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Multiple-locus variable-number tandem-repeat analysis of meticillin-resistant *Staphylococcus aureus* discriminates within USA pulsed-field gel electrophoresis types☆

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Summary

Many isolates of meticillin-resistant *Staphylococcus aureus* (MRSA) are indistinguishable when compared using the standard pulsed-field gel electrophoresis (PFGE) typing method. This may present a problem when investigating local outbreaks of MRSA transmission in a healthcare setting. It also impedes investigation of the widely disseminated community-acquired MRSA (USA 300-0114) in the inpatient setting, which is displacing other traditional hospital-acquired PFGE types. Combination of methods, including multiple-locus sequence typing (MLST), *spa* typing and staphylococcal cassette chromosome *mec* (SCC*mec*) typing, have been used with, or in place of, PFGE to characterise MRSA for epidemiological purposes. These methods are technically challenging, time-consuming and expensive and are rarely feasible except in large laboratories in tertiary care medical centres. Another method, which is simpler and with faster turnaround time, is multiple-locus variable-number tandem-repeat analysis (MLVA). We investigated the utility of MLVA to distinguish common PFGE types. The results suggest that MLVA can be used to identify unrelated strains with identical PFGE patterns or confirm close genetic composition of linked isolates. MLVA could potentially be used in conjunction with PFGE to validate relationships, but further prospective evaluation of these relationships will be required in order to define the proper role, if any, for use of this method in hospital epidemiology.

Keywords

Meticillin-resistant *Staphylococcus aureus*; Multiple-locus variable-number tandem-repeat analysis; Nosocomial infections; Pulsed-field gel electrophoresis

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Conflict of interest statement

None declared.

Introduction

Meticillin-resistant *Staphylococcus aureus* (MRSA) has become a major cause of infections acquired by patients in the healthcare setting. It is usually resistant to multiple antimicrobial agents and is now endemic in many healthcare facilities. In recent years, MRSA has also been reported as the cause of community-acquired (CA) infections in persons with no known contact with any healthcare setting. MRSA infections known to be associated with community acquisition are often designated CA-MRSA to distinguish them from healthcare-associated infections (HA-MRSA). The CA-MRSA strains have shown a predilection for skin and soft tissue infections.¹ In addition, these strains have been reported to contain genes for Panton–Valentine leukocidin (PVL) and the mobile genetic element staphylococcal cassette chromosome *mec* (SCC*mec*) type IV which appears to be absent in most HA-MRSA strains.²

Many MRSA strains are indistinguishable when compared using the standard pulsed-field gel electrophoresis (PFGE) typing method.^{3–5} This may present a problem when using molecular techniques to investigate local outbreaks of MRSA transmission in a healthcare setting.⁶ Combinations of methods including multiple-locus sequence typing (MLST), *spa* typing and SCC*mec* typing have been used to characterise MRSA for epidemiological purposes but none has supplanted PFGE as a reference method for bacterial typing.⁷

Multiple-locus variable-number tandem-repeat analysis (MLVA) is a method for bacterial typing based on the detection of short sequence repeats that vary in copy number, such as variable numbers of tandem repeats (VNTR) at different regions of the microbial genome. These VNTRs are often highly polymorphic, with variation in both the number of repeat units and by sequence heterogeneity among individual units.^{8,9} Tenover *et al.* recently compared MLVA of MRSA to PFGE using a collection of archived *S. aureus* isolates obtained from several regions over an unstated time period.⁷ Although MLVA did not predict PFGE type, the authors suggested that it might be valuable in distinguishing within identical PFGE types.⁷ The aim of this study was to evaluate whether MLVA provides a means of discrimination within CA-MRSA and HA-MRSA strains collected from a single geographic location over a defined time-period spanning six years, and found to be identical by PFGE.

Methods

MRSA

Isolates from clinical specimens (skin and soft tissue infection; respiratory; blood; cerebrospinal fluid; urine) as part of ongoing studies of MRSA at our institution were analysed using PFGE (400 adults, 67 children, 30 neonates).^{1,10,11} The adult and neonates were housed in the University of Alabama Hospital and the children in The Children's Hospital of Alabama located on the same campus. From these we selected strains with identical PFGE patterns (50 adults, 30 children, eight neonates). No individual was represented more than once. Isolates were classified as HA-MRSA based upon medical record review if they came from patients with a history of hospitalisation; residence in a long-term care facility (e.g. nursing home); dialysis or surgery within one year before the date of specimen collection; growth of MRSA from a culture obtained 48 h or more after admission; presence of a permanent indwelling catheter; percutaneous device at the time of culture; prior positive MRSA culture and neonatal age group. CA-MRSA was defined as isolates from patients without any of these risk factors. Adult HA-MRSA were collected from 2000 to 2004, isolates from children during 2004 and 2005, and neonatal isolates in 2005. None of the isolates from adults and children was linked to any another epidemiologically. The neonatal isolates were collected during an outbreak of MRSA

infections in the neonatal intensive care unit. PFGE reference strains (USA100, USA200, USA300, USA400, USA500, USA600, USA700, and USA800) were obtained through the Network on Antimicrobial resistance in *S. aureus* (NARSA) programme.

PFGE

Preparation of agarose plugs—Isolates were grown overnight on Blood Agar and a suspension prepared in Tris–EDTA (TE) buffer. Agarose plugs (0.9%) were made by adding 4 µg lysostaphin and an equal volume of 1.8% PFGE grade agarose (Bio-Rad, Hercules, CA, USA) in TE and formed in disposable moulds, incubated overnight at 37 °C in EC lysis buffer followed by Proteinase K (1 mg/mL) digestion at 50 °C overnight followed by four washes in TE buffer, then stored at 4 °C in TE buffer.

Restriction enzyme digestion and gel electrophoresis—A plug slice, ~2 × 7 mm, was digested with 30 U *Sma*I at room temperature (~25 °C) for 2–3 h or overnight. PFGE grade agarose (1% w/v) was prepared in 0.5 × TBE. PFGE was performed on a CHEF Mapper (Bio-Rad) using the following running parameters: 200 V (6 V/cm); temperature 14 °C; initial switch 5 s; final switch 40 s; time 22 h. After electrophoresis the gel was stained with ethidium bromide.

Data analysis—Gel images were captured using a GelDoc2000 (Bio-Rad) and stored as TIFF files. Each gel included *S. aureus* NCTC 8325 in the first and last wells which was used to normalise patterns for analysis with Bionumerics v5.01 software (Applied Maths, Sint-Martens-Latem, Belgium). Dendrograms were produced using a band tolerance of 0.6% and the unweighted pair group method with arithmetic averages based on Dice coefficients.

MLVA

DNA isolation—DNA was extracted from overnight growth on sheep blood agar. Colonies were suspended in 1 mL of polymerase chain reaction (PCR) grade water to a McFarland 0.5 standard turbidity and centrifuged for 5 min. The supernatant was removed, and 200 µL of Proteinase K was added to the tube.¹² The tube was mixed and placed in a heating block for 1 h at 60 °C followed by 10 min in a 90 °C heating block and immediately stored at –80 °C until used.

Multiplex PCR—The method of Sabat *et al.* was used with modifications as noted below.¹³ The PCR reaction mixture consisted of 1 × GoTaq[®] Green Master Mix (Promega), 1 µmol/L each of ClfA-F and ClfA-R, 0.5 µmol/L each of ClfB-F/ClfB-R, CdrCDE-F, CdrCDE-R, Spa-F, Spa-R, *SspA*-F and *SspA*-R (TIBMolbio, Berlin, Germany) and 5 µL of the DNA extract in a final volume of 50 µL.¹³ Amplification was performed in a Perkin–Elmer Cetus model 480 thermocycler or an AB Biosoftware 9700 thermocycler with the following conditions: 94 °C for 5 min, 20 cycles of 94 °C, 30 s, 55 °C for 30 s, 72 °C for 30 s with a final extension at 72 °C for 5 min. PCR products were analysed with a 2% agarose gel in 1 × TBE at 95 V along with molecular weight standards. The ethidium bromide-stained gel image was captured as a TIFF file and analysed as noted above for PFGE using Bionumerics software (Applied Maths).

Results

PFGE types were determined for all 497 isolates and grouped by USA type. Those with identical patterns were evaluated further using MLVA to determine whether this tool could be used in conjunction with PFGE in the investigation of transmission. The most common type was USA300-0114 which was found in children (CA: 25; HA: 5) and adults (CA: 13; HA: 7). However, USA100 from adults (CA: 3; HA: 16) and neonates (HA: 8), USA200

from adults (HA: 12), and USA600 from adults (HA: 11) were also found in numbers large enough for analysis. A standard for designating similarity among strains with MLVA does not exist. For our purposes we choose 80% similarity since it has been recommended as the cut-off to define clonality using PFGE (Table I).¹⁴

MLVA proved to be useful in distinguishing between unrelated USA100, USA200, and USA300-0114 (Table I). There are two USA100 patterns shown in Figure 1A and B which represent unrelated isolates from adults. Figure 1C represents PFGE USA100 isolates from an outbreak in the neonatal intensive care units. MLVA patterns are identical in six of the eight. The remaining two isolates collected four months apart have two MLVA patterns that are distinct from the six (38% similarity) and differed from each other (78% similarity) only in a single band. These are clearly related by both PFGE and MLVA. This is in contrast to isolates from adults and children that are not linked and have a more diverse number of MLVA types. Figure 2 shows the analysis of USA200 strains isolated from adults. These were predominantly classified as HA and there are no paediatric isolates with this PFGE type. The most prolific PFGE type is USA300-0114, found in large numbers in both adults and children and in both HA and CA isolates (Figure 3). This pattern was found only once in the neonates (data not shown). USA600 types did not amplify with either *spa* or *sspA* primers, yielding too few bands for analysis, but resulting in a pattern unique compared with the other PFGE types (Figure 4).

Discussion

MRSA infections remain an important cause of healthcare-associated infections and intense efforts to limit the spread of these organisms have been employed, such as screening patients on admission.¹⁵ In order to identify transmission vectors or breaks in infection control practice, however, it may be necessary to provide evidence of transmission using strain typing. The reference method for molecular typing of bacteria has been PFGE.⁴ The predominance of only a few PFGE types in our institution caused us to investigate alternative methods for discriminating within PFGE types.^{6,10} There have been efforts to substitute MLVA for PFGE because of the rapid turnaround time, lower cost, and the use of instrumentation found in many laboratories. Sabat *et al.* first described the MLVA methods used in this report and suggested that it was a simple and reproducible means for typing MRSA and a possible replacement for PFGE.¹³ Tenover *et al.* evaluated MLVA to determine if it could predict PFGE types.⁷ They concluded that it could not be used to predict PFGE types, but that it did show some discrimination among isolates within a PFGE type and that further investigation would be needed to determine the value of this approach.

We evaluated MLVA for the ability to distinguish genetic relatedness of bacterial strains with identical PFGE patterns. The MRSA collection from a six-year period evaluated in this study contained strains from adults, children and neonates classified as either CA or HA. Isolates from adults and children were from two different facilities and there was no evidence that they were epidemiologically linked. A small number of isolates from neonates was obtained at a time when transmission among neonates in an intensive care unit was occurring and these were assumed to be from a single outbreak of MRSA. Using a cut-off of 80% similarity, the adult and child isolates with identical PFGE patterns were separated, resulting in several distinct clusters and unique MLVR patterns. As expected, this showed a greater diversity than indicated by PFGE typing. In contrast, eight isolates from neonates with identical PFGE USA100 patterns were placed in one cluster; according to MLVA typing, six isolates were identical and two others had unique patterns. Since these were thought to be epidemiologically linked in time and location, MLVA supported linkage, in contrast to strains from adults and children separated by both time and location which showed a greater genetic diversity. The one case in which the MLVA pattern did predict the

PFGE type was for USA600 isolates. These consistently gave patterns without either the *sspA* or *spa* bands. This is the one exception where it would be possible to predict a PFGE type using MLVA but only applicable if USA600 was a known problem in the institution.

This study suggests that MLVA can be used to identify unrelated strains that have identical PFGE patterns or to confirm the close genetic composition of linked isolates. MLVA could be used in conjunction with PFGE to validate relationships, but further prospective evaluation of these relationships will be required in order to define the proper role, if any, for use of this method in hospital epidemiology.

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References

1. Kowalski T, Berbari E, Osmon D. Epidemiology, treatment, and prevention of community-acquired methicillin-resistant *Staphylococcus aureus* infections. *Mayo Clin Proc.* 2005; 80:1201–1208. [PubMed: 16178500]
2. Harbarth S, Pittet D. Methicillin-resistant *Staphylococcus aureus*. *Lancet Infect Dis.* 2005; 5:653–663. [PubMed: 16183520]
3. Tenover FC, McDougal LK, Goering RV, et al. Characterization of a strain of community-associated methicillin-resistant *Staphylococcus aureus* widely disseminated in the United States. *J Clin Microbiol.* 2006; 44:108–118. [PubMed: 16390957]
4. McDougal LK, Steward CD, Killgore GE, Chaitram JM, McAllister SK, Tenover FC. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. *J Clin Microbiol.* 2003; 41:5113–5120. [PubMed: 14605147]
5. Berglund C, Mölling P, Sjöberg B, Söderquist B. Predominance of staphylococcal cassette chromosome mec (SCCmec) type IV among methicillin-resistant *Staphylococcus aureus* (MRSA) in a Swedish county and presence of unknown SCCmec types with Panton–Valentine leukocidin genes. *Clin Microbiol Infect.* 2005; 11:447–456. [PubMed: 15882194]
6. Patel M, Waites KB, Hoesley CJ, Stamm AM, Canupp KC, Moser SA. Emergence of USA300 MRSA in a tertiary medical center: implications for epidemiologic studies. *J Hosp Infect.* 2008; 68:208–213. [PubMed: 18289726]
7. Tenover FC, Vaughn RR, McDougal LK, Fosheim GE, McGowan JE Jr. Multiple-locus variable-number tandem-repeat assay analysis of methicillin-resistant *Staphylococcus aureus* strains. *J Clin Microbiol.* 2007; 45:2215–2219. [PubMed: 17494714]
8. Cho S, Boxrud DJ, Bartkus JM, Whittam TS, Saeed M. Multiple-locus variable-number tandem repeat analysis of *Salmonella enteritidis* isolates from human and non-human sources using a single multiplex PCR. *FEMS Microbiol Lett.* 2007; 275:16–23. [PubMed: 17692097]
9. Malachowa N, Sabat A, Gniadkowski M, et al. Comparison of multiple-locus variable-number tandem-repeat analysis with pulsed-field gel electrophoresis, *spa* typing, and multilocus sequence typing for clonal characterization of *Staphylococcus aureus* isolates. *J Clin Microbiol.* 2005; 43:3095–3100. [PubMed: 16000419]
10. Patel M, Kumar RA, Stamm AM, Hoesley CJ, Moser SA, Waites KB. USA300 genotype community-associated methicillin-resistant *Staphylococcus aureus* as a cause of surgical site infections. *J Clin Microbiol.* 2007; 45:3431–3433. [PubMed: 17670923]
11. Moser, SA.; Waites, KB.; Loeb, DJ.; Morris, JL.; Patel, P.; Reynolds, C. Comparison of PFGE and MLVR typing methods applied to community associated and hospital associated MRSA.

106th General Meeting of the American Society for Microbiology; 21–25 May 2006; Orlando, FL. [Abstract]

12. Blanchard A, Hentschel J, Duffy L, Baldus K, Cassell GH. Detection of ureaplasmas in the urogenital tract of adults, in amniotic fluid, and in the respiratory tract of newborns. Clin Infect Dis. 1993; 17:S148–S153. [PubMed: 8399906]
13. Sabat A, Krzyszton-Russjan J, Strzalka W, et al. New method for typing *Staphylococcus aureus* strains: multiple- locus variable-number tandem repeat analysis of polymorphism and genetic relationships of clinical isolates. J Clin Microbiol. 2003; 41:1801–1804. [PubMed: 12682193]
14. Singh A, Goering RV, Simjee S, Foley SL, Zervos MJ. Application of molecular techniques to the study of hospital infection. Clin Microbiol Rev. 2006; 19:512–530. [PubMed: 16847083]
15. Chaberny IF, Schwab F, Ziesing S, Suerbaum S, Gastmeier P. Impact of routine surgical ward and intensive care unit admission surveillance cultures on hospital-wide nosocomial methicillin-resistant *Staphylococcus aureus* infections in a university hospital: an interrupted time-series analysis. E pub 1 Sept 2008. J Antimicrob Chemo.

Figure 1. Dendrogram calculated using the multiple-locus variable-number tandem-repeat analysis (MLVA) data. (A) MLVA analysis of identical USA100a pulsed-field gel electrophoresis (PFGE) from adults. (B) MLVA analysis of USA100b PFGE strains which differ from those in (A). (C) MLVA analysis of USA100 PFGE isolates from neonates. Isolates SA are from University of Alabama at Birmingham hospital. The first two digits are the year followed by the isolate number. HA, hospital-acquired; CA, community-acquired.

Figure 2. Multiple-locus variable-number tandem-repeat analysis (MLVA) of USA200 isolates from adults. Dendrogram calculated using MLVA data. Isolates SA are from University of Alabama at Birmingham hospital. The first two digits are the year followed by the isolate number. HA, hospital-acquired.

Figure 3.

Multiple-locus variable-number tandem-repeat analysis (MLVA) of USA300-0114 isolates from adults and children. Isolates AMT are from children and SA are from University of Alabama at Birmingham hospital. The first two digits are the year followed by the isolate number. HA, hospital-acquired; CA, community-acquired.

Figure 4. Multiple-locus variable-number tandem-repeat analysis (MLVA) of USA600 isolates. Isolates SA are from University of Alabama at Birmingham hospital. The first two digits are the year followed by the isolate number. HA, hospital-acquired.

Table I

Number of multiple-locus variable-number tandem-repeat analysis (MLVA) types found within identical pulsed-field gel electrophoresis patterns

USA type (<i>N</i>) ^a	MLVA types 80%		
	Total ^b	Clusters ^c	Unique ^d
100a (10)	6	3	3
100b (9)	3	1	2
100c (8)	3	1	2
200 (12)	7	2	5
300 (50)	14	9	4

^aNumber of strains evaluated.

^bNumber of different MLVA patterns.

^cNumber of the total with two or more representatives.

^dNumber of unique patterns.