2 are duplicated in lanes 9 and 15, respectively. Lane 4, genomic DNA from S. aureus ATCC 12600; lane 16, genomic DNA from S. intermedius.
interpret in the absence of more definitive epidemiological data, it should be noted that most of the typing methods evaluated in the previous study grouped the pseudo-outbreak strains either within the same type or as subtypes distinguished by relatively minor differences (6). Taken together, these results suggest that the identical fingerprint that we observed with eight of nine pseudo-outbreak strains may represent the appropriate inclusion of related strains.

The single exception among the pseudo-outbreak strains was strain SA-14 (Fig. 1). Again, the uncertainty associated with the SA isolates makes it difficult to draw any conclusion with regard to our exclusion of isolate SA-14. However, all but one of the methods used in the previous study also distinguished SA-14 from the other pseudo-outbreak strains. Moreover, the only method that did not distinguish SA-14 from the other pseudo-outbreak strains (coa PCR) was among the least discriminatory of all of the techniques evaluated in the previous study (6). Taken together, these results suggest that our exclusion of SA-14 may represent the appropriate exclusion of an unrelated strain.

Our protocol successfully excluded only four of the nine group SA strains that were originally considered unrelated to the pseudo-outbreak strain (Fig. 1). Included among these four isolates were S. aureus ATCC 12600 (isolate SA-4) and S. intermedius ATCC 49052 (isolate SA-16). The exclusion of S. intermedius was based on the absence of any hybridization signal (Fig. 1). The pattern observed with S. aureus ATCC 12600 (Fig. 1, lane 4) was identical to the pattern observed when DNA from the same strain was hybridized as part of the group SB (see Fig. 2, lane 7) and group SC (see Fig. 3, lane 3) strains. Importantly, this level of reproducibility was achieved with only 5 of the 13 methods evaluated in the previous study (6).

The remaining seven strains in the SA group were isolated from seven different states (6). Using our protocol, we could not distinguish five of these isolates (isolates SA-6, -7, -12, -18, and -20) from the pseudo-outbreak strain (Fig. 1). Although these strains had no obvious relationship to each other or to the pseudo-outbreak strain, it should be noted that a number of techniques evaluated in the previous study also tended to group these strains together (6). For instance, strains SA-12, SA-18, and SA-20 were identical on the basis of the 8 of 13 typing techniques (6). Nevertheless, on the basis of the absence of any epidemiological link between these strains, we must conclude that our inability to distinguish these strains from the pseudo-outbreak strain represents a failure to exclude unrelated strains.

Characterization of group SB isolates. Using our fingerprinting protocol, we observed a total of nine hybridization patterns among the 20 group SB strains (Fig. 2). The patterns observed with 6 strains (strains SB-7, -8, -9, -11, -13, and -17) were unique both with respect to each other and with respect to the patterns observed with the other 14 SB strains. Five of the six unique patterns were observed with strains that had no clear epidemiological link to other strains. The sixth pattern was observed with strain SB-11, which was one of four strains isolated during the course of an outbreak (outbreak II) of postsurgical infections associated with a contaminated anesthetic (1). The other three strains (strains SB-2, -4, and -6) all had identical fingerprints that were unlike that of any other strain (Fig. 2). Although these results suggest that our method falsely excluded a related strain, it should be noted that strains SB-2, SB-4, and SB-6 were isolated from the same patient, while strain SB-11 was isolated from a different patient (6). Moreover, SB-11 was distinguished from the outbreak II strain by 7 of the 13 typing techniques used in the previous study (6). Taken together, these results suggest that this strain may not be related to the outbreak II strain and that our results may not constitute the exclusion of a related strain.

Of the 13 strains isolated from the hospital in which outbreak I occurred, only 2 strains (strains SB-8 and SB-17) were clearly distinguished from the outbreak I strain (Fig. 2). The remaining 11 isolates all exhibited an identical hybridization pattern (Fig. 2). Included among these 11 isolates were the seven outbreak I strains as well as four strains (strains SB-1, -14, -16, and -18) that were originally judged by plasmid profile analysis to be unrelated to the outbreak strain (6). However, although it was after the defined outbreak period, two of these strains (strains SB-1 and SB-16) were obtained from patients admitted to the same surgical service in which outbreak I occurred (6). Additionally, with the exception of phage typing, the SB-1 and SB-16 isolates were identical to each other in every respect and matched the predominant pattern observed with the outbreak I strain by 8 of the 13 typing methods used in the previous study (6). These results strongly suggest that SB-1 and SB-16 are related to each other and that they may be epidemiologically related to the outbreak I strain.

Strains SB-14 and SB-18 could not be distinguished from the outbreak I strain (Fig. 2), even though they had no relationship to the outbreak other than having been isolated from the same hospital. In an attempt to resolve this apparent discrepancy, we performed an additional Southern blot analysis with HindIII-digested genomic DNA and an hlb gene probe. This blot was done on the basis of our previous demonstration that HindIII-digested genomic DNA was more discriminatory (i.e., revealed more distinct patterns) than HaeIII-digested genomic DNA when hlb was used as a probe (5). However, in this case, the use of HindIII-digested DNA did not allow us to distinguish strains SB-14 and SB-18 from each other or from the outbreak I strain (data not shown). Our inability to distinguish between SB-14 and SB-18 is relevant because the same strains differed from each other by 11 of the 13 typing techniques used in the previous study (6). However, in six cases, the distinctions were relatively minor, with the two strains being classified as subtypes of each other rather than distinct strains (6). Similarly, SB-14 was clearly distinguished (i.e., distinguished as a distinct type rather than a subtype) from the outbreak I strain by only 3 of the 13 assays, while SB-18 was clearly distinguished from the outbreak strain by only 1 of the 13 assays (6). Nevertheless, on the basis of the available epi-
Tenover et al. (6) compared 13 different typing methods using a set of 59 relatively well-defined strains of *S. aureus*. These methods were evaluated on the basis of typeability, reproducibility, discriminatory power, ease of interpretation of results, and ease of use (6). With the exceptions of typeability and reproducibility, these parameters are subject to some degree of subjective interpretation. That is particularly true with regard to discriminatory power, since it is difficult, if not impossible, to definitively establish the relatedness of any two bacterial strains. It is therefore imperative that any new typing technique be evaluated by direct comparison to existing techniques. To that end, we used our protocol to characterize the set of 59 *S. aureus* isolates examined by Tenover et al. (6).

As in the previous study, these 59 strains were examined in three groups of 20 strains each. The epidemiological relationships within one of these groups (group SA) were unclear, while the remaining groups (groups SB and SC) contained 29 strains from one of four relatively well-defined outbreaks (6).

With regard to the outbreak strains, our protocol failed to include two strains (strains SB-11 and SC-13) that were thought to belong to an epidemiologically related group and failed to exclude five strains (strains SB-1, SB-16, SB-14, SB-18, and SC-3) that were originally judged to be unrelated to any of the outbreak strains. However, as discussed above, we believe there is reason to question the original classification of three of the seven misidentified strains. Specifically, we believe that strains SB-1 and SB-16 may be related to the outbreak I strain, while strain SB-11 may not be related to the outbreak II strain. If that is true, then our protocol misidentified only 4 of 40 strains in the SB and SC groups. In all but one case (strain SC-13), the misidentification involved the failure to exclude an unrelated strain. Although the epidemiological relationships among the SA strains were unclear, we could not distinguish five SA strains from each other or from the pseudo-outbreak strain, even though these five strains were isolated from diverse geographical regions and had no apparent epidemiological link. On the basis of these results, we conclude that our genomic fingerprinting protocol is relatively accurate with respect to the identification of epidemiologically related strains but that it has a tendency to falsely include unrelated strains within epidemiologically related groups.

To provide a more direct comparison between our method and the methods evaluated by Tenover et al. (6), we summarized our results using the numerical system used in the previous study. Given the uncertainty associated with the SA strains, this comparison was restricted to the relatively well-defined outbreaks in the SB and SC groups and was based on the number of related strains (\( n = 29 \)) that were correctly identified as outbreak related and the number of unrelated strains (\( n = 11 \)) that were distinct by comparison to all four outbreak strains (6). All assumptions made in the previous report with regard to the relatedness of individual strains (6) were maintained in our analysis. More specifically, we ignored the possibilities that SB-1 and SB-16 may be related to the outbreak I strain and that SB-11 may not be related to the outbreak II strain. However, we did introduce two modifications into the scoring system. First, all differences were taken as an indication of nonidentity. Put more directly, subtype distinctions made in the previous report (6) were not made in our analysis. As an example, Tenover et al. (6) reported that 28 of 29 outbreak strains were correctly identified by immunoblotting with pooled human serum. However, three strains were distinguished from the relevant outbreak strain at what Tenover et al. (6) defined as the subtype level. In our analysis, we have included these subtype distinctions as distinguishing characteristics such that the immunoblotting protocol correctly
identified only 25 of 29 related strains. Although somewhat arbitrary, we believe this approach is justified because (i) it is virtually impossible to consistently define subtypes when comparing diverse typing techniques and (ii) any attempt to categorize strains on the basis of minor differences will introduce a degree of subjectivity into the analysis that will inevitably affect reproducibility, particularly when the technique is applied in different laboratories. We also believe that our approach is valid as long as it is equally applied. Second, Tenover et al. (6) reported the three primary parameters of (i) total number of valid typing, (ii) number of related strains observed among all 60 strains, (iii) total number of unrelated strains in the groups S. aureus and SC (n = 29) that were correctly identified as outbreak related, and (iii) total number of unrelated strains in groups S. aureus and SC (n = 11) that were incorrectly identified as being related to one of the four outbreak strains. To derive a single numerical indicator of accuracy, we changed the third parameter to reflect the total number of unrelated strains that were correctly identified as being unrelated to any outbreak. This parameter was easily derived by subtracting the number of misidentified strains given in the previous report (6) from the total number of unrelated strains (n = 11). This change allowed us to summarize the results on the basis of the total number of strains (n = 40) that were correctly identified.

On the basis of this scoring system, the overall scores ranged from 26 (biotyping) to 36 (genomic fingerprinting with mecA, agr, Tn554, and aph(2')-acc(6') probes, hereinafter referred to as RFLP analysis) (Table 1). The overall score obtained with our protocol was 33. Seven techniques (bacteriophage typing, ribotyping with Clai-digested genomic DNA, RFLP analysis, PFGE, FIGE, immunoblotting with pooled human serum, and MLEE) had overall scores equal to or greater than that obtained with our protocol (Table 1). On the basis of this comparison, we conclude that our protocol is relatively reliable but not to the extent that its accuracy would, by itself, dictate its use to the exclusion of other protocols. However, we do believe that our protocol offers certain advantages over many of the techniques that yielded comparable overall scores. For example, our protocol is based exclusively on chromosomal genes that, by comparison to phage typing, antibiogram analysis, and plasmid profiles, should be relatively invariant over time. Additionally, with the exception of cna, our analysis involves chromosomal loci that are present in all S. aureus strains (35). The typeability of our protocol is therefore much higher than that of the alternative RFLP analysis protocol (6). Indeed, in the alternative protocol, only the agr probe hybridized with genomic DNA from all S. aureus strains (6). We also believe that, from a technical perspective, our protocol is less demanding and requires less specialized equipment than PFGE, FIGE, immunoblotting, or MLEE, all of which had overall scores equal to or greater than those obtained with our protocol (Table 1). Finally, our technique was highly reproducible, as judged by repetitive blots with independently processed preparations of genomic DNA (data not shown). Indeed, our protocol was one of only six techniques (the other five were bacteriophage typing, biotyping, Clai ribotyping, insertion sequence [IS] typing, and cou PCR) that correctly identified all of the repetitive strains included among the 60 strains examined (6).

From a technical perspective, four methods evaluated by Tenover et al. (6) are directly comparable to our protocol. Two of these (RFLP analysis and Clai ribotyping) had overall scores comparable to those obtained with our protocol, while the other two (HindIII ribotyping and IS typing with an IS257/43I probe) had a lower overall score of 30 (Table 1). Although the IS typing protocol had a relatively high overall score, we believe that it can be discounted as a general typing protocol because an excessive number of strains (24 of 60) examined in the previous study did not yield a hybridization signal (6). Similarly, the only probe used in the RFLP analysis protocol that yielded a hybridization signal with all S. aureus strains was agr (6). These results illustrate that the RFLP protocol is based largely on negative characteristics and suggests that it may not be useful in all situations. However, the three probes that did not yield hybridization signals in all strains are associated with antibiotic resistance, which suggests that the typeability of the protocol may be highest in precisely those situations in which epidemiological characterization is most important (i.e., in situations involving multiply resistant organisms). That is particularly true in the case of the mecA and Tn554 probes, both of which are closely associated with methicillin resistance (4). On the other hand, the previous study revealed only two mecA patterns, four Tn554 patterns, and two aph(2')-acc(6') patterns among the 60 strains examined (6). The relatively small number of patterns, particularly among geographically related isolates (6), suggests that fingerprinting with these probes offers relatively little discriminatory power and that its usefulness may be limited in at least some situations. In contrast, we have demonstrated a great deal of variability in the cna, fnbA, fnbB, and hlb target genes during the course of this study and an earlier study in which we used our fingerprinting protocol (5). For example, 22 of 46 strains examined in the previous study were identical and were judged to be the same strain (5). Taking these 22 strains as a single strain, the total number of strains examined in the previous study was 25. Using the cna, fnbA, fnbB, and hlb probes in combination with each other, we observed a total of 16 distinct hybridization patterns among these 25 strains (5). Moreover, the total number of strains examined in the previous study is probably an overestimate since some of these strains may have been related to each other (i.e., the only distinction made in the previous study was whether these strains were related to the outbreak strain). Furthermore, we observed seven additional patterns among the isolates evaluated during the course of this study. Taken together, these results suggest that our protocol has a degree of discriminatory power that exceeds that of most other protocols. That observation, together with the fact that the target genes for every probe other than cna are highly conserved (3), strongly suggests that our genomic fingerprinting protocol will have wide applicability for the epidemiological typing of S. aureus clinical isolates. The observations that the ribotyping protocol with Clai-digested genomic DNA had an overall score identical to that obtained with our protocol (Table 1) and yielded a signal for all S. aureus strains examined suggests that this protocol may have similar utility. It should also be noted that, like our genomic fingerprinting protocol, the Clai ribotyping protocol correctly identified all of the internal control strains (6). In contrast, the HindIII ribotyping protocol had a lower overall score (Table 1) and did not correctly identify all of the internal controls (6).

Tenover et al. (6) suggested that the epidemiological typing of S. aureus might require a combination of methods. Specifically, it was suggested that the initial analysis could be done by a relatively simple method that was likely to identify all potentially related strains, while a second method could be used for the more detailed analysis of individual isolates (6). On the basis of technical considerations and our experience with the NICU outbreak, we believe that antibiogram analysis and/or plasmid profile analysis may be most appropriate for the former. For the reasons discussed above, we also believe that our genomic finger-
printing protocol is among the most viable alternatives for the latter. Moreover, the continued development of rapid DNA isolation procedures, the availability and relatively low cost of the required equipment, the potential to amend the protocol to take advantage of rapid blotting systems, the availability of highly sensitive, nonradioactive detection methods, and the availability of software appropriate for the automated analysis of fingerprint profiles are all factors that can be addressed in an effort to facilitate the use of our protocol in clinical microbiology laboratories.

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