Severe *Staphylococcus aureus* Infections Caused by Clonally Related Community-Acquired Methicillin-Susceptible and Methicillin-Resistant Isolates

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We investigated the genetic relatedness of 5 community-acquired (CA) *Staphylococcus aureus* isolates obtained from 4 consecutive pediatric patients presenting with sepsis syndrome and severe pneumonia during a 3-week period in 2000. Two patients were infected with methicillin-susceptible *S. aureus* (MSSA), and 2 were infected with methicillin-resistant *S. aureus* (MRSA). The pulsed-field gel electrophoresis patterns for the 2 CA-MRSA isolates were identical to each other, as were the patterns for the 3 CA-MSSA isolates. A 2-band difference reflecting the presence of a staphylococcal cassette chromosome *mec* (SCCmec) element distinguished the CA-MRSA isolates from the CA-MSSA isolates. The small, mobile type IV SCCmec element was present in the CA-MRSA isolates. These data suggest that an insertion or, less likely, a deletion of the SCCmec type IV element occurred in a highly virulent *S. aureus* background. Staphylococcal toxin genes *sea*, *seh*, *lukS-PV*, and *lukF-PV* were detected in all isolates. Also, in all isolates, was a partial homolog of *seo* (*seo′*). The relationship among these patient isolates strengthens the assumption that CA-MRSA infections may be caused by isolates closely related to MSSA isolates.

Methicillin-resistant *Staphylococcus aureus* (MRSA) infection is no longer confined to patients with known risk factors or to health care–associated settings. Reports of community-acquired (CA) MRSA in patients without traditional risk factors from several regions in the United States and elsewhere [1–12] document that the emergence of CA-MRSA has had important clinical implications. Unlike the well-described MRSA isolates that have been circulating in health care environments for many years, CA-MRSA isolates have distinctive properties: often they are resistant only to methicillin, with implied cross-resistance only to other β-lactam compounds, and they are usually associated with clinical syndromes resembling those caused by methicillin-susceptible *S. aureus* (MSSA) [1, 13, 14]. CA-MRSA can also cause serious or even fatal infections, as shown by 4 pediatric deaths reported from Minnesota and North Dakota [15].

In MRSA isolates, the *meca* gene encodes a protein designated “penicillin-binding protein 2a” or “penicillin-binding protein 2” that has transpeptidase activity necessary for peptidoglycan synthesis in *S. aureus*. Despite its designation, it actually has decreased binding affinity for β-lactam antibiotics and allows peptidoglycan synthesis even in the presence of β-lactam antibiotics.

*meca* is carried on a mobile genetic element called "staphylococcal cassette chromosome mec" (SCCmec) [16]. Three SCCmec types (I, II, and III) were reported among hospital-acquired MRSA isolates obtained from...
several countries and that differed in size, the presence of a deletion in the genes regulating mecA expression, and cassette recombinase gene allotypes [16]. In SCCmec type I, no antibiotic resistance determinants except mecA are found. In contrast, types II and III contain multiple determinants for non-β-lactam antibiotic resistance and provide a molecular explanation for the multiple-drug resistance often documented in MRSA isolates circulating in hospital environments. The large size of SCCmec types II and III probably limits their horizontal transfer.

We recently demonstrated a novel SCCmec type called “type IV” that is smaller in size than types I–III and, like SCCmec type I, lacks resistance determinants other than mecA [17, 18]. SCCmec type IV has been found in most CA-MRSA isolates reported to date [17–19], which explains why CA-MRSA isolates are most often resistant only to β-lactam compounds [1, 9, 20].

The origin of strains that contain the SCCmec type IV element remains unclear. For example, they might have originated from hospital-acquired MRSA strains and have undergone deletions of antibiotic resistance genes under lower antibiotic selective pressure in the community. Alternatively, the SCCmec element may have been transferred to an MSSA genetic background. Initial reports indicated that the majority of CA-MRSA isolates circulating were of 1 clonotype [9, 20, 21]. However, SCCmec type IV bears intact ccr genes [18], and molecular analysis of 11 CA-MRSA isolates from Chicago, Illinois, has shown that SCCmec type IV can now be found in diverse S. aureus genetic backgrounds, which suggests that the SCCmec type IV element can readily be mobilized and is capable of horizontal transfer [17].

We recently observed an unusual cluster of severe CA S. aureus infections among 4 children hospitalized at our institution. To evaluate the relationship between these 4 cases, we undertook various molecular analyses.

**CASE HISTORIES**

Severe illness and “sepsis syndrome” occurred in 4 pediatric patients who presented during a period that spanned 20 days in July and August 2000 (table 1). At presentation, the patients were 15, 27, 1, and 2 months old. Patients 1A and 2A were siblings who were hospitalized on the same day. Patient 1A died. Prompted by the recognition of severe sepsis in these children, surveillance was initiated in the intensive care unit for similar cases. The 2 additional patients, 1B and 2B, were hospitalized in the intensive care unit within the subsequent 3-week period. Thus, these 4 cases represented consecutive presentations of severe S. aureus sepsis syndrome in our intensive care unit. All 4 patients lived in the south side of Chicago. Patients 1B and 2B lived ~1 mile apart from each other and ~1 mile from patients 1A and 2A, who were siblings. Patient 1B was a full-term neonate discharged shortly after birth. Patient 2B was a full-term neonate with intraventricular hemorrhage and hyperbilirubinemia who received care in the intensive care nursery at our institution. However, MRSA isolates were not known to be circulating in the intensive care nursery at that time. Sibling patients 1A and 2A had no previous hospitalizations and were considered to be healthy before this illness.

Severe pneumonia complicated by empyema or pleural effusion was evident in all 4 patients. Hypotensive episodes in all 4 patients required intensive medical intervention. Skin rash or erythroderma was initially noted in only 1 patient (patient 2A). However, subsequent desquamation occurred in 3 patients. Patient 1A did not have dermatologic findings, but she died 8 h after arrival at the hospital. S. aureus was isolated from clinically important site(s) from all 4 patients (table 2). Two were infected with MSSA isolates, and 2 were infected with MRSA isolates. Two were bacteremic. One had evidence of renal dysfunction, and 3 had abnormal findings of urinalyses. Two patients had evidence of hepatic dysfunction. All had evidence of coagulopathy and thrombocytopenia, and 1 had chemical evidence of myositis (table 1).

**MATERIALS AND METHODS**

**Bacterial strains.** S. aureus isolates were identified by typical colony morphology, typical Gram stain appearance, and positive results of a Staphaurex test (Murex Biotech). CA-MRSA isolates CA05 and 8/6-3P were used as prototype strains for SCCmec type IVa and IVb, respectively, as described elsewhere [18]. Isolate MW2, a CA-MRSA isolate (SCCmec type IVa) recovered from a child with fatal septicemia and septic arthritis for which the whole genome sequence has been published, was obtained from the National Institutes of Health–sponsored Network on Antimicrobial Resistance in Staphylococcus aureus (http://www.narsa.net).

**Antimicrobial susceptibility.** The Vitek system (bioMérieux Vitek) was used for initial susceptibility testing in the University of Chicago Clinical Microbiology Laboratory. The isolates were further evaluated by disk diffusion and broth microdilution, using methods described by the NCCLS, if they were methicillin resistant [22]. The antimicrobial agents tested included penicillin, methicillin, clindamycin, erythromycin, gentamicin, rifampin, trimethoprim-sulfamethoxazole, and vancomycin.

**PFGE.** Whole-cell DNA was prepared and digested in agarose plugs with Smal, as described elsewhere [23, 24]. Restriction fragments were resolved using a CHEF DR III apparatus (Biorad Laboratories) with the following settings: voltage, 6 V/cm; temperature, 14°C; initial time, 5 s; final time, 40 s; and duration, 20 h. The relatedness of strains was determined by comparison of the restriction fragment–length polymorphism,
Table 1. Characteristics of 4 pediatric patients with invasive *Staphylococcus aureus* infection.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1A</th>
<th>2A</th>
<th>1B</th>
<th>2B</th>
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<tbody>
<tr>
<td>Age, months</td>
<td>15</td>
<td>27</td>
<td>1</td>
<td>2</td>
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<tr>
<td>Sex</td>
<td>Female</td>
<td>Female</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Clinical feature</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Hypotension</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Pleural effusion or empyema</td>
<td>Empyema</td>
<td>Bilateral pleural effusion</td>
<td>Right-side pleural effusion</td>
<td>Empyema</td>
</tr>
<tr>
<td>Skin involvement</td>
<td>None&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Erythematous palms, soles, and perineum, with subsequent peeling</td>
<td>Peeling on day 10 of hospitalization</td>
<td>Desquamation and peeling on day 7 of hospitalization</td>
</tr>
<tr>
<td>Microbiological feature</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>S. aureus bacteremia</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
</tr>
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<td>Other site of isolation</td>
<td>Pleural fluid</td>
<td>Endotracheal tube, wound drainage</td>
<td>Endotracheal tube</td>
<td>Pleural fluid</td>
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<td>Laboratory finding (value)</td>
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<td></td>
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<tr>
<td>Creatinine level (mg/dL)</td>
<td>Increased (1 mg/dL)</td>
<td>Normal (0.7 mg/dL)</td>
<td>Normal (0.4 mg/dL)</td>
<td>Normal (0.1 mg/dL)</td>
</tr>
<tr>
<td>Urinalysis findings (H+)</td>
<td>P, H</td>
<td>ND</td>
<td>P, H</td>
<td>P, H</td>
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<tr>
<td>Serum CPK level (U/L)</td>
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<td>Normal (32 U/L)</td>
<td>Normal (27 U/L)</td>
<td>Increased (117 U/L)</td>
</tr>
<tr>
<td>Serum AST level (U/L)</td>
<td>Increased (122 U/L)</td>
<td>Normal (16 U/L)</td>
<td>Normal (21 U/L)</td>
<td>Normal (34 U/L)</td>
</tr>
<tr>
<td>Serum ALT level (U/L)</td>
<td>Normal (16 U/L)</td>
<td>Normal (8 U/L)</td>
<td>Normal (21 U/L)</td>
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<td>PT, s</td>
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<td>Prolonged, 14.5 s</td>
<td>Prolonged, 15.2 s</td>
<td>Prolonged, 16.3 s</td>
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<tr>
<td>APTT, s</td>
<td>&gt;100</td>
<td>46.8</td>
<td>66.1</td>
<td>45</td>
</tr>
<tr>
<td>Thrombocytopenia (platelet count, platelets/mm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>Present (37,000)</td>
<td>Present (123,000)</td>
<td>Present (62,000)</td>
<td>Present (122,000)</td>
</tr>
<tr>
<td>Outcome</td>
<td>Died</td>
<td>Survived</td>
<td>Survived with neurological deficit&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Survived</td>
</tr>
</tbody>
</table>

**NOTE.** ALT, alanine aminotransferase; APTT, activated thromboplastin time; AST, aspartate aminotransferase; CPK, creatine phosphokinase; H, hematuria; ND, not done; P, proteinuria; PT, prothrombin time.

<sup>a</sup> Died within 8 h after hospital admission.

<sup>b</sup> Highest value is shown when >1 abnormal value was obtained.

<sup>c</sup> Poor feeding, lateral gaze palsy, and hypertonicity.
in accordance with the guidelines published by Tenover et al. [25]. PFGE patterns resulting in 2–3 band differences were considered to be closely related, those with 4–6 band differences were considered to be possibly related, and those with ≥7 band differences were considered to be unrelated.

**Multilocus sequence typing (MLST).** MLST was performed as described elsewhere [26]. The allelic profile of *S. aureus* isolates was obtained by sequencing internal fragments of 7 “housekeeping” genes and submitted to the MLST home page (http://www.mlst.net), where 7 numbers depicting the allelic profile were assigned that defined the MLST type.

**Southern blot testing.** Southern blotting of PFGE gels was performed as described elsewhere [27]. Membranes were probed, as described elsewhere [1], with the use of a PCR-generated fragment containing the mecA gene labeled with the [α-32P]-dNTP RadPrime DNA labeling system (GibcoBRL).

**SCCmec typing.** SCCmec typing was performed by PCR with sets of region-specific primers (figure 1), as described elsewhere [17, 18].

**PCR amplification of toxin genes.** The synthetic oligonucleotide primers for the PCR amplification of the *tst*, *sea*, *seb*, *sec*, *sed*, *seh*, and *seo* genes encoding TSST-1, SEA, SEB, SEC, SED, SEE, and SEO, respectively, have been described elsewhere [28, 29]. The primers for the PCR amplification of the lukS-PV and lukF-PV genes encoding Panton-Valentine leukocidin (PVL) components PVL-S and PVL-F were designed as follows: PVL-1, 5′-CTGTTGGCATTCTAGTTATCTGTA-3′; and PVL-2, 5′-CGATATCGTTGCTATCACA-3′. PCR was also performed to detect the 189-bp truncated *seo* fragment (*seo*) found in CA-MRSA isolate MW2 [30]. The primers used to detect this fragment were as follows: SEO-3, 5′-ATGATATGATTTCGGAAAACGAG-3′; and SEO-4, 5′-GAAAATAGATCATCTATATGTAATTATTTGGG-3′. Control strains that harbored the relevant enterotoxin- and toxic shock syndrome (TSS)–associated toxin genes were ATCC 13565 (SEA), ATCC 14458 (SEB), ATCC 19095 (SEC), ATCC 23235 (SED), ATCC 27664 (SEE), NRS123 (SEH), N315 (SEO), ATCC 51650 (TSST-1), and ATCC 49775 (PVL components S and F).

**RESULTS**

The *Smal* restriction fragment patterns (figure 2) of the 5 *S. aureus* isolates recovered from 4 patients were compared visually. The fragment patterns of the 3 CA-MSSA isolates recovered from patients 1A and 2A were identical (figure 2). The fragment patterns of the 2 CA-MRSA isolates recovered from patients 1B and 2B were identical to each other (figure 2) and to isolate MW2 (data not shown). Importantly, the *Smal* pattern of the CA-MRSA isolates differed from that of the CA-MSSA isolates by only 2 bands. Specifically, a band in the CA-MRSA isolates that migrated just below the 194-kb marker was missing in the CA-MSSA isolates, and a band in the MSSA isolates that migrated just above the 145-kb marker was missing from the CA-MRSA isolates. Southern blot hybridization, using the mecA probe, demonstrated that these differences were due to the presence of a SCCmec element in the chromosome of the CA-MRSA isolates that produced a shift in the molecular weight of the 145-kb fragment to 194 kb (figures 2 and 3).

MSSA isolate 2352 from patient 2A and MRSA isolate 2355 from patient 1B were selected as representative isolates for MLST typing. The allelic profile of isolate 2352 (from patient 2A) was identical to that of isolate 2355 (from patient 1B) except for a 1-bp change in the *tpi* locus. Thus, both isolates were assigned to MLST type 1.

The 2 CA-MRSA isolates contained type IV SCCmec, as exemplified by having type 2 *crr* genes and the class B mec complex (Ψ1272-ΔmecRI-mecA-IS431) [17, 18]. However, PCR amplification of the left extremity of SCCmec type IV (L-C region)
Figure 1. Map depicting the structure of type IVa and type IVb staphylococcal cassette chromosome mec (SCCmec). PCR amplification of 4 regions that define SCCmec was accomplished with sets of primers as follows: the region from the left extremity to the ccr genes (L–C region) was amplified with primers cLs1 (type IVa) or cL2* (type IVb) and a5. The region from upstream of ccr to ΔmecR1 (C–M region) was amplified with primers a6 and mcR8. The region from upstream of the mecA gene complex to mecA (M–I region) was amplified with primers is4 and mA2. The primers mA3 and cR1 were used to amplify the region from upstream of IS431mec to orfX (I–orfX region).

by 2 primer pairs, cLs1/a5 and cL2/a5, revealed distinct products that differed from those we described elsewhere [17, 18]. With the primer pairs cLs1/a5, the amplified fragment from the prototype SCCmec IVa–containing isolate, CA05, was 9.9 kb, but no product was detected in the 2 CA-MRSA isolates. With the primer pair cL2/a5, the amplified fragment from the prototype SCCmec IVb isolate, 8/6-3P, was 9 kb, but it was slightly larger (>9 kb) in the 2 CA-MRSA isolates 2355 and 2356 (data not shown). The EcoRV restriction patterns of the >9-kb fragment of these 2 CA-MRSA isolates were identical to each other but differed from that of the 9-kb fragment of isolate 8/6-3P (figure 4).

The I–orfX region (figure 1), amplified by the primer set mA3/cR1, of CA-MRSA isolates 2355 and 2356 was also different from previously characterized CA-MRSA isolates carrying SCCmec type IV. The 6.4-kb I–orfX amplified fragment from isolates CA05 and 8/6-3P was absent from isolates 2355 and 2356, but 2 new bands (8 kb and 4 kb) were demonstrable.

Genes lukS-PV and lukF-PV, encoding LukS and LukF, respectively, the 2 protein components of the synergohymenotropic PVL, were detected in all 5 patient isolates, as were ser and seh. The see gene was detected only in the CA-MSSA isolates, and the sec gene was detected only in the CA-MRSA isolates. The tst, sed, see, and intact see genes were not detected in any of the 5 tested S. aureus clinical isolates. The unique see' fragment found in strain MW2 by Baba et al. [30] was present in all 5 isolates recovered from our patients.

Prompted by the observation that unique features of the MW2 genome, the seh gene encoding staphylococcal enterotoxin H and see', were present in all 5 of our patient isolates, as well as the highly virulent CA-MRSA isolate MW2, and also by the observation that these 6 isolates had a similar or identical pulsotype (designated “type G” in our laboratory), we screened 12 CA-MRSA isolates reported elsewhere [17] for seh and see', as well as 5 additional SCCmec type IV CA-MRSA isolates (CA06, CA09, CA13, 2309, and 2310). The 12 previously reported isolates represented 5 PFGE pulsotypes, 4 coagulase types, and 2 ribotypes. The 5 additional CA-MRSA isolates were chosen because they had SCCmec IV and were pulsotype G. seh and see' were uniformly present in MW2 and all of the pulsotype G isolates and were uniformly absent from all CA-MRSA isolates of other PFGE types.

Figure 2. PFGE of SmaI-digested genomic DNA of Staphylococcus aureus isolates recovered from 4 pediatric patients. Lane 1, Molecular weight Pulse Marker D 2476. Lane 2, Strain 2352 (recovered from patient 2A). Lane 3, Strain 2353 (recovered from patient 1A). Lane 4, Strain 2354 (recovered from patient 2A). Lane 5, Strain 2355 (recovered from patient 1B). Lane 6, Strain 2356 (recovered from patient 2B). All 5 isolates have an identical PFGE pattern, except for a 2-band difference reflecting the presence of staphylococcal cassette chromosome mec in the community-acquired methicillin-resistant S. aureus isolates.
DISCUSSION

Our patient isolates demonstrate that a prevalent, virulent CA-MRSA strain likely arose from insertion of SCCmec type IV into a virulent MSSA strain. The pulsotypes of the 5 S. aureus isolates recovered from our 4 patients presenting with sepsis syndrome were closely related to each other and to the sequenced CA-MRSA isolate MW2 that caused similar, severe disease. MW2 and our CA-MRSA patient isolates were indistinguishable. The only discernible difference in the PFGE banding patterns of our CA-MRSA isolates and our CA-MSSA isolates was the presence of an SCCmec element in a fragment of the CA-MRSA isolates, as demonstrated by Southern blot hybridization. Moreover, unique toxin gene sequences of the MW2 genome, seh and sed, were present in all 5 of our patient isolates, as well as in MW2. These genetic elements are absent from the 5 other completely sequenced S. aureus genomes, although an intact copy of sea can be found in N315 [31]. Furthermore, identical results of MLST typing of 1 MRSA and 1 MSSA isolate reinforced the notion of a close genetic relationship among our patient isolates.

Two phenomena could theoretically explain the close genetic relationship between these CA-MRSA and CA-MSSA patient isolates, despite the differences in methicillin susceptibility. It is possible that SCCmec IV inserted into a CA-MSSA isolate, thereby converting it into a CA-MRSA strain like MW2. Circumstantial evidence for such in vivo transfer of a large SCCmec element, likely type II or type III according to our classification system [16–18], was also documented in a Dutch infant [32]. Such horizontal transfer of SCCmec was once thought to be relatively rare, as evidenced by the few ancestral clones that accounted for MRSA clinical isolates worldwide [33]. More recently, it has been realized that the diversity in lineages of MRSA isolates indicates that horizontal transfer of SCCmec into MSSA recipient backgrounds has been relatively frequent [34, 35]. In support of this, SCCmec IV has been found in multiple S. aureus backgrounds by our groups [17].

It is also possible that the SCCmec element was lost from a CA-MRSA isolate to yield the MSSA clone. In vitro spontaneous loss of methicillin resistance has been described elsewhere [36–39], usually in association with deletion of SCCmec [39]. However, to date, such spontaneous loss has been described only in isolates that have also undergone deletion of the plasmid encoding the structural β-lactamase gene and its regulatory elements, rendering the subclones susceptible to methicillin and also penicillin. The methicillin-susceptible isolates recovered from our patients produced β-lactamase and were resistant to penicillin. Thus, spontaneous loss similar to the previously described in vitro occurrences is unlikely to explain the relationships between our isolates. On the other hand, preliminary data suggest that there may be a barrier to the introduction of a mecA complex into a naïve, susceptible S. aureus background [40].

The recent publication of the whole genome sequence of MW2, a virulent CA-MRSA isolate with a PFGE pattern identical to that of our CA-MRSA isolates, suggested that MW2 differed from other MRSA isolates for which whole genomic sequence data are available [30]. Multiple unique toxin genes not present in other MRSA genomes, such as N315, Mu50, E-MRSA16 (strain 252), and COL, were identified in MW2. This suggests that the origin of MW2 is distinct from that of “hospital circulating” MRSA isolates. Indeed, Okuma et al. [41] noted the similarity between MW2 and isolate 476, a highly virulent MSSA strain. We speculate that the available data are most consistent with the notion that SCCmec IV, an element substantially smaller than SCCmec I–III [18], is able to readily transfer into multiple MSSA backgrounds with successful resultant MRSA clones of pathogenic potential. Such a scenario would explain the relationship between our patient isolates, as well as our recent observation that a SCCmec IV element was found among 11 CA-MRSA isolates of diverse genetic backgrounds, as evidenced by PFGE, coagulase typing, and ribotyping [17].

All of our patients had an invasive pulmonary infection, and their S. aureus isolates harbored genetic elements encoding the S and F proteins that assemble to form the synergohemolytic PVL. Recently, PVL was detected in a majority of isolates associated with severe necrotic hemorrhagic pneumonia and skin infection in France [42, 43]. This toxin was also dem-

Figure 3. Southern blot of the pulsed-field agarose gels containing Smal-digested genomic DNA and using the mecA gene as a probe, demonstrating the presence of staphyloccocal cassette chromosome mec in community-acquired methicillin-resistant Staphylococcus aureus isolates isolated from pediatric patients. Lane 1, Strain 2356 (recovered from patient 2B). Lane 2, Strain 2355 (recovered from patient 1B). Lane 3, Strain 2354 (recovered from patient 2A). Lane 4, Strain 2353 (recovered from patient 1A).
shown among 14 CA-MRSA isolates with the same clonality in France [21].

None of our patients had cases that met the definition of TSS established by the Centers for Disease Control and Prevention (CDC) in 1981 [44], strictly speaking. However, the term “toxic shock–like illness” was used to describe conditions in a subset of patients who had a compatible illness but whose illnesses did not meet this case definition. Parsonnet [45] proposed that the CDC case definition should be revised to incorporate laboratory findings that are useful in diagnosis of TSS, including production of TSS-associated toxins. However, in the absence of an agreed-upon, revised definition, we have used the term “sepsis syndrome” to describe the multisystem clinical manifestations observed in our patients.

The gene encoding TSST-1, the toxin isolated in the majority of staphylococcal TSS cases, was absent from our patient isolates. However, other toxins capable of causing sepsis syndrome or TSS-like illness were identified. One example is staphylococcal enterotoxin H, which was present in MW2 and in all 5 of our patient isolates; this can cause a staphylococcal TSS-like illness in rabbits [46]. Abundant SEH has been detected in isolates carrying this gene [47]. Indeed, SEH binds with high affinity to human major histocompatibility complex class II and exhibits potent mitogenic activity in human T cells [48].

We also found the enterotoxin B gene (seb) in the CA-MSSA isolates and the enterotoxin C gene (sec) in both CA-MRSA isolates. Products of both genes have been implicated as causes of nonmenstrual severe TSS [49–51]. seb and sec are encoded on different pathogenicity islands, SaPI3 and SaPI4, respectively [52], which suggests, despite evidence indicating close epidemiologic and genetic relatedness among our isolates, that they have been recipients of distinct staphylococcal pathogenicity islands.

The seD fragment found in all 5 patient isolates, MW2, and the other clonally related CA-MRSA isolates we studied is distinctive in its sequence and has relatively low homology (63.5%) with the intact seO gene found in the genome of N315 [31]. This 189-bp fragment aligned with the 3′ end of the intact seO gene from strains N315 [31] and A900322. The lack of an upstream Shine-Delgarno sequence suggests that a truncated gene product with toxin activity is unlikely.

We previously reported that SCCmec type IV was present in multiple, unrelated CA-MRSA backgrounds. Our data extend this observation by documenting that seh and seD were not found in the non–type G CA-MRSA backgrounds we screened and, thus, were signature molecular features of the highly virulent MW2 [30], our sepsis syndrome patient isolates, and other type G CA-MRSA isolates. Whether they are markers for highly virulent S. aureus isolates awaits further clarification.

SCCmec type IV was identified in the 2 CA-MRSA patient isolates. However, variation in the PCR amplicon sizes from the L–C and I–orfX regions precluded their classification into the subtypes IVa, IVb [18], and IVc [53]. Thus, CA-MRSA strains documented in this report harbor a different SCCmec subtype than that of strain MW2 (SCCmec type IVa), despite having an identical pulsotype. These data suggest 2 possible models for the evolution of these isolates: either they were derived from a single progenitor that diverged after the introduction of the SCCmec element, or different isolates having the same MSSA background were independent recipients of distinct SCCmec elements. Distinguishing these possibilities is a current focus in our laboratory.
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