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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[▽]

Abdullah Kilic,[1,3] Haijing Li,[1] Charles W. Stratton,[1,2] and Yi-Wei Tang[1,2]*

Departments of Medicine[1] and Pathology,[2] Vanderbilt University Medical Center, Nashville, Tennessee 37232, and Gulhane Military Medical Academy, Ankara 06018, Turkey[3]

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Antimicrobial susceptibility patterns, Panton-Valentine leukocidin (PVL) occurrence, and staphylococcal cassette chromosome *mec* (SCC*mec*) 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 SCC*mec*-II, and 847 (64.4%) were SCC*mec*-IV. More SCC*mec*-IV isolates were recovered from children than SCC*mec*-II isolates (424 [50.1%] versus 50 [11.2%]; odds ration [OR] = 7.98; $P < 0.000001$). The PVL gene was detected in 93.6% of SCC*mec*-IV isolates, in contrast to 0.2% in SCC*mec*-II isolates. Within SCC*mec*-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, SCC*mec*-II strains showed greater resistance than SCC*mec*-IV strains to clindamycin, erythromycin, levofloxacin, gentamicin, rifampin, minocycline, and trimethoprim-sulfamethoxazole. Both SCC*mec*-II and SCC*mec*-IV strains recovered from adults were more resistant to these antibiotics than those recovered from children. SCC*mec*-II strains were predominantly recovered from the respiratory tract, whereas SCC*mec*-IV strains were predominantly recovered from skin, soft tissue, abscesses, and surgical wounds. These data indicate that SCC*mec*-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 leucocidin (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* (SCC*mec*) (2, 28). To date, five different SCC*mec* elements have been identified in MRSA. The SCC*mec* typing provides strong evidence for the independent deviation of HA-MRSA and CA-MRSA clones (28). The SCC*mec* types I, II, and III are predominantly found in HA-MRSA strains, whereas the SCC*mec* 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 SCC*mec* 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.

* Corresponding author. Mailing address: Molecular Infectious Disease Laboratory, Vanderbilt University Hospital, 4605 TVC, Nashville, TN 37232-5310. Phone: (615) 322-2035. Fax: (615) 343-8420. E-mail: yiwei.tang@vanderbilt.edu.

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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

Variable	No. (%) of SCC*mec*-II strains (n = 398)	No. (%) of SCC*mec*-IV strains (n = 423)	P
Isolation site			
Bloodstream	83 (20.9)	31 (7.3)	<0.000001
Respiratory	151 (37.9)	28 (6.6)	<0.000001
Skin, soft tissue, abscess, and post surgical wounds	127 (31.9)	352 (83.2)	<0.000001
Others	37 (9.3)	12 (2.8)	<0.0001
Antibiotic resistance			
Methicillin, penicillin, amoxicillin Clavulanate, cefazolin	398 (100.0)	423 (100.0)	NS[a]
Erythromycin	393 (98.7)	381 (90.1)	<0.000001
Clindamycin[b]	386 (97.0)	56 (13.2)	<0.000001
Gentamicin	29 (7.3)	4 (0.9)	<0.00001
Levofloxacin	386 (97.0)	70 (16.8)	<0.000001
Minocycline	3 (0.8)	0 (0.0)	NS
Rifampin	17 (4.3)	0 (0.0)	<0.0001
Trimethoprim-sulfamethoxazole	9 (2.3)	8 (1.9)	NS
Vancomycin	0 (0.0)	0 (0.0)	NS
PVL gene occurrence	0 (0.0)	377 (89.1)	<0.000001

[a] NS, not significant.
[b] Includes inducible resistance.

TABLE 2. Characteristics and antibiotic resistance profiles of MRSA strains recovered from children at Vanderbilt University Medical Center, 15 November 2004 to 14 November 2005

Variable	No. (%) of SCC*mec*-II strains (n = 50)	No. (%) of SCC*mec*-IV strains (n = 424)	P
Isolation site			
Bloodstream	6 (12.0)	9 (2.1)	<0.001
Respiratory	31 (62.0)	18 (4.2)	<0.000001
Skin, soft tissue, abscess, and post surgical wounds	12 (24.0)	390 (92.0)	<0.000001
Others	1 (2.0)	7 (1.7)	NS[a]
Antibiotic resistance			
Methicillin, penicillin, amoxicillin-clavulanate, cefazolin	50 (100.0)	424 (100.0)	NS
Erythromycin	47 (94.0)	387 (91.3)	NS
Clindamycin[b]	43 (86.0)	34 (8.0)	<0.000001
Gentamicin	0 (0.0)	1 (0.2)	NS
Levofloxacin	39 (78.0)	18 (4.2)	<0.000001
Minocycline	0 (0.0)	0 (0.0)	NS
Rifampin	0 (0.0)	1 (0.2)	NS
Trimethoprim-sulfamethoxazole	0 (0.0)	1 (0.2)	NS
Vancomycin	0 (0.0)	0 (0.0)	NS
PVL gene occurrence	1 (2.0)	416 (98.1)	<0.000001

[a] NS, not significant.
[b] Includes inducible resistance.

Bacterial strains. From 15 November 2004 through 14 November 2005, all clinical MRSA strains isolated in the Clinical Microbiology Laboratory at VUMC were included in the study. To avoid overrepresentation, only the first isolate from each patient during the study period was included. Computerized culture data included the sex, age, and site of isolation of each isolate. The isolates were identified by standard phenotypic procedures and stored at −70°C (36). Children and adults included in the study were defined as <18 and ≥18 years old, respectively.

Antimicrobial susceptibility testing. In vitro antimicrobial susceptibility testing for amoxicillin-clavulanate, cefazolin, clindamycin, erythromycin, gentamicin, levofloxacin, minocycline, penicillin, rifampin, trimethoprim-sulfamethoxazole (SXT), and vancomycin was determined by a disc diffusion method in accordance with Clinical and Laboratory Standards Institute standards (4). Inducible clindamycin resistance was identified as a D-shaped inhibition zone by the clindamycin-erythromycin double-disk test (34).

SCC*mec* typing and PVL detection. A loopful of each purified bacterial isolate was placed into 1 ml of distilled water and heated at 95°C for 7 min. The supernatant was used for PCR amplification. A real-time TaqMan PCR was performed on the 7700 ABI Prism Sequence Detector (Applied Biosystems Foster City, CA) to determine the SCC*mec* types I, II, III, and IV and to detect the PVL gene as described previously (10), with modifications. In brief, 1 μl of the extracted nucleic acid was added to 24 μl of reaction mixture containing 0.8 μM of each primer and 0.4 μM fluorophore probe (final concentration), and the solution was mixed with 25 μl of TaqMan Universal PCR Master Mix (Applied Biosystems). The TaqMan cycling conditions were a 2-min degradation of the preamplified templates at 50°C and then 40 cycles of denaturation at 95°C for 15 s and annealing and extension at 58°C for 60 s (21). The primers and fluorophore TaqMan probes for SCC*mec* types I, II, III, and IV and the PVL gene were modified from those published previously (10) (SCC*mec*-I, 5′-TTT GGC ACG TAA TAC TTC CGA TT-3′, 5′-AAA ATT CAA CAT TTT GGC GAT GA-3′, and 5′–6-carboxyfluorescein [FAM]–TTA CAA TCG TCG AAG AAC-MGB-3′; SCC*mec*-II, 5′-AAC GAG ACG TGC CCA AGA AG-3′, 5′-CAT CAG TTC ATG TTT ACT ATT AGG TAT TTT GTC-3′, and 5′-VI C-ATT TGC CGC TGG GCT-minor groove binder [MGB]-3′; SCC*mec*-III, 5′-GCA GAA CAG ATA ATC GAA CAG GCT AT-3′, 5′-GCG ATA ACA ACA TAA TAC GTC ACA TTG-3′, and 5′-FAM-AAC GCA TCC AAC AA A-MGB-3′; SCC*mec*-IV, 5′-GAA CAG ACC TGA GCT CCA ACG T-3′, 5′-GGT TTG TYT TGT AKA YCA TAA CAC A-3′, and 5′-VIC-AAG ATG CAA AAG AAG GCA ATA-MGB-3′; PVL, 5′-AAA ATG CCA GTG TTA TCC AGA GGT A-3′, 5′-TTT GCA GCG TTT TGT TTT CG-3′, and 5′-FAM-CTT CAA TCC AGA ATT TAT TGG TGT-MGB-3′; K = G or T; Y = C or T).

Statistical analysis. Statistical comparisons were performed with Epi Info software (version 6; Centers for Disease Control and Prevention, Atlanta, GA). Associations between *SCCmec*-II and SCC*mec*-IV MRSA for patient demographics, antibiotic resistance, PVL occurrence, and culture site were analyzed using the χ^2 test or the Student's t test. $P \leq 0.05$ 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 SCC*mec*-II, 847 (64.4%) were SCC*mec*-IV, 2 (0.2%) were mixed SCC*mec*-II/IV, and 18 (1.4%) were nontypeable isolates. Fifty (11.2%) SCC*mec*-II isolates and 424 (50.1%) SCC*mec*-IV isolates were recovered from children (odds ratio [OR], 0.13; $P < 0.000001$). Since MRSA isolates from VUMC predominantly carried either SCC*mec*-IV or SCC*mec*-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 SCC*mec*-II and SCC*mec*-IV, respectively. More SCC*mec*-II isolates were recovered from an older population (49.3 ± 21.8 years) than the SCC*mec*-IV isolates (22.4 ± 20.3 years; $P < 0.000001$). The demographic parameters between the SCC*mec*-II and SCC*mec*-IV MRSA strains in child and adult patients are listed in Tables 1 and 2.

The PVL gene was detected in 93.6% of SCC*mec*-IV isolates, in contrast to 0.2% in SCC*mec*-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-

TABLE 3. PVL-positive proportions in SCC*mec*-IV isolates recovered from children and adults

Patient category	Total			Source of isolate											
				Bloodstream[a]			Respiratory tract[a]			SSASW[b]			Other[c]		
	No. tested	No. positive	% Positive	No. tested	No. positive	% Positive	No. tested	No. positive	% Positive	No. tested	No. positive	% Positive	No. tested	No. positive	% Positive
Child	424	416	98.1	9	9	100.0	18	13	72.2	390	388	99.5	6	6	100.0
Adult	423	377	89.1	31	19	61.3	18	18	100.0	352	333	94.6	12	7	58.3

[a] For children versus adults, $P = 0.028$ in bloodstream and $P = 0.018$ in respiratory tract.
[b] SSASW, skin and soft tissues, abscesses, and surgical wounds.
[c] Includes 29 urine specimens, only 3 of which were positive for PVL. The three PVL-positive specimens were SCC*mec*-IV isolates.

rence was detected from SCC*mec*-IV strains recovered from the bloodstream in children (9/9; 100.0%) than in adults (19/31; 61.3%) ($P < 0.028$), while PVL occurrence was higher among SCC*mec*-IV strains recovered from respiratory sites in adults (100.0%) than in children (72.2%) ($P < 0.018$) (Table 3).

SCC*mec*-II MRSA strains were predominantly recovered from the respiratory tract (40.6%) and from skin, soft tissue, abscesses, and postsurgery wounds (31.0%), while SCC*mec*-IV strains were predominantly isolated from skin, soft tissue, abscesses, and postsurgery wounds (87.6%) (Tables 1 and 2). A total of 129 MRSA isolates (10.0%) were bloodstream isolates, among which 114 (88.4%) were from adults and 15 (11.6%) were from children. SCC*mec*-II strains were recovered more frequently from the respiratory tract of children (62.0%) 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 SCC*mec*-II and SCC*mec*-IV strains are compared in Tables 1 and 2. MRSA strains recovered from adults were more resistant to clindamycin (SCC*mec*-II, $P < 0.000001$; SCC*mec*-IV, $P = 0.0137$) and levofloxacin (SCC*mec*-II, $P < 0.00001$; SCC*mec*-IV, $P < 0.000001$) than those recovered from children, and this trend remained the same in both SCC*mec*-II and SCC*mec*-IV strains (Tables 1 and 2). SCC*mec*-II MRSA strains showed greater resistance than SCC*mec*-IV strains to clindamycin, erythromycin, levofloxacin, gentamicin, rifampin, minocycline, 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 SCC*mec* types in MRSA isolates from middle Tennessee. Among 1,315 MRSA isolates, 34.1% were SCC*mec*-II and 64.4% were SCC*mec*-IV. The results of this study demonstrate that the SCC*mec*-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 SCC*mec*-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 SCC*mec*-II strains. Our data demonstrated that 19.9% of SCC*mec*-II and 4.7% of SCC*mec*-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 SCC*mec*-II and SCC*mec*-IV isolates. While SCC*mec*-IV isolates recovered in middle Tennessee were still mainly from abscess, surgical, and skin and soft-tissue specimens, our study discovered that SCC*mec*-II strains were recovered more frequently from the respiratory tract of children than adults, indicating that more respiratory-site infections are caused by SCC*mec*-II strains in children (15). In general, SCC*mec*-II isolates were mainly recovered from the respiratory tract as well as skin, soft tissue, abscesses, and postsurgery wounds, while SCC*mec*-IV isolates were recovered dominantly from the latter site(s). These data, especially higher numbers of SCC*mec*-II isolates recovered from the respiratory tract in children, support recent findings that the nosocomial spread of MRSA happens mainly via the nasal route (5, 20, 26). 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 SCC*mec*-IV strains, in contrast to 0.2% of SCC*mec*-II isolates. Within SCC*mec*-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 SCC*mec*-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 SCC*mec* 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 SCC*mec*-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 SCC*mec*-IV strains isolated from children and adults, respectively. SCC*mec*-II isolates possessed significantly greater resistance than SCC*mec*-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 SCC*mec*-II isolates in the past, a high rate of fluoroquinolone resistance in SCC*mec*-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. SCC*mec* 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 strains carrying SCC*mec* type IV or V are now being introduced from their community site of origin into the hospital setting with the potential to cause nosocomial spread (27, 30, 33). MRSA isolates carrying SCC*mec* type I, II, or III can eventually be acquired and spread in communities or vice versa. SCC*mec* 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.

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