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Blinded Comparison of Repetitive-Sequence PCR and Multilocus Sequence Typing for Genotyping Methicillin-Resistant 
Staphylococcus aureus Isolates from a Children’s Hospital in St. Louis, Missouri

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Received 31 March 2006/Accepted 10 April 2006

We performed a blinded study to compare repetitive-sequence PCR and multilocus sequence typing for genotyping hospital- and community-acquired methicillin-resistant Staphylococcus aureus (MRSA). The MRSA strains that were sequence type 8 (ST8), staphylococcal cassette chromosome mec (SCCmec) type IV, and Panton-Valentine leukocidin-positive clustered separately from those that were ST5 and SCCmec type II.

Until recently, Staphylococcus aureus infections in the community were caused mostly by methicillin-susceptible S. aureus (MSSA) strains, and the dissemination of hospital-acquired methicillin-resistant S. aureus (HA-MRSA) into the community was infrequent (21). However, since the mid-1990s, there have been increased reports of community-acquired MRSA (CA-MRSA) infections in otherwise healthy community-dwelling adult and pediatric patients who do not share the known risk factors for MRSA colonization (13, 22). CA-MRSA isolates have been shown to contain a unique genetic element called staphylococcal cassette chromosome mec (SCCmec) type IV and V (17). These isolates are frequently clindamycin susceptible and have a narrower antibiotic resistance pattern than HA-MRSA (21). In addition, CA-MRSA isolates are isolated commonly from skin and soft-tissue infections (13) and possess genes encoding the Panton-Valentine leukocidin (PVL) toxin (25).

Twenty-eight clinical isolates of S. aureus were obtained from individual pediatric patients between January 2002 and January 2004. These isolates were identified as S. aureus by the clinical laboratory with standard methods, using Gram’s stain, catalase, and Staphaurex latex agglutination (Remel, Lenexa, KS) (1). Susceptibility testing was performed by the clinical laboratory using the CLSI (formerly NCCLS) guidelines for disk diffusion susceptibility testing (15). For the purpose of this study, two definitions of CA and HA were used. One definition was based on the susceptibility phenotype, in which clindamycin-susceptible strains, including those shown to have inducible clindamycin resistance, were considered to be CA. Clindamycin-resistant strains were assumed to be HA. The second definition was based on day of acquisition. For the latter, MRSA strains were considered to be CA if the cultures were positive in an outpatient setting or within 48 h of hospitalization. Strains that were recovered from patients after 48 h of hospitalization were defined as HA. The study set was chosen to contain representative CA and HA strains based on either definition.

Prior to PCR genotyping, individual clinical isolates were grown overnight in trypticase soy broth. The cells were centrifuged, and the DNA was purified from the pellets using the extraction method described by Kalai et al. (9) in combination with a QIAamp genomic DNA extraction kit (QIAGEN, Inc., Chatsworth, CA). Repetitive-sequence PCR (rep-PCR) typing using the RW3A primer sequences was performed according to the procedure of Del Vecchio et al. (4) and with Ready-To-Go randomly amplified polymorphic DNA analysis beads (Amersham Biosciences, Piscataway, NJ). To investigate the prevalence of resistance determinants, PCR amplification for known resistance genes was performed using Ready-To-Go randomly amplified polymorphic DNA analysis beads (Amersham Biosciences). PCR detections of the ermA (10), ermC (10), msrA (12), aacA-aphD (24), mecA (24), and lukS-PV-lukF-PV (8) genes were performed using the primers and thermal cycling profile previously described. Typing of the staphylococcal chromosome mec element was done using a multiplex real-time PCR assay described by Francois et al. (7) using an Applied Biosystems 7300 real-time PCR system (Applied Biosystems, Foster City, CA). For multilocus sequence typing (MLST), PCRs using S. aureus genomic DNA isolates were performed as described previously (6). PCR fragments of the seven housekeeping genes, arcC, arcE, glpF, gmk, pta, tpi, and yqIL, were obtained from chromosomal DNA and were directly sequenced. The sequencing reactions were cycled in a PTC-225 (MJ Research, Waltham, MA) and loaded onto a 3730xl DNA analyzer (Applied Biosystems, Foster City, CA).

Rep-PCR genotyping was performed blinded to the source of the study isolates or their susceptibility profiles, MLST sequence types (STs), or SCCmec types. Each strain was classi-
fied using the nomenclature put forward by Enright et al. of naming MRSA clones according to their MLST-resistance phenotype-SCCmec types (5). The majority of the 28 strains of MRSA belonged to two epidemic clones that were identified as ST5 (1-4-1-4-12-1-10)-MRSA-II (9 strains) and ST8 (3-3-1-1-4-4-3)-MRSA-IV (15 strains). The two MSSA strains that were included in this study were also ST8. Historical designations previously given to ST8-MRSA-IV and ST5-MRSA-II are New York/Japan and Clone V (or New York clone V), respectively. Three additional SCCmec type II strains of MRSA (Sa229, Sa235, and Sa386) had allelic profiles that represented two novel ST entries in the database. These novel profiles, 1-36-1-4-12-1-10 and 1-4-1-4-12-1-10, most closely resembled the allelic profile for ST5 after a comparison utilizing the MLST website (http://saureus.mlst.net). A single MRSA strain, Sa221, was shown to be ST72 (1-4-1-8-4-4-3) using MLST. Although this strain was positive for the mec gene, it could not be SCCmec typed. RW3A rep-PCR of the 28 MRSA strains produced two distinct clusters of strains that correlated strongly with MLST-SCCmec type (Fig. 1) and the presence or absence of the msrA and erm resistance genes. Quantitative analyses of the RW3A rep-PCR products show that the clusters shared less than 60% similarity.

One of the two clusters contained all 15 of the ST8-MRSA-IV strains. The strains of this ST8-MRSA-IV cluster also included all of the PVL-positive strains with the exception of a single strain, Sa384. These ST8-MRSA-IV strains have an overall similarity of 92% based on the comparison of amplified RW3A rep-PCR products (Fig. 1). Ninety percent of the ST8-MRSA-IV strains were determined to be from abscesses from the lower extremities.

The second cluster was composed of the single ST72 strain and the 12 SCCmec type II strains that included the 9 ST5-SCCmec-II strains plus the 3 strains with closely related but novel ST entries (Sa229, Sa235, and Sa386). The strains of this SCCmec type II cluster were uniformly PVL negative and shared 88% similarity (Fig. 1). Ten of the 13 strains in the SCCmec type II cluster were from respiratory specimens. Strain Sa221 (ST72-SCCmec nontypeable) shared only 80% similarity with the other strains in the PVL-negative SCCmec type II cluster.

The 28 strains that compose the SCCmec type II and ST8-MRSA-IV clusters also correlated strongly with the presence or absence of the msrA and erm resistance genes. The msrA gene was present in 13 of the 15 ST8-MRSA-IV strains, compared to 0 of the 13 SCCmec type II strains. In contrast, the erm gene was present in all 13 strains of the SCCmec type II cluster, although 4 of the ST8-MRSA-IV strains also possessed erm.

MLST is a powerful genotyping method that is usually applied to the evaluation of evolutionary relationships because the variation that is quantified accumulates more slowly as a
result of the neutral mutations. As a consequence, isolates with the same ST may be identified as members of the same clone but have very different pulsed-field gel electrophoresis (PFGE) patterns (6). PCR-based genotyping methods, such as RW3A rep-PCR, and PFGE detect genetic events in MRSA that occur on a shorter time scale (23). While rep-PCR has been shown to have high discriminatory power as well as reproducibility for genotyping MRSA, the method is still regarded as having less discriminatory power than PFGE (23). As a consequence, there have been very few direct comparisons between rep-PCR and MLST for genotyping bacteria. This blinded study compared a collection of 28 HA- and CA-MRSA strains using rep-PCR and MLST. The RW3A rep-PCR genotyping method was reproducible and generated highly discriminatory fragment patterns. The success obtained with rep-PCR in separating HA and CA-MRSA strains was due in large part to the fact that the ST8-MRSA-IV (Clone V) and ST5-MRSA-II (New York/Japan) clones have been shown to have very different chromosomal backgrounds (20).

Nine of the 13 strains that composed the SCCmec type II cluster, as determined by RW3A rep-PCR, were ST5-MRSA-II. ST5-MRSA-II is a well described pandemic clone of HA-MRSA and is the dominant clone in hospitals in Miami, New York, New Jersey, Pennsylvania, and Connecticut (19). The 13 strains in the SCCmec type II cluster shared several traits that are commonly attributed to HA-MRSA clones (22): having clindamycin resistance and causing serious invasive infections. All 13 of the strains in the SCCmec type II cluster were clindamycin resistant; 4 of these strains were also resistant to gentamicin. Additionally, 10 of these strains were isolated from respiratory specimens, which is a common body site for hospital-acquired infections. Of note, half of the strains belonging to the SCCmec type II cluster could not be distinguished as possible HA-MRSA strains from a determination based on day of acquisition. Thus, the epidemiology associated with these 28 strains of MRSA proved to be less reliable than the information obtained by PCR and genotyping for making a determination of CA- versus HA-MRSA infections.

Strain Sa221 was determined to be ST72 by using MLST and shared only 80% similarity to the ST5-MRSA-II strains. Unfortunately, the SCCmec element for Sa221 could not be determined; however, ST72-MRSA-IV has been described as a cause of CA-MRSA infections in Miami (3), San Francisco (2), and Mississippi (18). ST72-MRSA-IV belongs to CC8 and has also been designated pulsed-field type USA700 (14).

RW3A rep-PCR grouped all 15 MRSA strains determined to be ST8 into the ST8-MRSA-IV cluster. The ST8-MRSA-IV strains shared several traits known to be common to CA-MRSA clones (25). Fourteen of the 15 ST8-MRSA-IV strains were isolated from skin and soft-tissue infections and possessed the PVL toxin. In addition, 13 of these 15 ST8-MRSA-IV strains, 12 of which were susceptible to clindamycin, possessed the msrA gene. Interestingly, two strains of PVL-negative, ST8-MRSA-IV strain, Sa384. Despite the fact that the PVL gene has been described as a stable genetic marker of CA-MRSA strains (25), the current understanding of SCCmec type IV CA-MRSA dictates that its detection worldwide must consider that not all community-acquired MRSA strains will possess the PVL gene (11). Interestingly, the Sa384 strain shared the same MLST allelic profile as the other ST8-MRSA-IV strains in the SCCmec type IV cluster, indicating that these strains share a common evolutionary pathway. Strain Sa384 differed from the CA-MRSA ST8-MRSA-IV strains in being obtained from a lower-respiratory specimen (bronchoalveolar) and having multidrug resistance to clindamycin, gentamicin, and trimethoprimsulfamethoxazole (18, 25). To date, most SCCmec IV CA-MRSA isolates have had MLST allelic profiles that are not found in studies of HA-MRSA (18). However, CA-MRSA has the potential to move into the hospital setting and cause outbreaks (16). This study reiterates that the detection of SSOmec type IV CA-MRSA does not guarantee PVL production or predictable resistance profiles.

Other investigators have reported that the relative frequencies of S. aureus erm genes are not the same worldwide or static over time and that different erm genes have been found in different S. aureus clones (26). Among our MRSA isolates, there was a strong predominance of the ermA over the ermC determinant. In our study, the only 2 MRSA strains that possessed the ermC gene, Sa221 and Sa384, were remarkably different from the other strains in their respective clusters, and ermC could be used as a marker for genetic variation in this group of CA-MRSA isolates.

The results of MLST and SCCmec typing and the detection of genes that encode PVL and macrolide/lincosamide resistance were very encouraging in suggesting the strength of rep-PCR for separating this collection of strains into HA- and CA-MRSA. RW3A rep-PCR should be investigated further to determine whether this inexpensive and rapid method of genotyping can be used by individual laboratories to follow the dissemination of CA-MRSA SCCmec type IV isolates.

REFERENCES


