Successful Multiresistant Community-Associated Methicillin-Resistant \textit{Staphylococcus aureus} Lineage from Taipei, Taiwan, That Carries Either the Novel Staphylococcal Chromosome Cassette \textit{mec} (SCC\textit{mec}) Type V\textsubscript{T} or SCC\textit{mec} Type IV

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Methicillin-resistant \textit{Staphylococcus aureus} (MRSA) isolates carry the methicillin resistance gene (\textit{mec}) on a horizontally transferred genetic element called the staphylococcal chromosome cassette \textit{mec} (SCC\textit{mec}). Community-acquired MRSA (CAMRSA) isolates usually carry SCC\textit{mec} type IV. We previously reported that 76\% of 17 CAMRSA isolates (multilocus sequence type 59) obtained from pediatric patients with skin and soft tissue infections (SSTIs) from Taipei did not carry SCC\textit{mec} types I to IV. We used DNA sequence analysis to determine that the element harbored by these nontypeable isolates is a novel subtype of SCC\textit{mec} V called SCC\textit{mec} V\textsubscript{T}. It contains a \textit{ccrC} recombinase gene variant (\textit{ccrC2}) and \textit{mec} complex C2. One SSTI isolate contained molecular features of SCC\textit{mec} IV but also contained \textit{ccrC2} (a feature of SCC\textit{mec} V\textsubscript{T}), suggesting that it may harbor a composite SCC\textit{mec} element. The genes \textit{lukS-PV} and \textit{lukF-PV} encoding the Panton-Valentine leukocidin (PVL) were present in all CAMRSA SSTI isolates whether they contained SCC\textit{mec} type IV or V\textsubscript{T}. SCC\textit{mec} V\textsubscript{T} was also present in 5 of 34 (14.7\%) CAMRSA colonization isolates collected from healthy children from Taipei who lacked MRSA risk factors. Four (80\%) of the these isolates contained \textit{lukS-PV} and \textit{lukF-PV}; as did 1 of 27 (3.7\%) SCC\textit{mec} IV-containing colonization isolates. A total of 63\% (10 of 16) of the SSTI isolates and 61.7\% (21 of 34) of the colonization isolates tested were resistant to at least four classes of non-\beta-lactam antimicrobials. SCC\textit{mec} V\textsubscript{T} is a novel SCC\textit{mec} variant that is found in multiply resistant CAMRSA strains with sequence type 59 in Taipei in association with the PVL leukotoxin genes.

Methicillin-resistant \textit{Staphylococcus aureus} (MRSA) was first recognized as a healthcare-associated pathogen in the 1960s (22). Since the early reports of community-associated MRSA (CAMRSA) (13, 27), infections caused by MRSA in patients lacking traditional MRSA risk factors with onset outside health care settings have been increasing globally (36). Clinical syndromes caused by these CAMRSA isolates have ranged from skin and soft tissue infections (SSTIs) to necrotizing pneumonia (1, 4, 9, 12, 25, 33), severe sepsis (1, 4, 25), and necrotizing fasciitis (24). Asymptomatic colonization with MRSA among healthy subjects, considered to be rare until the 1960s (22). Since the early reports of community-associated MRSA, considered to be rare until the 1960s (22). Since the early reports of community-associated MRSA (CAMRSA) (13, 27), infections caused by MRSA in patients lacking traditional MRSA risk factors with onset outside health care settings have been increasing globally (36). Clinical syndromes caused by these CAMRSA isolates have ranged from skin and soft tissue infections (SSTIs) to necrotizing pneumonia (1, 4, 9, 12, 25, 33), severe sepsis (1, 4, 25), and necrotizing fasciitis (24). Asymptomatic colonization with MRSA among healthy subjects, considered to be rare until recently, has also been documented in various populations, especially from the same geographic location from where CAMRSA infections have been reported (10, 14, 37). In CAMRSA infections in children without predisposing risk, the clinical syndromes resemble those of CA methicillin-susceptible \textit{S. aureus} (MSSA) infections (13), and the responsible isolates lack multiple resistance to antimicrobials other than \beta-lactams (13, 41).

Two important genotypic characteristics have been associated with CAMRSA. The staphylococcal chromosome cassette \textit{mec} (SCC\textit{mec}) (15, 16, 21), the genetic element that carries the methicillin resistance gene, \textit{mecA}, integrates into the \textit{orfX} gene in the \textit{S. aureus} genome in a site specific manner. SCC\textit{mec} type IV (21) has been associated with CAMRSA in a variety of genetic backgrounds (23, 41). Outbreaks in the United States have been associated with isolates with sequence types (ST) 8 and 1 (23), determined by multilocus sequence typing (MLST) (11). Also associated with CAMRSA are the \textit{lukS-PV} and \textit{lukF-PV} genes that encode the two subunits that comprise the Panton-Valentine leukocidin (PVL), a synergohymenotropic cytotoxin associated with furunculosis, severe necrotizing hemorrhagic pneumonia, necrotizing fasciitis, and other lesions involving the skin or mucosa in both CAMRSA and CAMSSA strains (12, 18, 24). A high carriage rate of the \textit{lukS-PV} and \textit{lukF-PV} genes (i.e., the PVL locus) among CAMRSA has been documented in isolates associated with SSTIs, severe sepsis, necrotizing fasciitis, and necrotizing pneumonia (12, 24, 25, 41). Notably, the PVL locus is infrequently found among healthcare-associated MSSA (18) or MRSA isolates (41). Few studies have documented the prevalence of the PVL locus among CAMRSA colonizing asymptomatic individuals in non-outbreak settings.

We previously analyzed 17 CAMRSA isolates obtained from patients with SSTIs from the Tri-Services General Hospital (TSGH), a tertiary care, military medical school-affiliated institution in Taipei, for antimicrobial susceptibility patterns, genotyping by MLST and SmaI genomic fingerprinting, the presence of the PVL locus (42), and the SCC\textit{mec} type. The resistance phenotypes differed from CAMRSA described from other locations in that the isolates uniformly had the constitutive macrolide, lincosamide, and streptogramin B resistance.
We examined here the SCCmec type in 13 of the 14 nontypeable SSTI isolates. This was accomplished by sequencing the \( mec \), \( orfX \), and \( ccr \) complexes contained in one of the CAMRSA SSTI isolates and by screening the remaining isolates by PCR. This was accomplished by sequencing the \( mec \) complex C2 and the \( orfX \) target is a unique product of SCCmec \( V_T \) that is formed when this primer is used alone. The multiplex PCR involved the use of one forward universal primer (\( \beta 2 \)) with three reverse primers (\( \alpha 2 \), \( \alpha 3 \), and \( \gamma R \)) to target multiple genes. The individual targets are listed with their respective reverse primers.

\(^{b}\) Only one single primer is required because the \( mec \) complex C2 is flanked by complementary inverted repeats of 15431.

\(^{c}\) This target is a unique product of SCCmec \( V_T \)

\(^{d}\) The target primer \( orfXprobe \) was used with two reverse primers (\( orfX \) and \( ccr \) CDS5-R) to target the same gene.

\(^{e}\) The forward primer \( \gamma F \) was used with two reverse primers (\( \gamma R \) and \( CDS5-R \)) to target the same gene.

\(^{f}\) The reference or source is for both the forward and the reverse primers. A reference is given for the forward primer only if it was used with more than one reverse primer, and one is also given for each single primer. For the multiplex PCR, the primers were described previously, and the assay conditions are described in this study.

\(^{g}\) Predicted size of PCR product when the indicated reverse primer is used in conjunction with the given forward primer.

\(\text{Product size (kb) without decay} + \text{Source or reference}^b\)

\(\text{Product size when this reverse primer is used in conjunction with} \ \gamma F\)

\(\text{Product size when this reverse primer is used in conjunction with} \ \beta 2\)

\(\text{Statistical comparisons were performed by the use of chi-square test with a web-based chi-square calculator (http://www.georgetown.edu}}\)

\(\text{Isolates were frozen in skim milk (Difco) at} ± 70^\circ \text{C. Two groups of CAMRSA isolates from Taipei were studied. The first group, TSGH 1 to 17, were consecutive CAMRSA isolates from children hospitalized at TSGH for SSTIs during the 5-year period from September 1997 to August 2002 (42). A case was considered community acquired if the isolate was obtained from a patient within 72 h of admission to TSGH. None of the SSTI patients had any of the selected risk factors for MRSA infection as described previously (42) as follows: (i) hospitalization within 6 months of the date of MRSA isolation, (ii) history of any surgical procedure, (iii) antimicrobial therapy within 6 months of the date of MRSA isolation, and (iv) household contact with an individual with an identified risk factor or a worker in a healthcare environment. One isolate (TSGH 6) was mecA negative and was excluded.}

\(\text{The CAMRSA colonization isolates (C1 to C48) were obtained in a 1-year period (January to December 2003) by culturing the nares of 640 healthy children. Subjects enrolled were 12 years of age or younger with no acute medical problem who either presented for a well-child healthcare visit or attended one of three kindergartens in Taipei near the TSGH. Isolates were stratified by whether they lacked SCCmec types I to IV.}

\(\text{We also examined the SCCmec type in 13 of the 14 nontypeable SSTI isolates. This was accomplished by sequencing the} \ ccr \text{ and} \ mec \text{ complexes contained in one of the CAMRSA SSTI isolates and by screening the remaining isolates by PCR using type- and subtype-specific primers. The “nontypeable” isolates harbor a variant of the newly described SCCmec} \ V \text{ element (16) that we have called SCCmec} \ V_T. \text{We also examined antibiotic resistance profiles, SCCmec types and PVL locus prevalence among 48 CAMRSA isolates asymptomatically colonizing healthy children from Taipei.}}\)
TABLE 2. Percentage of isolates with resistance to the indicated antimicrobials among the SSTI and colonization isolates

<table>
<thead>
<tr>
<th>Isolates (n)</th>
<th>PCR (mecA)</th>
<th>% Isolates resistant to:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OXA</td>
<td>ERY</td>
</tr>
<tr>
<td>SSTI (16)</td>
<td>100</td>
<td>81.3</td>
</tr>
<tr>
<td>Col (34)*</td>
<td>100</td>
<td>97.1</td>
</tr>
</tbody>
</table>

*Colonization (Col) isolates included were the subset from patients without MRSA risk factors. SXT was tested by disk diffusion. The following antimicrobials were tested by using Vitek 2: oxacillin (OXA), ERY, CLI, ciprofloxacin (CIP), GEN, TET, CHL, and RIF.

Southen hybridization. Pulsed-field gels were blotted onto GeneScreen Plus Hybridization transfer membranes, and a 10× SSC buffer (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) (2). The gel was washed in 0.25 M HCl for 15 min, then 1.5 M NaCl-0.5 M NaOH for 30 min, and in 3 M NaCl-0.5 M Tris for 30 min prior to blotting. Before hybridization, the gel was cross-linked using UV light with a UV C-linker PB-UVXL-1000 (Fisher Biotech/Fisher Scientific) and the “optimal cross-link” setting. The Promega Prime-a-Gene kit was used to radiolabel the appropriate probes with [α-32P]dATP (Amersham). Hybridization was performed at 70°C overnight with constant rotation in a hybridization buffer consisting of 7% sodium dodecyl sulfate, 1% bovine serum albumin, and 1 mM EDTA (pH 8) in 0.25 M Na2HPO4. Membranes were probed with a mecA gene probe produced by PCR with the primers mecAF and mecAR (Table 1). After overnight exposure on a Fuji Imaging Plate and scanning of the hybridization image with a PhosphorImager (Molecular Dynamics), membranes were stripped of the mecA probe by boiling in distilled water and rehybridized with an oriX gene probe produced by PCR, with the primer pair orIXprobeF-orIXprobeR. The oriX probe for detecting chromosomal DNA was produced by PCR with the same primers used to produce the araC probe for MLST analysis.

Screening for the mec complex and ccrC gene from strain TSGH 17. Sequencing was initiated by producing a XEMBL3 library from strain TSCH 17 and screening recombinant plasmids for the presence of mecA by hybridization. This was accomplished by isolating genomic DNA from strain TSCH 17 with the Genomic Tip kit (QIAGEN, Inc.) and producing a Sau3AI partial digestion (2), followed by purification of ~20-kb products after agarose gel electrophoresis using the Qiaex II gel extraction kit (QIAGEN). These products were inserted into the XEMBL3 cloning vector (LambaGEM-11 BamHI Arms; Promega) by using an in vitro packaging kit (Promega Packagene Extract) as recommended by the manufacturer with the bacterial host strain LE392. Plasmids containing the mecA gene were identified by hybridization with two [α-32P]dATP-labeled mecA gene probes produced by PCR with the primer pairs mecAF-mecAR and mecA aminoF-mecA aminoR (Table 1) in a final concentration of 2 μM in a standard PCR cocktail. DNA was isolated from mecA-hybridizing purified plasmids with the use of the Lambda Midi kit (QIAGEN) and phenol-chloroform-isooamyl alcohol extraction, followed by ethanol precipitation to concentrate the sample using standard procedures (2). Inserts from lambda DNA were subcloned into the Clal site of a dephosphorylated pBluescript (Stratagene) cloning vector by using T4 DNA ligase (NEB, Beverly, MA). Ligation products were transformed into Electromax STBL4 cells (Stratagene) by using a Bio-Rad Gene Pulser II and a 0.1-cm cuvette with settings of 1.2 kV, 25 μF, and 200 Ω. Transformed cells were plated onto LB agar supplemented with ampicillin (100 μg/ml) and overlaid with 100 μl of 2% X-Gal (5-bromo-4-chloro-3-indolylβ-D-galactopyranoside) dissolved in dimethyl formamide. Plasmid DNA from white colonies was isolated with the QIAprep Spin Miniprep kit (QIAGEN, Inc.) and sequenced with the M13/GC universal forward and reverse primers (5′-GTAAAACGACGGCCAGT-3′ and 5′-CAGGAGGAAACAGCTATGACCACAT-3′, respectively).

To obtain the sequence of the ccr complex, primers were designed from the sequences obtained from XEMBL3 subclones mentioned above in conjunction with ccrC-specific primers. In addition, primers were designed to extend the sequence obtained from the cloned fragments using a primer-walking strategy. Raw sequence data consisting of high-pressure liquid chromatography chromatograms were evaluated, edited, and assembled into contigs using software packaged within the VectorNTI suite (version 8; Informax, Inc., Bethesda, Md.). For completion of the sequence of the mecA complex, a primer-walking strategy with an initial set of mecA- and IS431-specific primers (V3, m2A, and m3A [16]) was used.

Screening for the PVL locus. Isolates were screened for the lukF-PV and lukS-PV genes encoding the PVL toxin by PCR amplification with the primer

Maths, Austin, TX) to compare the images with those in the national database as described previously (23).

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pair PVL-1 and PVL-2 (final concentration, 10 μM) that produces a 3.5-kb product (25) encompassing both lukF-PV and lukS-PV open reading frames (ORFs) and flanking DNA. The PVL locus was occasionally screened by using primer pair luk-PV-1 and luk-PV-2 (Table 1) (final concentration, 10 μM) that amplifies a product (25) encompassing both lukF-PV and lukF-PV ORFs. The thermocycler conditions used with the PVL-1–PVL-2 primer pair consisted of 94°C for 1 min, followed by 35 cycles of 94°C for 30 s, 63°C for 30 s, and 72°C for 1 min, with a final extension performed at 72°C for 7 min. The thermocycling conditions used with the luk-PV-1–luk-PV-2 primer pair were 95°C for 1 min and then 30 cycles of 95°C for 1 min and 68°C for 1 min, with a final extension performed at 68°C for 1 min.

GenBank accession numbers. The DNA sequences of the mec complex and the ccrC2 gene from TSGH 17 have been deposited in GenBank under accession numbers AY894415 and AY894416, respectively.

### RESULTS

**MRSA colonization among healthy children.** Of the 640 healthy children who were screened, 157 (24.5%) had a culture yielding *S. aureus*. Forty-eight (31%) of these were MRSA. Thus, the overall MRSA colonization rate was 7.5%. However, 14 of the isolates were from subjects that had at least one designated risk factor for MRSA; these were excluded from further analysis unless otherwise indicated. Thus, the MRSA colonization rate in subjects lacking the designated risk factors for MRSA was 5.3%.

**Antimicrobial resistance rates of SSTIs and colonization CAMRSA isolates.** To assess resistance to non-β-lactam antimicrobials, 16 SSTI CAMRSA isolates (TSGH 1 to 5 and TSGH 7 to 17 [excluding TSGH 6, which was methicillin susceptible]) and the colonization isolates were subjected to susceptibility testing by using the Vitek 2 system (Table 2). Consistent with our previous results obtained by disk diffusion (42), 100% of the SSTI isolates were resistant to ERY and CLI (Table 2). Of the 34 colonization isolates from patients lacking risk factors, 94.1 and 91.2% were resistant to ERY and CLI, respectively (Table 2).

Unlike the usual pattern for CAMRSA, resistance to the non-β-lactam antimicrobials was common among both groups of CAMRSA from Taiwan (Table 2). Consistent with our previous results obtained by disk diffusion (42), 100% of the SSTI isolates were resistant to ERY and CLI (Table 2). Of the 34 colonization isolates from patients lacking risk factors, 94.1 and 91.2% were resistant to ERY and CLI, respectively (Table 2).

Multiple resistance to non-β-lactam antimicrobials was also highly prevalent among the SSTI and colonization CAMRSA isolates (Fig. 1). Figure 1 shows the percentage of isolates with resistance to ≤2, 3, or ≥4 non-β-lactam antimicrobials. One (6.3%) SSTI isolate was resistant to five non-β-lactam antimicrobials. A total of 63% (10 of 16) of the SSTI isolates and 62% (21 of 34) of the colonization isolates were resistant to ≥4 non-β-lactam antimicrobials (counting the four quinolones as a single antibiotic) (Fig. 1). Using a less stringent definition, 94% (15 of 16) of the SSTI isolates and 85.3% (29 of 34) of the colonization isolates were resistant to ≥3 non-β-lactam antibiotics. Of the 34 colonization isolates, 1 (2.9%) was susceptible to all of the non-β-lactam agents tested. Both the SSTI and colonization isolates were uniformly susceptible to the fluoroquinolones and SXT.

**SCCmec typing.** Table 3 shows the results of SCCmec typing for the colonization and the SSTI isolates. The colonization
isolates that were from subjects that lacked an MRSA risk factor, a majority (27 of 34 [79.4%]) harbored SCCmec IV. SCCmec IV was also carried by all 14 colonization isolates that were from the subjects with at least one MRSA risk factor. Thus, 34% of the 41 SCCmec IV-containing colonization isolates were from subjects who had an MRSA risk factor.

One colonization isolate (2.9%) harbored SCCmec II, one (2.9%) harbored SCCmec III, and five (14.7%) harbored an element that was nontypeable when assessed for SCCmec types I to IV.

TABLE 4. Distribution of SCCmec types among colonization isolates

<table>
<thead>
<tr>
<th>ST</th>
<th>No. (%) of colonization isolates of SCCmec type:</th>
<th>Total no. (%) of isolates</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td>59</td>
<td>0</td>
<td>26 (76.4)</td>
</tr>
<tr>
<td>508</td>
<td>0</td>
<td>1 (2.9)</td>
</tr>
<tr>
<td>89</td>
<td>1 (2.9)</td>
<td>0</td>
</tr>
<tr>
<td>239</td>
<td>0</td>
<td>1 (2.9)</td>
</tr>
<tr>
<td>Total</td>
<td>1 (2.9)</td>
<td>1 (2.9)</td>
</tr>
</tbody>
</table>

* Includes only the subset of isolates from patients lacking MRSA risk factors. ST was determined by MLST as described in Materials and Methods.

Genotyping of colonization isolates. Among the 34 colonization isolates, the predominant genotype (91.2%) was ST 59 (Table 4). Only three of the isolates had unique backgrounds: ST 89, ST 508, and ST 239. In addition, all 14 isolates that were associated with an MRSA risk factor were ST 59.

The CDC has recently reported a system for normalizing pulsed-field patterns determined from different laboratories and assigning a PFT (designated with the prefix USA) based on similarity calculations and clustering with the use of BioNumerics software (23). To determine whether the PFT of the ST 59 isolates from Taipei clustered with ST 59 isolates in the U.S. national database, representative SmaI pulsed-field patterns from a sample of the colonization (every third isolate up to C28) and SSTI isolates (42) (Fig. 2B) were submitted to the CDC. The SSTI isolates and the colonization isolates clustered with PFT USA 1000 (73% similarity). ST 59 isolates from Taiwan were more closely related to each other (76% similarity). One pattern from the ST 59 lineage (isolate TSGH 5) did not match any known PFT in the national database and had >6 band differences compared with that of the other ST 59 isolates. The PFT of the ST 89 isolate did not correspond to any PFT in the CDC database.

Analysis of pulsed-field gels by Southern blotting. To gain insight into the size and architecture of the nontypeable SCCmec

![FIG. 2. Southern blotting analysis of ST 59 CAMRSA isolates containing either SCCmec VT or SCCmec IV. (A) Map illustrating the hybridization probes (black bars above and below the map), mecA and orfX. (B) Pulsed-field gel containing SmaI-digested DNA from ST 59 isolates containing SCCmec IV (lane 5, strain TSGH 5) or SCCmec VT (lanes 1, 2, 3, 4, 7, 8, and 9 represent strains TSGH 1, 2, 3, 4, 7, 8, and 9, respectively. Lane 6 contains DNA from strain TSGH 6, an MSSA isolate with ST 59. Lane 39 contains DNA from an MSSA healthcare-associated strain TSGH 39 that has a different ST. (C) Southern blot of the gel shown in panel B hybridized with the mecA probe. (D) Southern blot produced after the blot in panel C was stripped and rehybridized with a probe specific for orfX, the ORF adjacent to the right junction of SCCmec that contains the integration site. The faint bands appearing toward the bottom of the membrane are mecA-specific bands remaining after incomplete stripping of the membrane. Lane S contains DNA from a pulsed-field gel marker from Sigma. Lane Se contains DNA from Staphylococcus epidermidis.](http://jcm.asm.org/)
element, Southern blotting of SmaI-digested DNA from CAMRSA isolates from SSTIs and an ST 59 MSSA isolate was performed with mecA (Fig. 2C) and orfX (Fig. 2D) hybridization probes. The hybridization pattern was similar among all strains containing a nontypeable element, suggesting they all contained a similar element.

Two findings distinguished the SCCmec IV-containing isolates from those containing a nontypeable element. First, there was a striking difference between the sizes of the mecA- and orfX-hybridizing bands in the isolates carrying a nontypeable SCCmec (Fig. 2C and D, TSGH 1, 2, 3, 4, 7, 8, and 9). This is best illustrated in Fig. 2D, where the mecA-specific band is smaller than the orfX-hybridizing band. In contrast, the orfX and mecA gene probes cohybridized in the SCCmec IV-containing strains (Fig. 2D, lane 5).

The separation of the mecA and orfX hybridizing bands in the ST 59 strains containing a nontypeable element suggested that (i) the nontypeable SCCmec element was not inserted into the attB insertion site in the orfX gene, as are all other known SCCmec elements; (ii) the nontypeable element was present on a plasmid; or (iii) the nontypeable element contained a SmaI recognition sequence between mecA and orfX.

Southern blotting of undigested genomic DNA from one isolate (TSGH 3) demonstrated that the mecA gene cohybridized with the chromosomal gene probe aroE (data not shown). A plasmid could not be detected on agarose gels. These data suggested that the mecA and orfX Southern blotting results can be explained by the presence of an internal SmaI restriction site in the nontypeable element integrated into the genome and argued against the presence of an SCCmec element on a plasmid.

Characterization of the ccrC complex and mec complex of a new subtype of SCCmec V (SCCmec Vp). To determine the SCCmec type of the nontypeable isolates, the DNA sequences of the ccr and mec complexes of one of the SSTI isolates, TSGH 17, were determined (see Materials and Methods). By performing a BLAST search with sequences deposited in GenBank, one 5,753-bp contig was found to contain highly significant sequence similarity with the ccrC complexes from three other SCC elements (expectation scores ranged from 0 to 3.8 with sequence identities of 80 to 100%): (i) SCCmec III (strains 85/3907 and 85/2082), (ii) SCCmec V (strain WIS), and (iii) SCCcap1 (strain M). The last element does not contain mecA but does contain a ccrC homologue and encodes the type
capsular polysaccharide biosynthesis gene cluster (20). Individual nucleotide sequence alignments between the ccrC ORF from strain TSGH 17 and the corresponding 1,677-bp region from each of the SCC elements mentioned above revealed nucleotide identities of 97.7% (SCCmec III, 85/3907), 94.8% (SCCmec III, 85/2082), 90.3% (SCCmec V, WIS), and 88.6% (SCCcap1, strain M). Based on these data we designated these separate ccrC alleles as ccrC1 (strain WIS), ccrC2 (strain TSGH 17), ccrC3 (strains 85/2082), and ccrC4 (strain M).

The sizes of the ccrC ORFs differed among the five elements due to nucleotide polymorphisms that created stop codons in different locations (Fig. 3A). A deletion at nucleotide 1612 of the ccrC ORF in strain TSGH 17 abolished a stop codon and extended the ccrC ORF by 57 nucleotides compared to that in strain WIS (Fig. 3A), making the ccrC ORF from TSGH 17 the largest of the ccrC alleles described to date (1,677 bp). Interestingly, a truncated ccrC (ccrC') and a unique overlapping ORF at the 3' end are present in SCCmec III in strain 85/3907 but not in strain 85/2082 (Fig. 3A) due to a premature stop codon in the former.

A multiple sequence alignment was performed between the 1,677-bp ccrC ORF from strain TSGH 17 and the corresponding regions encompassing the ccrC homologues from all four SCC elements mentioned above (data not shown). The se-
sequence alignment tree (Fig. 3B) illustrates that the ccrC ORF from SCCmec V from strain WIS is most closely related to that of SCCcapI and more distantly related to SCCmec V.

The dendrogram in Fig. 3D is based on a multiple sequence alignment between four ccrC alleles and ccrA1, ccrA2, ccrA3, ccrB1, ccrB2, and ccrB3. This tree clearly illustrates how the four ccrC homologues form distinct branches in their cluster and how the ccrA, ccrB, and ccrC homologues form separate clusters.

The mec complex of strain TSGH 17 consists of mecAΔmecR1 flanked by IS431 elements positioned in opposite orientation that point toward the outside of the element (Fig. 4). This architecture conforms to that of mec complex class C2 (17), similar to that in SCCmec V of strain WIS (16, 17). The overall similarity between the mec complex of TSGH and that of strain WIS was 99.4%. However, a single nucleotide polymorphism in the IS431 transposase gene upstream of mecA (C144 in strain WIS is G144 in strain TSGH 17) converts a Tyr codon to a premature translational stop codon in strain TSGH 17. An additional distinguishing feature of the mec complex of strain TSGH 17 is the presence of an extra direct repeat unit (dru) in the intergenic hypervariable region upstream of the glycerophosphoryl diester phosphodiesterase gene (Fig. 4). This dru is responsible for the length polymorphism of SCCmec elements (34). There are four such dru’s in strain TSGH 17 and three in strain WIS.

Thus, the SCCmec element in TSGH 17 carries the ccrC2 recombinase gene and the mec complex C2, with signature molecular features in both ccrC2 and the mec complex that clearly distinguish this element from the SCCmec type V element in strain WIS. The presence of mec complex C2 in combination with a ccrC2 homologue indicates that TSGH 17 carries a variant of SCCmec V, which we call SCCmec V_T.

Determination of the SCCmec type in other SCCmec nontypeable isolates from Taiwan. To determine whether the remaining SCCmec nontypeable isolates contained SCCmec V_T, primers described by Ito et al. (16) to characterize SCCmec V (WIS) and those derived from the ccrC2 and mec C2 complexes of TSGH 17 were used. To detect the presence of ccrC, a forward primer, γF, was used in combination with either the reverse primer γR described by Ito et al. (16) or a reverse primer (CDS15-R) designed for the present study (Table 1). When using either of these primer pairs, products of the expected sizes (0.52 and 2.2 kb, respectively) were produced from strain WIS, strain TSGH 17, and all remaining nontypeable SSTI strains (Table 3). None of the prototype strains containing SCCmec types I to IV gave a product using these primers, validating the use of γF/CDS15-R as SCCmec V screening primers. In addition, no product was detected from the ST 59 strains that contained SCCmec II, III, or IV. To determine whether the ccrC ORF from the nontypeable isolates was similar to that of TSGH 17, the ccrC nucleotide sequence was determined for all nontypeable isolates starting at position 1249 of the ccrC ORF (relative to that of TSGH 17) and ending with the TAG stop codon (Fig. 3C). All but one of the sequenced strains had an identical ccrC sequence to that of TSGH 17 within this region, including the nucleotide polymorphisms that resulted in an extended ccrC ORF (Fig. 3C). The one different strain had only one nucleotide polymorphism (data not shown). These data strongly suggest that all 18 nontypeable strains (from both SSTI and colonization isolates) contain an element that is highly similar to SCCmec V_T.

To screen for the unique architecture of mec complex C2 (17), which is flanked by two complementary inverted repeats of IS431, we developed a PCR assay using a single IS431-specific primer (IS-5) (Table 1) that reads toward the inside of mec complex C2. A 5.5-kb product was produced from TSGH 17, all other nontypeable strains and, as expected, from WIS, the SCCmec V prototype strain (Table 3). This primer did not amplify a product from the prototype strains containing SCCmec types I to IV. Another distinguishing feature of the mec complex from strains TSGH 17 and strain WIS was the absence of the mecR1 membrane-spanning domain (MS) and the mecR1 penicillin-binding domain (PB) (Table 3). Similarly, a product for neither the mecR1 (MS) nor the mecR1 (PB) was detected from any of the nontypeable isolates in the present study (Tas-
ble 3). This is in contrast to mec complex class B that contains the mecR1 (MS), although it lacks the mecR1 (PB) (15, 17) (Table 3).

While amplifying templates for sequencing, we found a primer (ccrC-FR) (Table 1) that produced a 4-kb product when used as the only primer in a PCR with TS97 G17 genomic DNA. No product was formed from the prototype strain WIS containing SCCmec V (Table 3). ccrC-FR also produced a 4.0-kb product from all of the nontypeable strains but not from any of the SCCmec type I- to IV-containing isolates.

Thus, from the ccrC sequence data, the PCR screening assays, and the Southern blotting data, we conclude that the nontypeable elements in these Taiwan isolates are uniformly SCCmec V-T. Therefore, 5 (14.7%) of the 34 colonization isolates contained SCCmec V-T and 26 (76.4%) contained SCCmec IV (Table 4). In contrast, all 13 nontypeable SSTI isolates studied had SCCmec V-T (81.3%) and 2 SSTI isolates (12.5%) had SCCmec IV.

Table 4 also shows the STs of the colonization isolates stratified by the number of isolates containing each SCCmec type. Of the 31 ST 59 isolates, 26 (83.9%) carried SCCmec IV. All four SCCmec V-T isolates were ST 59. Each of the isolates with a unique ST carried a different SCCmec type: ST 89 (SCCmec II), ST 239 (SCCmec III), and ST 508 (SCCmec IV). Thus, one SCCmec IV-containing strain had an ST other than ST 59.

Evidence for a novel SCCmec type IV/T type VT composite element in TS97 10. TS97 10 contained mec complex type B (Table 3). In the ccr assays, both ccrC and ccrAB2-specific products were detected (Fig. 5). The entire sequence of the ccrC ORF from TS97 10 was determined and is identical to that of TS97 17. These data suggest that TS97 10 contains SCCmec IV (ccrAB2 complex and mec class B), but the detection of the ccrC gene from SCCmec V-T suggests the presence of a composite island.

Prevalence of the PVL locus. Only 5 (14.7%) of the 34 CAMRSA colonization isolates from healthy children harbored the PVL locus (Table 5). The PVL locus was more frequent among the colonization isolates that harbored SCCmec V-T (Table 5) (P = 0.0005 [Fisher exact test]). This is unlike the SSTI isolates, which all harbored the PVL locus (42) (Table 5) irrespective of whether they harbored SCCmec type IV or V-T.

DISCUSSION

We previously described the molecular epidemiology and resistance patterns of CAMRSA isolates that caused SSTIs in patients from the TS97 in Taipei (42). In that study we found that all of the SSTI CAMRSA isolates studied were of the ST 59 genetic background, and a majority harbored a nontypeable SCCmec element with a few harboring SCCmec IV. All of them carried the PVL locus associated with SSTIs, severe sepsis, necrotizing pneumonia, and necrotizing fasciitis. We have now designated the nontypeable element as SCCmec V-T, a variant of the SCCmec V element described recently (16). We have also identified SCCmec V-T-containing isolates among a group of CAMRSA colonization isolates from the same geographic location. This is the first report that documents a high prevalence of an SCCmec element other than SCCmec IV among a group of CAMRSA isolates (41).

The colonization isolates that carried SCCmec V-T were more likely to carry the PVL locus than those carrying SCCmec IV. This is also a departure from the situation elsewhere in which the PVL locus has been found exclusively in SCCmec IV-containing isolates (41) or in MSSA isolates. Among the SSTI isolates from Taipei, the PVL locus was uniformly present among both SCCmec types IV and V-T-containing isolates. It has been suggested that the PVL leukotoxin may be the determinant that is favoring the spread of MRSA isolates in the community (5) since the PVL locus has been less often

![FIG. 5. PCR assay demonstrating the presence of both ccrAB type 2 and ccrC2 in strain TS97 10. PCR products were resolved by agarose gel electrophoresis stained with ethidium bromide. Lanes contain PCR products produced with either the primer pair yF and CDS15-R to detect ccrC (ccrC) or the multiplex primers β2, α2, α3, and α4 for detecting ccrAB 1, 2, and 3. Lanes contain reactions from either SSTI isolates TS97 17 (lane 17) or TS97 10 (lane 16). DNA from control strains containing SCCmec I, II, III, and IV are in lanes labeled I (NCTC 10442/SCCmec I), II (strain N315, SCCmec II), III (strain 852082, SCCmec III), and IV (strain MW2, SCCmec IV) and contain ccrAB types 1, 2, 3, and 2, respectively. Lanes c, negative control lanes containing water instead of a DNA template.]

<table>
<thead>
<tr>
<th>SCCmec type</th>
<th>Colonization (n = 34)</th>
<th>SSTI (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVL</td>
<td>Total</td>
<td>PVL</td>
</tr>
<tr>
<td>II</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
<td>27b</td>
</tr>
<tr>
<td>V-T</td>
<td>4</td>
<td>14</td>
</tr>
</tbody>
</table>

a Data include only the 34 colonization isolates from patients that had no risk factors for MRSA.

b This value includes one isolate that has both SCCmec IV and a ccrC2 gene.
associated with nosocomial MRSA or MSSA infections. In the present study, a majority of CAMRSA isolates asymptotically colonizing healthy individuals lacked the PVL locus, suggesting that the PVL toxin is not required for the successful spread of CAMRSA, at least of the ST 59 genetic background. These data also suggest the need to design studies to determine whether CAMRSA isolated from patients with an SSTI, necrotizing pneumonia or necrotizing fasciitis are more likely to contain the PVL locus than CAMRSA that colonize asymptomatic individuals.

This is also the first report documenting such a high prevalence of a single genotype carrying an SCCmec V element. SCCmec V has also not been previously identified in the ST 59 (PFT USA 1000) genetic background or in an isolate from a continent other than Australia (6, 16). The predominant clone of CAMRSA currently circulating in the United States is from the ST 8 genetic background (PFT USA 300) (5). ST 1 (PFT USA 400) has also been reported among CAMRSA (41), especially among patients presenting with severe sepsis syndrome with necrotizing pneumonia (1, 25). We have also reported MSSA STI isolates in association with severe sepsis and necrotizing pneumonia (1, 25). MRSA isolates with ST 59 have been reported infrequently, mainly from San Francisco (3, 8, 32), and have usually contained SCCmec IV (3, 8). Sporadic ST 59 isolates with SCCmec II (3), SCCmec III (32) or a nontypeable SCCmec element (NT1) (3) have also been reported. Considering the high prevalence of ST 59 in CAMRSA isolates from Taiwan, it is tempting to speculate that the ST 59 isolate carrying the nontypeable element circulating in San Francisco might have originated from Taipei.

The genetic backgrounds that SCCmec V has been found in previously were all from Australia and are ST 45 (strain WIS, unpublished data), ST 8, and ST 152 (6). Thus, the ST 59 background is the fourth into which an SCCmec V-like element has been introduced (but the first in which SCCmec V has been found).

SCCmec V is characterized by the presence of a ccrC recombinase complex and mec complex type C2 (16). The SCCmec V variant we identified contains signature features in both its mec and ccr complexes. The nucleotide polymorphisms we found in the ccr region of SCCmec V extended the ccrC ORF compared with that found in strain WIS and led us to distinguish it from the other ccrC homologues by naming it ccrC2. We have also designated the other ccrC alleles as ccrC1 (SCCmec V), ccrC3 (SCCmec III), and ccrC4 (SCCcap1). Future studies will reveal whether the longer CcrC2 recombinase has a different activity than the other ccrC alleles. Interestingly, the sequence of the ccrC2 gene and flanking sequence is more closely related to that found in SCCmec III than to that in the prototype strain SCCmec V. Also, the ccrC1 gene in SCCmec V is more similar to that in SCCcap1. These observations suggest that SCCmec V and SCCmec V were formed by independent recombination events with ccr genes from separate sources or that the ccrC sequences underwent divergent evolution. In addition, in one isolate, we have provided evidence for the existence of a novel composite SCCmec element that contains all of the features of SCCmec IV but also contains a ccrC2 homologue. Whether the origins of SCCmec V will be found in that isolate is the subject of ongoing investigation.

SCCmec type IV was the fourth allotype of the integrated genomic island (SCCmec) found to carry the mecA gene (7, 21). The type IV element has associated with CAMRSA in multiple genetic lineages, even in narrow geographic locations (7), a finding that suggests ease of horizontal transfer, likely to be facilitated by its small size compared to the types I to III SCCmec allotypes (21). Since SCCmec IV is usually found among CAMRSA isolates, it is interesting that approximately one-third of the SCCmec IV-containing colonization isolates were associated with an MRSA risk factor.

The SCCmec V element described from strain WIS was similar to SCCmec IV in that it did not harbor any antibiotic resistance genes other than mecA and in its relatively small in size. In contrast, strain TSGH 17, the prototype SCCmec type VT strain, was one of the CAMRSA isolates resistant to four non-β-lactam agents (ERY, CLI, TET, and CHL). Completion of the sequence of SCCmec VT from this isolate will reveal whether it harbors any of these antibiotic resistance determinants.

The CAMRSA colonization isolates were obtained by screening 640 healthy children from Taipei at locations near the TSGH. The CAMRSA colonization rate among subjects lacking traditional risk factors was 5.3%, a high rate compared with 0.24% calculated in a meta-analysis (36), 0.6% among children in Chicago (14) in 1999, 3.5% among healthy people in southern Taiwan (19), and 1.4% in Hong Kong (31). It remains to be determined whether this high MRSA colonization rate is widespread in Taipei and other locations in Taiwan or can be found among adults from the same geographic location. We have avoided making direct comparisons between the SSTI and colonization isolates since the SSTI isolates were collected in a nonoverlapping time frame. Thus, any differences may simply reflect a temporal change in the epidemiology of CAMRSA isolates circulating in this area of Taipei. Nevertheless, the observation that five SCCmec V-containing MRSA isolates were among the colonization isolates indicates that the SCCmec V-containing clone was still circulating in 2003. The differences found in SCCmec V and PVL locus prevalence between the SSTI and colonization isolates warrant a comparative study in which the SSTI and colonization samples are gathered during the same time period.

The rate of multiple resistance to at least four non-β-lactam antimicrobials among the CAMRSA in this study population was unusually high for CAMRSA, which have tended to be “non-multiply resistant” to non-β-lactam agents in the United States, Europe, Australia, and elsewhere (6, 13, 28, 29, 41). This trend was true for both SSTI and colonization isolates in association with either SCCmec IV or SCCmec VT. These data are consistent with a recent report documenting high prevalence of multiple resistance in CAMRSA from Taiwan (19) and may reflect high antimicrobial usage in the community.

Despite the high rates of multiple resistance among our CAMRSA isolates, they were still less often multiply resistant than the healthcare-associated isolates from the same institution (42) and from colonization isolates reported elsewhere in Taiwan (19). Our isolates were uniformly susceptible to fluoroquinolones and SXT, and only one isolate was resistant to RIF. Although we also found uniform vancomycin susceptibility, Vitek 2 testing is unreliable for detecting intermediate or vancomycin resistance in S. aureus (39, 40).
Thus, the presence of the multiply resistant ST 59/PFT USA 1000 CAMRSA clone containing the PVL genes circulating in the community has the capability of sharply limiting therapeutic options should it become widespread.

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REFERENCES


ERRATUM

Successful Multiresistant Community-Associated Methicillin-Resistant *Staphylococcus aureus* Lineage from Taipei, Taiwan, That Carries Either the Novel Staphylococcal Chromosome Cassette *mec* (SCCmec) Type $V_T$ or SCCmec Type IV

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Volume 43, no. 9, p. 4719–4730, 2005. Page 4720, Table 1, footnote d: “15431” should read “IS431.”
Page 4722, Table 3, column 3, line 6, should read as follows: “C1 (5).”
Page 4722, column 1, lines 1 and 4: “(final concentration, 10 μM)” should read “(final concentration, 0.1 μM).”