Multiple-Locus Variable-Number Tandem-Repeat Assay Analysis of Methicillin-Resistant Staphylococcus aureus Strains

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Our objective was to determine if a multiple-locus variable-number tandem-repeat assay (MLVA) for Staphylococcus aureus could predict pulsed-field gel electrophoresis (PFGE) types (i.e., USA types), thus allowing us to replace PFGE with a simpler and more rapid typing method. One hundred three well-characterized isolates representing 13 major lineages of S. aureus were tested by both PFGE and MLVA. MLVA was performed using a rapid DNA extraction technique and PCR primers for sdrCDE, clfA, clfB, sspA, and spa. PFGE was performed with genomic DNA fragments generated using SmaI, as per CDC protocols. Banding patterns were analyzed both visually and with BioNumerics software. All isolates were typeable with MLVA and PFGE. MLVA patterns were highly reproducible. PFGE separated the isolates into 13 types with 42 subtypes. Using any band difference to designate a novel MLVA type, MLVA produced 45 types, including 9 clusters containing multiple isolates. Using BioNumerics and a cutoff of >75% relatedness, MLVA produced 28 types, 11 of which contained >1 isolate. Epidemiologically related outbreak isolates of USA300-0114 from five states clustered in one MLVA pattern. USA100 isolates were present in several unrelated (<40%) MLVA types. A cutoff of >80% separated outbreak strains of USA300-0114 into three distinct MLVA types. MLVA did not differentiate community methicillin-resistant S. aureus (MRSA) lineages (USA300, USA400, USA1000, and USA1100) from health care MRSA lineages (USA100, USA200, or USA500). The ability of MLVA to differentiate among strains that are indistinguishable by PFGE may be of epidemiologic value and warrants further study.

Infections caused by methicillin-resistant Staphylococcus aureus (MRSA) have traditionally been a problem in health care settings (8, 15). More recently, MRSA has been recognized as a cause of significant infections in community settings worldwide (2, 7, 14). Having access to an accurate strain typing method is critical for understanding the changing epidemiology of MRSA infections (12, 18) and for evaluating the efficacy of outbreak intervention and prevention strategies (20). The ability to type MRSA strains rapidly in clinical microbiology laboratories and to differentiate between the multiresistant MRSA strains associated with health care facilities and the more susceptible community-associated MRSA strains could improve public health and infection control efforts to reduce MRSA spread in hospitals and communities and to guide empirical therapy in community settings (14, 19).

A system of nomenclature for S. aureus pulsed-field gel electrophoresis (PFGE) results, known as USA types, has been adopted nationally and provides scientists with a common language for describing typing results (10, 11). The USA types have been validated using multilocus sequence typing (MLST) and spa typing (11). USA types determined by PFGE further differentiate the strains classified by MLST as ST5 or ST8 into epidemiologically and biologically distinct subgroups. For example, ST8 strains can be divided by PFGE into USA300, the most commonly isolated community-associated MRSA strain in the United States (7, 13, 17, 18), and USA500, a classic multidrug resistant health care-associated MRSA strain (11). These PFGE types have different antibiograms and toxin profiles. Similarly, PFGE types USA100 and USA800 are biologically distinct subgroups of ST5. Nonetheless, PFGE requires 72 h to perform, is expensive compared to PCR-based typing, requires specialized training for performance and interpretation, and is not readily available in most clinical microbiology laboratories.

Reports by Sabat et al. (16), Malachowa et al. (9), and Gilbert et al. (5) suggest that multiple-locus variable-number tandem-repeat assays (MLVA) could discriminate among MRSA strain types while providing results that paralleled the results of PFGE. The MLVA assay used by Sabat et al. targeted variable-number tandem repeats in the clfA, clfB, sdrCDE, spa, and ssp4 loci and used gel electrophoresis to analyze the banding patterns (16). The individual numbers of repeats in the fragments generated were not determined, which some investigators consider a drawback to the method (19). Francois et al. expanded the number of loci investigated and used a BioAnalyzer to improve the analysis of the PCR products (3). Others investigators have evaluated the staphylococcal interspersed repeat units for typing; however, this analysis required multiple agarose gels for comparisons (6).

The goal of our study was to determine whether MLVA was simple enough to perform in a routine clinical microbiology laboratory and whether MLVA patterns could predict PFGE
USA types. Thus, we assessed the technical performance of MLVA and compared the results of MLVA to the results of PFGE for a diverse set of MRSA and methicillin-susceptible S. aureus isolates, including subtypes from known outbreaks defined by epidemiologic and microbiologic data.

MATERIALS AND METHODS

Bacterial strains. A group of 103 community- and health care-associated MRSA isolates and two methicillin-susceptible isolates (USA900 and USA1200) were selected from the strain collections of the Centers for Disease Control and Prevention (CDC) and Project ICARE (4), the criteria established by Sabat et al. (where any band change was classified as a distinct MLVA type), MLVA produced 45 distinct patterns. Nine clusters of isolates shared the same MLVA patterns, while 36 organisms had unique MLVA patterns (data not shown). Using these stringent criteria for defining MLVA types (where each pattern is a separate MLVA type) epidemiologically defined outbreak strains of USA300-0114 were separated into four different clusters. MLVA also separated USA100 isolates into 9 distinct types, USA200 into 5 types, USA500 into 2 types, USA700 into 3 types, USA800 into 10 types, and the Iberian isolates into 2 types. Thus, visual analysis using any band difference to establish unique MLVA types was abandoned because it did not group together strains that were known to be epidemiologically related or highly related by PFGE.

Using BioNumerics software to facilitate analysis, two potential cutoff values of >80% relatedness and >75% relatedness were investigated in order to group the MLVA patterns into MLVA types that more closely matched the epidemiologic clustering. The former cutoff was selected because it is the gold standard for defining pulsed-field types (11). The latter cutoff was selected because on initial review it grouped epidemiologically related strains of the same PFGE subtype (USA300-0114) from five of six outbreaks together in a single MLVA cluster. No matter which cutoff was used, the USA type strains for USA300 and USA500 were indistinguishable by MLVA (Fig. 1), suggesting that MLVA may have difficulty grouping ST8 isolates (which include USA300, USA500, and the Iberian clone) into patterns that parallel the PFGE types, which was one of the goals of the study. Using the >80% relatedness cutoff, the number of MLVA types was reduced from 45 to 38 and included 11 clusters of >1 organism and 27 unique MLVA patterns (Table 1). At 80% relatedness, strains of USA300-0114 from well-defined outbreaks in California, Georgia, and Louisiana were separated into unique MLVA types, although they were still closely related (at approximately 77%). Outbreak strains from Colorado and Pennsylvania clustered in the same MLVA type with the isolates from Georgia. Three of the
four California outbreak isolates had a distinctly lower spa product (Fig. 2). Using the cutoff of >80% relatedness, only 17 of the 24 USA100 isolates had the same MLVA type; 5 other MLVA types, some at less than 40% relatedness, were also observed. Twenty-three USA300-0114 isolates grouped in one MLVA type, while 14 USA300 isolates of six highly related PFGE subtypes, including several USA300-0114 isolates, grouped in a second MLVA type. Interestingly, USA800 isolates, which, like USA100 isolates, are MLST ST5 (and part of MLST clonal complex 5), were separated into eight different MLVA types, six of which contained only a single organism. Thus, USA100 isolates were more homogeneous by MLVA typing than were USA800 isolates. Yet, one USA800 isolate and one USA100 isolate were clustered among the USA100 isolates by MLVA, even at the more stringent 80% similarity level. It would be particularly difficult to predict that an MRSA isolate belonged to the USA800 lineage based on its MLVA type.

Using a cutoff value of >75% relatedness, the number of isolates in each MLVA type, thus reducing the number of MLVA types from 38 to 28. Using the >75% cutoff with BioNumerics software clustered several MLVA patterns together that would likely have been designated as unrelated by visual categorization, such as the USA200 isolates (Table 1). Forty of the 45 USA300 isolates (including 8 different PFGE USA300 subtypes) clustered into a single MLVA type at 75% relatedness, including the USA300-0114 outbreak strains from California, Colorado, Georgia, Louisiana, and Pennsylvania. Interestingly, the USA300-0114 isolates from the Illinois outbreak still clustered separately in a different MLVA type, perhaps because of a smaller spa band (Fig. 2, second band from the right). The fact that MLVA differentiated among PFGE subtypes is especially apparent for the USA300-0047 isolates, which produced MLVA patterns that were <40% related (Fig. 3). In this case, the USA300-0047 isolates, although indistinguishable by PFGE, were all epidemiologically distinct. The ability of MLVA to differentiate among USA300-0114 outbreaks and among epidemiologically unrelated USA300-0047 isolates from different states (using the 80% cutoff value) may be of value in long-term epidemiologic studies, perhaps highlighting biological differences among the isolates that are masked by PFGE, while using the >75% cutoff may have more utility for local hospitals for screening isolates from potential hospital outbreaks.

To further assess the reproducibility of the MLVA method, DNA was extracted from two isolates, one USA100 and one USA300, on four consecutive days. Each lysate was used as DNA template for four separate MLVA typing experiments. Thus, there were 16 MLVA patterns generated for each organism. Although some variation in band intensity was noted, the MLVA patterns for each isolate were indistinguishable (data not shown). Thus, as previously noted by Sabat and coworkers, the intralaboratory reproducibility of the MLVA patterns was very high. This is reassuring for laboratories that would consider using MLVA as a rapid screening method for hospital outbreak studies.

Clearly, the utility of the MLVA procedure depends upon how the test is used. Using a cutoff of >75% relatedness, MLVA could have utility as a screening test to determine whether isolates collected over a relatively short period of time in a single hospital were likely to be part of an outbreak cluster. Among outbreak isolates from five states that clustered together, only one isolate, from the California outbreak, had a slightly different MLVA pattern (although, using >75% relatedness, it would still have been classified as the same MLVA type). Thus, the reproducibility of the method is high for known outbreak strains. The fact that many of the USA100 isolates clustered together in the same MLVA type is also an encouraging result. However, the fact that the USA800 isolates were split among six quite different MLVA types using a 75% relatedness cutoff suggests that the variability of the patterns observed may be strain dependent. Grouping of the MLVA patterns into MLVA types can be done visually, although using any band difference in a pattern to designate MLVA types

TABLE 1. Number of unique strain types defined by PFGE and MLVA

<table>
<thead>
<tr>
<th>USA type (n)</th>
<th>No. of PFGE subtypes</th>
<th>No. of MLVA types by &gt;80% cutoff</th>
<th>No. of MLVA types by &gt;75% cutoff</th>
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<tr>
<td>100 (24)</td>
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<td>6</td>
<td>3</td>
</tr>
<tr>
<td>200 (5)</td>
<td>4</td>
<td>3</td>
<td>1</td>
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<tr>
<td>300 (45)</td>
<td>7</td>
<td>6</td>
<td>4</td>
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<td>400 (1)</td>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>500 (3)</td>
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<td>2</td>
<td>1</td>
</tr>
<tr>
<td>600 (1)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>700 (3)</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>800 (12)</td>
<td>6</td>
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<td>900 (1)</td>
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<td>1000 (2)</td>
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<td>1200 (1)</td>
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<tr>
<td>Iberian (2)</td>
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</tr>
<tr>
<td>Pattern</td>
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<td>2</td>
<td>2</td>
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<td>0441 (2)</td>
<td></td>
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</tbody>
</table>

Total (103)  42   38   11   27   28   11   17  

Subtypes all show >80% relatedness by Dice’s coefficients using the unweighted-pair group method using average linkage clustering as defined by BioNumerics software.  
Clusters are groups of >1 organism that show related MLVA patterns as defined by BioNumerics software at the given cutoff value.
overdiscriminates among strains that are related epidemiologically by PFGE. Using strain typing software aids in clustering related isolates over time and for archiving and long-term storage of data.

In summary, MLVA would likely be helpful for identifying outbreaks in a hospital setting, where the major goal is to determine if isolates that are epidemiologically linked and collected over a relatively short period of time are related or
unrelated. Fifteen isolates can be typed in a single working day, the equipment required is relatively inexpensive compared to that required for PFGE, and only modest training is required to set up the assay. The results can be interpreted visually without the need for additional software; however, we found that for longitudinal studies, a software package greatly facilitated data management. MLVA would be particularly useful for laboratories already using PCR-based assays, since they already have the expertise for performing amplification assays. However, our data, using a set of geographically diverse MRSA strains isolated from across the United States, showed that MLVA clustered isolates collected over a period of several years in a manner different from PFGE. Thus, MLVA cannot be used to predict PFGE types The biological reasons for the differences in the patterns, such as those seen in the Illinois and California outbreaks, are intriguing and merit further study.

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The findings and conclusions in this publication are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

REFERENCES


