Antimicrobial Susceptibility Patterns and Staphylococcal Cassette Chromosome mec Types of, as Well as Panton-Valentine Leukocidin Occurrence among, Methicillin-Resistant Staphylococcus aureus Isolates from Children and Adults in Middle Tennessee

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Antimicrobial susceptibility patterns, Panton-Valentine leukocidin (PVL) occurrence, and staphylococcal cassette chromosome mec (SCCmec) types in methicillin-resistant Staphylococcus aureus (MRSA) strains isolated from children and adults at Vanderbilt University Medical Center during a 12-month period were evaluated. A total of 1,315 MRSA isolates were collected, of which 748 (36.7%) were recovered from children. Among all isolates, 448 (34.1%) were SCCmec-II, and 847 (64.4%) were SCCmec-IV. More SCCmec-IV isolates were recovered from children than SCCmec-II isolates (424 [50.1%] versus 50 [11.2%]; odds ratio [OR] = 7.98; P < 0.000001). The PVL gene was detected in 93.6% of SCCmec-II isolates, in contrast to 0.2% in SCCmec-IV isolates. Within SCCmec-IV isolates, a statistically higher PVL occurrence was noticed in children (98.1%) than in adults (89.1%) (OR = 6.34; P < 0.000001). Overall, SCCmec-II strains showed greater resistance than SCCmec-IV strains to clindamycin, erythromycin, levofloxacin, gentamicin, rifampin, minocycline, and trimethoprim-sulfamethoxazole. Both SCCmec-II and SCCmec-IV strains recovered from adults were more resistant to these antibiotics than those recovered from children. SCCmec-II strains were predominantly recovered from the respiratory tract, whereas SCCmec-IV strains were predominantly recovered from skin, soft tissue, abscesses, and surgical wounds. These data indicate that SCCmec-IV MRSA isolates frequently infect children in middle Tennessee and are likely to harbor the PVL gene.

Staphylococcus aureus is a frequent and important human pathogen that causes both hospital- and community-acquired infections (3, 6, 12, 25). Since methicillin-resistant S. aureus (MRSA) was first described in 1961 in England (18), it has become an important problem in hospitals around the world (6). MRSA became a problem in many European countries in the 1960s and in the United States in the 1970s (1, 6). In contrast to hospital-acquired MRSA (HA-MRSA), community-acquired MRSA (CA-MRSA) strains are isolated from healthy people in the community and are susceptible to a number of commonly used antibiotics (16, 24, 26). CA-MRSA causes predominantly skin and soft-tissue infections but can cause serious necrotizing pneumonitis. The increased virulence is due in part to the Panton-Valentine leukocidin (PVL) gene, which is generally present in CA-MRSA isolates. The presence of PVL along with superantigens can result in severe tissue necrosis (9, 25, 35). The CA-MRSA clone in the United States has resulted in several pediatric deaths (16, 23, 25), suggesting that children may have an increased risk of serious MRSA infections compared to adults.

Methicillin resistance in S. aureus is mediated by production of low-affinity penicillin binding protein 2a that is encoded by the mecA gene (3, 19). The gene is located on a mobile element, the staphylococcal chromosomal cassette mec (SCCmec) (2, 28). To date, five different SCCmec elements have been identified in MRSA. The SCCmec typing provides strong evidence for the independent deviation of HA-MRSA and CA-MRSA clones (28). The SCCmec types I, II, and III are predominantly found in HA-MRSA strains, whereas the SCCmec types IV and V are mainly associated with CA-MRSA throughout the world (2, 9, 15, 17).

The aim of this study was to determine the SCCmec types and occurrence of the PVL gene and to correlate these with phenotypic antibiotic susceptibility patterns for MRSA strains isolated from children and adults at Vanderbilt University Medical Center (VUMC) during a 12-month study period. We focused on the differences between children and adults because of the perception that children were having an increased incidence of serious staphylococcal infections (6, 11).

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MATERIALS AND METHODS

Patient demographics. Vanderbilt University Medical Center includes Vanderbilt University Hospital (501 beds) and Vanderbilt Children’s Hospital (304 beds). More than 700,000 patient visits occur each year, with approximately 35,000 patients being admitted. The ratio of adult visits/admissions to children’s visits/admissions is similar to the ratio of available beds, with 62% adults and 38% children.
TABLE 1. Characteristics and antibiotic resistance profiles of MRSA strains recovered from adults at Vanderbilt University Medical Center, 15 November 2004 to 14 November 2005

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%) of SCCmec-II strains (n = 398)</th>
<th>No. (%) of SCCmec-IV strains (n = 423)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bloodstream</td>
<td>83 (20.9)</td>
<td>31 (7.3)</td>
<td>&lt;0.0000001</td>
</tr>
<tr>
<td>Respiratory</td>
<td>151 (37.9)</td>
<td>28 (6.6)</td>
<td>&lt;0.0000001</td>
</tr>
<tr>
<td>Skin, soft tissue, abscess, and post surgical wounds</td>
<td>127 (31.9)</td>
<td>352 (83.2)</td>
<td>&lt;0.0000001</td>
</tr>
<tr>
<td>Others</td>
<td>37 (9.3)</td>
<td>12 (2.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Antibiotic resistance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methicillin, penicillin, amoxicillin, cefazolin</td>
<td>398 (100.0)</td>
<td>423 (100.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>393 (98.7)</td>
<td>381 (90.1)</td>
<td>&lt;0.0000001</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>386 (97.0)</td>
<td>56 (13.2)</td>
<td>&lt;0.0000001</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>29 (7.3)</td>
<td>4 (0.9)</td>
<td>&lt;0.0000001</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>386 (97.0)</td>
<td>70 (16.8)</td>
<td>&lt;0.0000001</td>
</tr>
<tr>
<td>Minocycline</td>
<td>3 (0.8)</td>
<td>0 (0.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Rifampin</td>
<td>17 (4.3)</td>
<td>0 (0.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>9 (2.3)</td>
<td>8 (1.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>NS</td>
</tr>
<tr>
<td>PVL gene occurrence</td>
<td>0 (0.0)</td>
<td>377 (89.1)</td>
<td>&lt;0.0000001</td>
</tr>
</tbody>
</table>

a NS, not significant.
b Includes inducible resistance.

c Statistical comparisons were performed with Epi Info software (version 6; Centers for Disease Control and Prevention, Atlanta, GA). Associations between SCCmec-II and SCCmec-IV MRSA for patient demographics, antibiotic resistance, PVL occurrence, and culture site were analyzed using the chi-square test or the Student's t test. P ≤ 0.05 was considered statistically significant.

RESULTS

A total of 2,740 consecutive Staphylococcus aureus isolates, of which 1,315 (48.6%) were MRSA, were collected for a full year from the Clinical Microbiology Laboratory at VUMC. Among the MRSA isolates, 482 (36.7%) were isolated from children. A total of 448 (34.1%) were SCCmec-II, 847 (64.4%) were SCCmec-IV, 2 (0.2%) were mixed SCCmec-II/IV, and 18 (1.4%) were nontypeable isolates. Fifty (11.2%) SCCmec-II isolates and 424 (50.1%) SCCmec-IV isolates were recovered from children (odds ratio [OR], 0.13; P < 0.000001). Since MRSA isolates from VUMC predominantly carried either SCCmec-II or SCCmec-II, analysis was focused mainly on these two groups of MRSA isolates. Among the 1,295 isolates, 241 (53.8%) and 399 (47.1%) were from males and carried SCCmec-II and SCCmec-IV, respectively. More SCCmec-II isolates were recovered from an older population (49.3 ± 21.8 years) than the SCCmec-IV isolates (22.4 ± 20.3 years; P < 0.000001). The demographic parameters between the SCCmec-II and SCCmec-IV MRSA strains in child and adult patients are listed in Tables 1 and 2.

The PVL gene was detected in 93.6% of SCCmec-IV isolates, in contrast to 0.2% in SCCmec-II (Tables 1 and 2). We further studied PVL presence proportions in variable culture sites in CA-MRSA isolates recovered from both children and adults (Table 3). The PVL presence proportion was statistically higher in children (416/424; 98.1%) than in adults (377/423; 89.1%) (OR, 6.34; P < 0.000001). A higher PVL occur-
The results of this study demonstrate that the SCCmec-IV isolates, 34.1% were SCCmec MRSA isolates from middle Tennessee. Among 1,315 MRSA isolates that were analyzed, there was no significant previous studies (6, 31, 37). When isolation site distribution of middle Tennessee possessed the characteristics reported in MRSA isolates indicated that the MRSA isolates recovered in MRSA isolates frequently infect children in middle Tennessee mec resistant to vancomycin (Tables 1 and 2).

All isolates were resistant to methicillin, to clindamycin, erythromycin, levofloxacin, gentamicin, rifampin, trimethoprim-sulfamethoxazole (SXT), and vancomycin was determined, and the resistance rates of the SCCmec-II and SCCmec-IV strains are compared in Tables 1 and 2. MRSA strains recovered from adults were more resistant to clindamycin (SCCmec-II, P < 0.000001; SCCmec-IV, P = 0.0137) and levofloxacin (SCCmec-II, P < 0.000001; SCCmec-IV, P < 0.000001) than those recovered from children, and this trend remained the same in both SCCmec-II and SCCmec-IV strains (Tables 1 and 2). SCCmec-II MRSA strains showed greater resistance than SCCmec-IV strains to clindamycin, erythromycin, levofloxacin, gentamicin, rifampin, trimethoprim, and SXT. All isolates were resistant to methicillin, amoxicillin-clavulanate, cefazolin, and penicillin. No isolate was resistant to vancomycin (Tables 1 and 2).

**DISCUSSION**

This is the first large-scale investigation of antimicrobial susceptibility patterns, PVL occurrence, and SCCmec types in MRSA isolates from middle Tennessee. Among 1,315 MRSA isolates, 34.1% were SCCmec-II and 64.4% were SCCmec-IV. The results of this study demonstrate that the SCCmec-IV MRSA isolates frequently infect children in middle Tennessee and are likely to harbor the PVL gene.

Exploration of age and culture site distribution of these MRSA isolates indicated that the MRSA isolates recovered in middle Tennessee possessed the characteristics reported in previous studies (6, 31, 37). When isolation site distribution of these MRSA isolates was analyzed, there was no significant difference in MRSA isolate numbers between those recovered from children and adults, except for a significantly higher rate of SCCmec-II isolates recovered from the respiratory tract of children (62%) than of adults (38%). Both children and adults were likely to have a staphylococcal bloodstream isolate with SCCmec-II strains. Our data demonstrated that 19.9% of SCCmec-II and 4.7% of SCCmec-IV strains were isolated from the bloodstream, which is consistent with previous findings that MRSA causes 5% to 19% of health care-associated bloodstream infections (7, 12).

There were significant differences regarding isolation sites between SCCmec-II and SCCmec-IV isolates. While SCCmec-IV isolates recovered in middle Tennessee were still mainly from abscess, surgical, and skin and soft-tissue specimens, our study discovered that SCCmec-II strains were recovered more frequently from the respiratory tract of children (62%) than of adults (37.9%) (OR, 2.67; p = 0.0011) (Tables 1 and 2).

Antibiotic susceptibility for amoxicillin-clavulanate, cefazolin, clindamycin, erythromycin, gentamicin, levofloxacin, minocycline, penicillin, rifampin, trimethoprim-sulfamethoxazole (SXT), and vancomycin was determined, and the resistance rates of the SCCmec-II and SCCmec-IV strains are compared in Tables 1 and 2. MRSA strains recovered from adults were more resistant to clindamycin (SCCmec-II, P < 0.000001; SCCmec-IV, P = 0.0137) and levofloxacin (SCCmec-II, P < 0.000001; SCCmec-IV, P < 0.000001) than those recovered from children, and this trend remained the same in both SCCmec-II and SCCmec-IV strains (Tables 1 and 2). SCCmec-II MRSA strains showed greater resistance than SCCmec-IV strains to clindamycin, erythromycin, levofloxacin, gentamicin, rifampin, trimethoprim, and SXT. All isolates were resistant to methicillin, amoxicillin-clavulanate, cefazolin, and penicillin. No isolate was resistant to vancomycin (Tables 1 and 2).

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CA-MRSA has now been introduced from its site of origin in the community into the hospital setting (27, 31). It has been reported that CA-MRSA strains cause skin infections and pneumonia (6, 22).

The PVL gene was present in 93.6% of SCCmec-IV strains, in contrast to 0.2% of SCCmec-II isolates. Within SCCmec-IV strains, a significantly higher incidence of the PVL gene was detected in children than in adults. First discovered in 1932 (29), PVL is a biocomponent synergohymenotropic toxin that is present in the majority of CA-MRSA carrying SCCmec-IV (9, 25). An association between PVL-containing strains of MRSA and virulent necrotizing pneumonia mainly in previously healthy children has been reported (14, 16, 23). Therefore, rapidly determining PVL presence/absence in the early clinical stage may improve patient outcomes and guide proper therapy, such as immunoglobulin administration (13, 32).

In contrast to the multidrug resistance usually seen in HA-MRSA, antibiotic resistance in CA-MRSA strains is often limited to β-lactams (6). In our study, the SCCmec typing correlated well with major antimicrobial susceptibility patterns. Antimicrobial susceptibility results in MRSA strains included in this study were consistent with previous findings, in that
most SCCmec-IV isolates remain susceptible to tetracycline, minocycline, clindamycin, gentamicin, rifampin, and SXT (25). However, in comparison to these antibiotics, 4.2% and 16.8% levofloxacin resistance was noticed in SCCmec-IV strains isolated from children and adults, respectively. SCCmec-II isolates possessed significantly greater resistance than SCCmec-IV isolates to several commonly used antibiotics, especially clindamycin, erythromycin, and levofloxacin. Similar emerging fluoroquinolone resistance has been reported in other parts of the world, such as Australia (27). Considering fluoroquinolone resistance spread rapidly in SCCmec-IV isolates in the past, a high rate of fluoroquinolone resistance in SCCmec-IV strains can be predicted in the near future.

Our study did not define these MRSA isolates as hospital acquired or community acquired based on patient history. SCCmec types are considered an independent deviation of HA-MRSA and CA-MRSA clones (28). The term “community-acquired,” however, may need to be modified, since MRSA HA-MRSA and CA-MRSA clones (28). The term “community-acquired or community acquired based on patient history.

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versa. SCC

acquired or community acquired based on patient history. This eventually be acquired and spread in communities or vice versa. SCCmec typing is not reliable for determining either HA- or CA-MRSA clonal spread. Other molecular techniques with higher discriminatory power, including pulsed-field gel electrophoresis, spa gene sequencing, and multilocus sequence typing (8, 36) as well as epidemiologic information, should be used to determine the epidemiologic relatedness of a group of MRSA isolates recovered in the hospital and/or community.

ACKNOWLEDGMENTS


