A Novel Methicillin-Resistance Cassette in Community-Acquired Methicillin-Resistant \textit{Staphylococcus aureus} Isolates of Diverse Genetic Backgrounds

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Until recently, it has been unclear whether community-acquired (CA) methicillin-resistant \textit{Staphylococcus aureus} (MRSA) isolates represent the spread of hospital MRSA isolates into the community. In 2 CA-MRSA isolates, a novel genetic element, designated staphylococcal cassette chromosome \textit{mec} (SCC\textit{mec}) type IV, was found; it differs from SCC\textit{mec} types I–III in its small size and absence of non-\textit{\beta}-lactam genetic-resistance determinants. To study the prevalence of type IV SCC\textit{mec}, polymerase chain reaction characterization of SCC\textit{mec} was performed on DNA from 12 CA-MRSA isolates. The 12 CA-MRSA isolates were from diverse genetic backgrounds, as evidenced by their stratification into 5 pulsed-field gel electrophoresis types, 4 coagulase types, and 2 ribotypes. Eleven of the 12 isolates contained the novel SCC\textit{mec} type IV element. Ten were resistant only to \textit{\beta}-lactam antibiotics. SCC\textit{mec} type IV is present on the genome of CA-MRSA isolates. Its relatively small size and presence in isolates of diverse genetic backgrounds suggest that it may spread among \textit{S. aureus} isolates.

The notion that methicillin-resistant \textit{Staphylococcus aureus} (MRSA) is confined to patients with well-described risk factors in the hospital environment [1] has been challenged recently with the discovery of community-acquired (CA) MRSA isolates in children and adults who lacked these predisposing risk factors. At the University of Chicago Children’s Hospital (UCCH; Chicago), the prevalence of CA-MRSA isolates in children without identified predisposing risk increased from 10 cases/100,000 admissions in 1988–1990 to 259 cases/100,000 admissions in 1993–1995 [2]. In a follow-up study in 1998–1999, the prevalence of CA-MRSA at UCCH was 208 isolates/100,000 admissions. Some of the infected children in 1998–1999 had risk factors for MRSA infection, although the temporal acquisition of their isolates fit our definition of community acquisition (obtained ≤3 days into hospitalization) [3].

Other researchers also have recognized infection with these CA-MRSA isolates as an emerging clinical problem [1, 4–6]. Such CA-MRSA infections may be severe; 4 children in Minnesota and North Dakota had fatal CA-MRSA disease [7], and 2 children recently were hospitalized at our institution with CA-MRSA-mediated toxic shock syndrome (authors’ unpublished data).

Transmission of CA-MRSA isolates also has been documented in day-care settings after hospitalization of index patients [4, 5] and among members of a high school wrestling team [8]; CA-MRSA colonization also has been documented in otherwise healthy children who were using an acute-care facility at our center and among children seeking care at an inner-city well-child clinic [6].

The MRSA isolates from patients in the community setting are noteworthy in their tendency to be susceptible to most non-\textit{\beta}-lactam antimicrobials, in contrast to hospital-acquired MRSA isolates, and are most often resistant only to methicillin. Methicillin resistance in \textit{S. aureus} implies cross-resistance to all \textit{\beta}-lactam antibiotics and is mediated by the production of a penicillin-binding protein, called “PBP 2a” or “PBP 2a,” that has decreased binding affinity for \textit{\beta}-lactam antibiotics [1]. The gene encoding PBP 2a, \textit{mecA}, permits cell-wall synthesis despite the presence of \textit{\beta}-lactam antibiotics. \textit{mecA} is carried on a large, mobile genetic element called “staphylococcal cassette chromosome \textit{mec}” (SCC\textit{mec}) [9] that is integrated into the chromosome of MRSA isolates obtained from various countries. All MRSA isolates studied to date carry the \textit{mecA} gene and contain an SCC\textit{mec} element [9].

Three SCC\textit{mec} types, I–III, have been assigned on the basis of DNA sequencing and polymerase chain reaction (PCR) analyses of the element from a collection of 38 epidemic MRSA isolates obtained from 20 countries [9]. The 3 SCC\textit{mec} types (table 1) share an identical chromosomal integration site, conserved terminal inverted repeats and direct repeats at the integration junction points, conserved genetic organization around
the mecA gene, and cassette chromosome recombinase (ccr) genes responsible for the horizontal transfer of SCCmec. Genetic elements encoding resistance to other antibiotics were commonly found in 2 of the SCCmec types described to date (II and III). Among 38 MRSA isolates from 20 countries, most collected during the 1980s, all contained an SCCmec element; 34 could be grouped into 1 of these 3 types.

We wondered whether this newly recognized change in CA-MRSA epidemiology reflected the movement of hospital MRSA isolates into the community or whether the CA-MRSA isolates reflected new clones circulating in the community. Accordingly, we subjected some of our isolates to SCCmec typing by PCR, to assess their epidemiologic relatedness to other MRSA isolates.

Initial examination of 2 of these isolates suggested the presence of a novel SCCmec element, designated type IV [10]. This element differed from SCCmec types I–III in that it was smaller and contained no resistance determinants except for the mecA gene. To determine the prevalence of this type IV SCCmec element among CA-MRSA isolates, we used a PCR mapping strategy to characterize the SCCmec region for the 2 isolates that we sequenced and 10 other CA-MRSA isolates chosen from our collection.

Materials and Methods

Bacterial strains. A CA-MRSA isolate was defined as one cultured from a patient within 72 h of admission to the hospital or from a patient who was not hospitalized. The 12 CA-MRSA isolates in this study are depicted in table 2 [10]. Isolates with the designations CAxx were chosen from CA-MRSA isolates that were collected during prospective surveillance of MRSA isolates from patients hospitalized at UCCH in 1998–1999 (UCCH isolates) [3]. The other 6 isolates (ER isolates) were obtained from a study conducted in 1996 that identified MRSA isolates from the nasopharynx and skin of children attending the emergency room at our institution [6]. These isolates were recovered from skin or mucous membranes of patients, none of whom were seeking care for staphylococcal disease syndromes. Isolates from both studies were chosen to represent the diversity of pulsed-field gel electrophoresis (PFGE) profiles we have encountered among CA-MRSA isolates (authors’ unpublished data).

PFGE. Whole-cell DNA was prepared and digested in agarose plugs with Smal, as described elsewhere [11, 12]. Restriction fragments were resolved using a CHEF DR III apparatus (BioRad).

Antimicrobial susceptibility testing. Broth dilution MIC testing for oxacillin was performed as described elsewhere [11]. NaCl (2%) was included in the medium when assaying the MIC of oxacillin, as recommended by the National Committee for Clinical and Laboratory Standards [13]. The Pasco MIC gram-positive panel (Difco) was used to determine the susceptibility profile of the ER isolates for non-β-lactam antimicrobials, and the Vitek system (bioMérieux Vitek) was used for susceptibility testing of the UCCH isolates. The antimicrobial agents tested included penicillin, oxacillin, clindamycin, erythromycin, gentamicin, rifampin, trimethoprim-sulfamethoxazole, ciprofloxacin, and vancomycin.

The assignment of SCCmec type was based on PCR priming of the various regions of the SCCmec element, as described elsewhere [9, 10]. Primers used were identical to those we reported elsewhere [9, 10], except that the primer set is4/mA2 was used to define a region of the SCCmec element that we now designate M-I, and the primer set mA3/cR1 was used to cover the right extremity from IS431 to orfX. The results of this PCR definition of the SCCmec element allowed for the assignment of the ccr and mec complex types (table 1) [10] and, thus, the assignment of the appropriate SCCmec type (I–IV).

Additional molecular definition of MRSA isolates. Coagulase typing was performed according to the method described by Ushioda et al. [14], using a coagulase typing kit (Denka Seiken) and neutralizing rabbit antisera specific to coagulase types I–VIII (Denka Seiken). In brief, cells of the test strain were grown in brain-heart infusion broth overnight; 0.1 mL of the culture supernatant was added to 0.1 mL of each anticoagulase antiserum or normal rabbit serum and was incubated at 37°C for 1 h. The diluted rabbit plasma (0.2 mL) was added to each tube and incubated at 37°C. Coagulation was judged by visual inspection after incubation for at least 1 h.

Probe-based ribotyping was performed according to the procedure described by Yoshida et al. [15]. DNA (2 mg) was digested with the HindIII restriction enzyme and analyzed by electrophoresis on 0.8% submarine agarose gels in a Tris-acetate EDTA buffer. Southern transfer of DNA from the agarose gel to a nylon membrane A (Paul BioSupport) was performed as described elsewhere [15]. The 23S rDNA of 669 bp, which was amplified from a subclone carrying the HindIII-EcoR1 fragment of pOH4 with primers M4 and RV, was used as a probe. The probe was labeled with digoxigenin using a DNA-labeling kit (Boehringer). Hybridization took place in a hybridization solution containing 20 ng of digoxigenin-labeled probe per milliliter at 42°C overnight. Washing of the filter and visualization of the signal was achieved using a detection kit (Boehringer), according to the method recommended by the manufacturer.

Results

The 12 isolates could be classified into 5 PFGE types and 4 coagulase types—I (n = 3), II (n = 4), III (n = 4), and VII (n = 1) (table 2)—that did not conform to the PFGE stratification. Stratification by ribotyping produced 2 patterns, A (n = 9) and C (n = 3) (table 2). Of the 6 UCCH isolates, 5 were associated with clinical disease, and 1 was colonizing the

Table 1. Characteristics of staphylococcal cassette chromosome mec (SCCmec) types.

<table>
<thead>
<tr>
<th>SCmec type</th>
<th>Gene type</th>
<th>mec complex</th>
<th>Size, kb</th>
<th>RE type</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>IS1272</td>
<td>mecI/PM/MS</td>
<td>+/+/+</td>
<td>B 34.3</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
<td>+/+/-</td>
<td>A 53.0</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>3</td>
<td>+/+/-</td>
<td>A 66.9</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>2</td>
<td>+/-/-</td>
<td>B 20.9-24.3</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. ccr. Cassette chromosome recombinase; MS, membrane-spanning domain; PB, penicillin-binding domain; RE, right extremity of SCCmec element; +, positive; −, negative.
skin (table 2). The 6 ER isolates were all colonizing isolates obtained from a naris or skin and were not associated with any clinical syndrome caused by S. aureus. MRSA risk factors were variably present among these 6 patients (table 2).

Antimicrobial susceptibility testing revealed that 10 of the 12 isolates were susceptible to all non-β-lactam antimicrobial agents tested. Of the remaining 2 isolates, 1 had intermediate resistance to erythromycin. The other, CA07, was resistant to clindamycin, erythromycin, gentamicin, trimethoprim-sulfamethoxazole, and ciprofloxacin but susceptible to vancomycin. This isolate was obtained in July 1999 from a 15-year-old patient with diabetes who had undergone repair of a slipped femoral epiphysis 2 years earlier. Four months prior to admission, in March 1999, she had her left pinna pierced and experienced intermittent swelling and redness in the ensuing months. Doxycycline was initially prescribed, without improvement. Surgical drainage and oral ciprofloxacin also yielded no improvement, and cervical lymphadenopathy developed, anatomically proximal to the inflamed pinna. A visit to the UCCH emergency department ensued, where physical examination revealed an erythematous, swollen, fluctuant superior surface of the pinna with bloody discharge from the broken skin in the inflamed area. Isolate CA07 was recovered from this bloody discharge.

Genetic elements were found in 11 of the 12 isolates that were similar to those we described for SCCmec type IV [10] (tables 1 and 2). All 12 had mecA gene sequences. All 12 had the 5′ terminus of mecRI. The 11 SCCmec type IV–containing isolates had IS1272 and lacked mecI and the 3′ terminus of mecRI. Thus, the elements defining the class B mec complex were present in 11 of the 12 isolates. Typing of the ccr gene revealed that all 12 CA-MRSA isolates tested were type 2 and that the fragment spanning ccr and mecRI was identical to the corresponding region of the type IV SCCmec. All 12 isolates were right extremity of SCCmec element type ii (table 2).

### Discussion

Our data indicate that the SCCmec elements of 11 of the 12 CA-MRSA isolates we studied are similar or identical to one another. SCCmec elements from 2 of these 12 isolates were sequenced in their entirety and assigned to a novel type [10] on the basis of the genetic elements identified. Our data extend this observation and are consistent with the presence of the type IV element in all but 1 of these 12 CA-MRSA isolates.

SCCmec type IV elements carry the type 2 ccr gene complex, which is also found in the type II SCCmec. They also carry the class B mec gene complex, which also is found in the type I SCCmec. The presence of ccr and mec complexes in the type IV element that also were found in other SCCmec types strongly suggests that genetic recombination events must have occurred, resulting in the emergence of the type IV element. The novel type IV SCCmec elements we sequenced were 24,309 and 20,921 bp in size, substantially smaller than the types I–III SCCmec elements, which were 34–67 kb in size, as described elsewhere [9].

In a previous report regarding SCCmec [9], 34 of 38 MRSA isolates could be classified into SCCmec types I, II, and III. The other 4 isolates could not be classified into these 3 groups. However, 3 of these isolates had class B mec complexes, and 2 had type 2 ccr complexes. Thus, in retrospect, 2 of these 4 “untypeable” isolates contained genetic elements identical to those in type IV SCCmec elements. Additional characterization of these isolates is in progress to define how closely the SCCmec elements in these MRSA isolates from Japan resemble the small type IV SCCmec elements we found in our CA-MRSA isolates years later in Chicago.

The 12 CA-MRSA isolates we studied are of diverse genetic backgrounds, as defined by PFGE, coagulase typing, and ribotyping—the 3 nucleic acid–based procedures we used to stratify our isolates. This observation indicates that multiple genetic
backgrounds are associated with CA-MRSA isolates. Thus, the presence of SCCmec type IV in 11 of the 12 isolates suggests that this element can spread in a promiscuous manner, although the genetic strategy by which horizontal spread of these elements occurs remains to be elucidated. The smaller size of the type IV element might facilitate transfer on a plasmid or bacteriophage to a susceptible recipient strain.

Our data suggest that MRSA isolates circulating in the community with the characteristics we have described do not reflect simple transfer of hospital-milieu MRSA isolates into the community. Rather, they suggest that these novel type IV SCCmec elements, which are smaller than SCCmec elements types I–III, likely have increased mobility, compared with their larger SCCmec counterparts and, therefore, may have greater propensity for transfer to diverse S. aureus genetic backgrounds on plasmids, bacteriophages, and perhaps even by currently-undefined independent means.

Ten of the 12 isolates we examined were uniformly susceptible to all the non-β-lactam antimicrobials tested. Until now, the explanation as to why CA-MRSA isolates have tended not to be multiply resistant to unrelated non-β-lactam antimicrobial agents has not been clear but is now explained by the lack of other antimicrobial resistance determinants in the SCCmec type IV elements we sequenced. This property of SCCmec type IV differs from SCCmec types II and III, which carry resistance determinants to non-β-lactam antimicrobials within the boundaries of the SCCmec element.

Among our 12 CA-MRSA isolates, CA07 was unusual in its resistance to multiple non-β-lactam antimicrobials, although it was susceptible to rifampin and vancomycin. This isolate was obtained from a patient with multiple risk factors for MRSA infection, and we now rely on clindamycin and vancomycin in this regard.

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References