JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2005, p. 4719–4730 Vol. 43, No. 9
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.9.4719–4730.2005

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

Susan Boyle-Vavra,[1]* Ben Ereshefsky,[1] Chih-Chien Wang,[2] and Robert S. Daum[1]

Department of Pediatrics, Section of infectious Diseases, University of Chicago, Chicago, Illinois,[1] and Department of Pediatrics, Tri-Services General Hospital, National Defense Medical Center, Taipei, Taiwan[2]

Received 11 February 2005/Returned for modification 27 March 2005/Accepted 22 May 2005

Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates carry the methicillin resistance gene (*mecA*) on a horizontally transferred genetic element called the staphylococcal chromosome cassette *mec* (SCC*mec*). Community-acquired MRSA (CAMRSA) isolates usually carry SCC*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 (SSTI) from Taipei did not carry SCC*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*mec* V called SCC*mec* V_T. It contains a *ccrC* recombinase gene variant (*ccrC2*) and *mec* complex C2. One SSTI isolate contained molecular features of SCC*mec* IV but also contained *ccrC2* (a feature of SCC*mec* V_T), suggesting that it may harbor a composite SCC*mec* element. The genes *lukS-PV* and *lukF-PV* encoding the Panton-Valentine leukocidin (PVL) were present in all CAMRSA SSTI isolates whether they contained SCC*mec* type IV or V_T. SCC*mec* V_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 *lukS-PV* and *lukF-PV*, as did 1 of 27 (3.7%) SCC*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-β-lactam antimicrobials. SCC*mec* V_T is a novel SCC*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 *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 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 *S. aureus* (MSSA) infections (13), and the responsible isolates lack multiple resistance to antimicrobials other than β-lactams (13, 41).

Two important genotypic characteristics have been associated with CAMRSA. The staphylococcal chromosome cassette *mec* (SCC*mec*) (15, 16, 21), the genetic element that carries the methicillin resistance gene, *mecA*, integrates into the *orfX* gene in the *S. aureus* genome in a site specific manner. SCC*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 *lukS-PV* and *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 *lukS-PV* and *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*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

* Corresponding author. Mailing address: University of Chicago, MC6054, 5841 S. Maryland Ave., Chicago, IL, 60637. Phone: (773) 702-6176. Fax: (773) 702-1196. E-mail: sboyle@peds.bsd.uchicago.edu.

TABLE 1. Primers used in this study

Assay type and gene target	Primer	Sequence	Product size (kb)[a]	Source and/or reference[b]
Single-target PCR[c]				
mecA	mecAF	5′-CTTTGCTAGAGTAGCACTCG-3′		
	mecAR	5′-GCTAGCCATTCCTTTATCTTG-3′	0.5	13
mecA	mecAaminoF	5′-AGTTGTAGTTGTCGGGTTTGG-3′		
	mecAaminoR	5′-GGGACCAACATAACCTAATAGAT-3′	0.74	This study
mecI	mI3	5′-CAAAAGGACTGGAGTCCAAA-3′		
	mI4	5′-CAAGTGAATTGAAACCGCCT-3′	0.18	38
mecR1 (MS)	mcR3	5′-ATCTCCACGTTAATTCCATT-3′		
	mcR4	5′-GTCGTTCATTAAGATATGACG-3′	0.3	38
mecR1 (PB)	mcR1	5′-CGCTCAGAAATTTGTTGTGC-3′		
	mcR5	5′-CAGGGAATGAAAATTATTGGA-3	0.32	38
mec complex C2[d]	IS-5	5′-CTGCATCAATGGCACGATATAA-3′	5.5	This study
orfX	orfXprobeF	5′-GGAAGCAAGCCATAGCAGAA-3′		
	orfXprobeR	5′-TGCTTCTCCACGCATAATCTT-3′	0.4	This study
ccrAB4	ccrA4-F	5′-ATGGGATAAGAGAAAAAGCC-3′		
	ccrB4-R	5′-TAATTTACCTTCGTTGGCAT-3′	1.4	This study
ccrC	γF[g]	5′-CGTCTATTACAAGATGTTAAGGATAAT-3′		16
	γR	5′-CCTTTATAGACTGGATTATTCAAAATAT-3′	0.5[h]	16
	CDS15-R	5′-GTATGCGGGTTGTTCTTGTTCAT-3′	2.2[h]	This study
V_T unique[e]	*ccrC*-FR	5′-CCAACAAATTAAAGCAAAACAAGC-3′	4.0	This study
PVL locus	PVL-1	5′-CTGGTGCGATTCATGGTA-3′		
	PVL-2	5′-CGATATCGTGGTCATCACA-3′	3.5	2.5
Multiplex PCR				
Multiple[f]	β2	5′-ATTGCCTTGATAATAGCCITCT-3′		15; this study
ccrAB1	α2	5′-AACCTATATCATCAATCAGTACAT-3′	0.7[i]	15; this study
ccrAB2	α3	5′-TAAAGGCATCAATGCACAAACACT-3′	1.0[i]	15; this study
ccrAB3	α4	5′-AGCTCAAAAGCAAGCAATAGAAT-3′	1.6[i]	15; this study

[a] Predicted size of PCR product when the indicated reverse primer is used in conjunction with the given forward primer.
[b] 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.
[c] For each gene target, the first primer listed is the forward primer, and the subsequent primer listed is the reverse primer.
[d] Only a single primer is required because the mec complex C2 is flanked by complementary inverted repeats of 15431.
[e] This target is a unique product of SCC*mec* V_T that is formed when this primer is used alone.
[f] The multiplex PCR involved the use of one forward universal primer (β2) with three reverse primers (α2, α3, and α4) to target multiple genes. The individual targets are listed with their respective reverse primers.
[g] The forward primer γF was used with two reverse primers (γR and CDS15-R) to target the same gene.
[h] Product size when this reverse primer is used in conjunction with γF.
[i] Product size when this reverse primer is used in conjunction with β2.

(MLS_{Bc}) phenotype. Furthermore, the CAMRSA isolates were uniformly from the ST 59 genetic background and carried the PVL locus. The SmaI genome fingerprints of these CAMRSA isolates were similar to each other but differed from those of healthcare-associated isolates from the same institution. Moreover, only 3 of the 17 SSTI isolates studied carried SCC*mec* IV. The remaining isolates were nontypeable in that they lacked SCC*mec* types I to IV.

We examined here the SCC*mec* type in 13 of the 14 nontypeable SSTI isolates. This was accomplished by sequencing the *ccr* and *mec* complexes contained in one of the CAMRSA SSTI isolates and by screening the remaining isolates by PCR with type- and subtype-specific primers. The "nontypeable" isolates harbor a variant of the newly described SCC*mec* V element (16) that we have called SCC*mec* V_T. We also examined antibiotic resistance profiles, SCC*mec* types and PVL locus prevalence among 48 CAMRSA isolates asymptomatically colonizing healthy children from Taipei.

MATERIALS AND METHODS

Bacterial strains. All isolates were confirmed as *S. aureus* by a positive agglutination reaction using the Staphaurex Plus system (Remel) and by Gram staining. Isolates were frozen in skim milk (Difco) at −70°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.

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 or not they were from subjects with one or more of the risk factors listed above.

Strains ATCC 29213 (methicillin susceptible) and ATCC 43300 (methicillin resistant) were used as controls for oxacillin susceptibility testing. Control strains used for SCC*mec* typing—NCTC10442 (SCC*mec* I), N315 (SCC*mec* II), 85/2082 (SCC*mec* III), and WIS (SCC*mec* V)—were kindly provided by Keiichi Hiramatsu and Teruyo Ito (Juntendo University, Juntendo, Japan). Strain MW2 (SCC*mec* IV) was obtained from the Network for Antimicrobial Resistance in *S. aureus* (www.narsa.net).

Statistical methods. Statistical comparisons were performed by using the chi-square test with a web-based chi-square calculator (http://www.georgetown.edu

TABLE 2. Percentage of isolates with resistance to the indicated antimicrobials among the SSTI and colonization isolates

Isolates (*n*)	PCR (*mecA*)	% Isolates resistant to:								
		OXA	ERY	CLI	CIP	GEN	TET	CHL	SXT	RIF
SSTI (16)	100	81.3	100	100	0	12.5	87.5	62.5	0	0
Col (34)[a]	100	97.1	94.1	91.2	0	35.3	44.1	67.6	0	14.7

[a] 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.

/faculty/ballc/webtools/web_chi.html). In the case of a comparison group with ≤5, the Fisher exact test (two-tailed) was used. Comparisons were considered significant if the *P* value was ≤0.05.

Susceptibility testing. Strains were tested for susceptibility using the Vitek 2 system (bioMérieux Vitek, Inc., Hazelwood, MO) with a gram-positive card according to the manufacturer's recommendations in the Clinical Microbiology Laboratories at The University of Chicago Hospitals. When oxacillin susceptibility testing was performed by broth MIC analysis, procedures recommended by the Clinical and Laboratory Standards Institute (CLSI [formerly the National Committee for Clinical Laboratory Standards]) (30) were used. Trimethoprim-sulfamethoxazole (SXT) testing was performed by disk diffusion according to CLSI guidelines (30). The non-β-lactam antibiotics in the Vitek 2 panel were erythromycin (ERY), clindamycin (CLI), fluoroquinolones (ciprofloxacin, norfloxacin, ofloxacin, and levofloxacin), gentamicin (GEN), tetracycline (TET), chloramphenicol (CHL), and rifampin (RIF).

SCC*mec* typing. PCR was performed to detect *mecA* using the primer pair mecAF and mecAR as described previously (13) (Table 1). SCC*mec* elements were distinguished by the molecular architecture of the *ccr* and *mecA* complexes as described previously (15, 25, 26). PCR typing of SCC*mec* types I to IV was performed under conditions previously described (21, 26). SCC*mec* type II (*ccrAB* complex type 2 and *mec* complex class A), SCC*mec* type III (*ccrAB* complex type 3 and *mec* complex class A), and SCC*mec* type IV (*ccrAB* complex type 2 and *mec* complex class B) were assigned according to previously described criteria (21). PCR primers used to detect *mecI* (primers mI3 and mI4), the *mecR1* membrane-spanning region (MS) (primers mcR3 and mcR4), and the *mecR1* penicillin-binding region (PB) (primers mcR1 and mcR5) were originally reported by Suzuki et al. (38) (Table 1). Screening for *ccrAB* complex types 1, 2, and 3 (*ccrAB* 1, 2, and 3) was accomplished with a new multiplex PCR assay which uses a mixture of four primers consisting of a common forward primer (β2) and reverse primers, α2, α3, and α4 specific for *ccrAB* complexes 1, 2, and 3 (15). Thermocycler conditions used were as follows: 94°C for 1 min, followed by 30 cycles of 94°C for 30 s, 63°C for 1 min, and 72°C for 1 min, followed in turn by a single extension at 72°C for 5 min. The presence of the *ccrAB* gene complex type 4 (*ccrAB4*) was assessed in a separate reaction that used the primer pair ccrA4F and ccrB4R (Table 1). Screening for the *ccrC* complex (*ccr5*) was performed by using a forward primer (γF) in combination with either the reverse primer γR described by Ito et al. (16) or CDS15-R designed in the present study (Table 1). Prototype strains used for SCC*mec* typing were NCTC10442 (SCC*mec* I), N315 (SCC*mec* II), 85/2082 (SCC*mec* III), MW2 (SCC*mec* IV), and WIS (SCC*mec* V). The control strain used for detection of *ccrAB4* was *S. epidermidis* strain ATCC 12228 that contains *ccrAB4* in the non-*mec* containing SCC composite island we recently described (26).

MLST. MLST was performed by PCR amplification and sequencing of seven housekeeping genes using the primer pairs designed by Enright et al. (11). Denville Taq-Pro Complete (Denville Scientific) or the *Taq* DNA polymerase (Promega) was used for the PCRs. PCR products were evaluated on an agarose gel and purified by using Millipore 96-well Montage plates according to the manufacturer's instructions. The purified templates were sequenced at the University of Chicago Core Sequencing Facility. Each sequence was submitted to the MLST database website (http://www.mlst.net) for assignment of the allelic profile and sequence type.

PFGE. Agarose plugs were prepared containing intact bacterial cells and digested with SmaI as reported (7). The restriction fragments were resolved on a Chef DR-III pulsed-field gel electrophoresis (PFGE) apparatus (Bio-Rad) as described previously (23). On each gel, a SmaI digest of *S. aureus* strain 8325 was the molecular size standard. The USA pulsed-field type (PFT) to which the isolates belonged was determined by submitting images of pulsed-field gels in tagged image file format (Tiff) to Linda McDougal at the Centers for Disease Control and Prevention (CDC), who used Bionumerics software (Applied Maths, Austin, TX) to compare the images with those in the national database as described previously (23).

Southern hybridization. Pulsed-field gels were blotted onto GeneScreen Plus Hybridization transfer membranes overnight in 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, in 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 blot was cross-linked using UV light with a UV Cross-linker FB-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-^{32}P]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 Na_2HPO_4. 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 *orfX* gene probe produced by PCR, with the primer pair orfXprobeF-orfXprobeR. The *aroE* probe for detecting chromosomal DNA was produced by PCR with the same primers used to produce the *aroE* template for MLST analysis.

Sequencing the *mec* complex and *ccrC* gene from strain TSGH 17. Sequencing was initiated by producing a λEMBL3 library from strain TSGH 17 and screening recombinant plaques for the presence of *mecA* by hybridization. This was accomplished by isolating genomic DNA from strain TSGH 17 with the Genomic Tip kit (QIAGEN, Inc.) and performing 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 λEMBL3 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. Plaques containing the *mecA* gene were identified by hybridization with two [α-^{32}P]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 plaques with the use of the Lambda Midi kit (QIAGEN) and phenol-chloroform-isoamyl alcohol extraction, followed by ethanol precipitation to concentrate the sample using standard procedures (2). Inserts from lambda DNA were subcloned into the ClaI site of a dephosphorylated pBluescript (Stratagene) cloning vector by using T4 DNA ligase (NEB, Beverley, MA). Ligation products were transformed into Electromax STBL4 cells (Stratagene) by using a Bio-Rad Gene Pulser II and a 0.l-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-indolyl-β-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/pUC universal forward and reverse primers (5′-GTAAAACGACGGCCAGT-3′ and 5′-CACACAGGAAACAGCTATGACCAT-3′, respectively).

To obtain the sequence of the *ccr* complex, primers were designed from the sequences obtained from λEMBL3 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 IS*431*-specific primers (V3, mA2, and mA3 [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

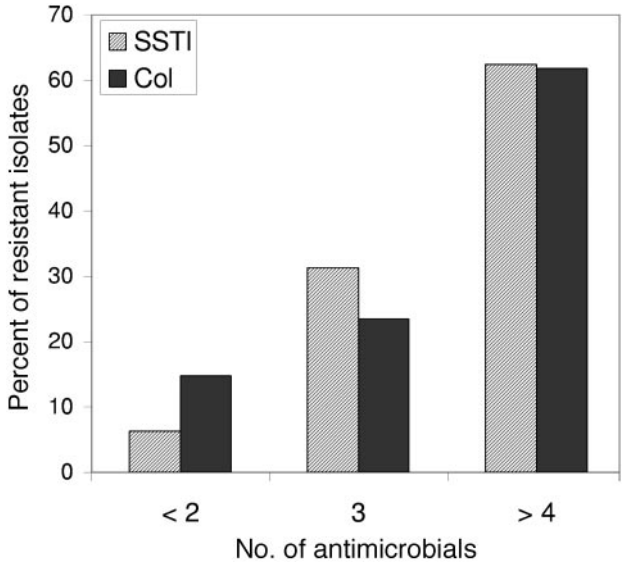

FIG. 1. Multiple resistance to non-β-lactam antimicrobials among SSTI and colonization isolates. The percentages of isolates with resistance to ≤2, 3, and ≥4 non-β-lactam antimicrobials in SSTI and colonization CAMRSA from Taipei are shown. The antimicrobials tested are listed in Table 2.

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 433-bp product (18) that includes a portion of both the *lukS-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).

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.

SCC*mec* typing. Table 3 shows the results of SCC*mec* typing for the colonization and the SSTI isolates. Of the colonization

TABLE 3. Summary of SCC*mec* typing results in colonization and SSTI isolates from Taiwan[a]

SCC*mec* type	PCR product typing								No. (%) of isolates	
	mecA	*ccr* genes (complex type[c])	*mecI*	*mecR1*		*ccrC*		*mec* complex C2[d]	Col (n = 34)	SSTI (n = 16)
				PB	MS	γF/CDS15-R	*ccrC*-FR			
IV	+	AB2 (2)	−	−	+	−	−	−	27 (79.4)	2 (12.5)
V_T	+	C2 (5)	−	−	−	+	+	+	5 (14.7)	13 (81.3)
II	+	AB2 (2)	+	+	+	−	−	−	1 (2.9)	0
III	+	AB3 (3)	+	+	+	ND	ND	ND	1 (2.9)	0
IV/*ccrC*	+	AB2/C2 (2/5)	−	−	+	+	−	−	0	1 (6.3)
V[b]	+	C1 (1)	−	−	−	+	−	+	0	0

[a] Reflects data from the 34 colonization (Col) isolates that were from patients that did not have MRSA risk factors. SCC*mec* typing was performed by PCR as described in Materials and Methods. Primers for *mecA*, *ccr* complex, *mecI*, *mecR1* (PB), and *mecR1*(MS) are shown in Table 1 and are described in Materials and Methods. ND, not determined.

[b] V refers to the SCC*mec* V element in strain WIS from Australia (16).

[c] *ccr* complexes 1, 2, and 3 each contain a *ccrA* and *ccrB* gene, whereas the *ccr5* complex consists of either *ccrC1* (present in the SCC*mec* V prototype strain WIS) or *ccrC2* (present in the *SCCmec* V_T prototype strain).

[d] Primer IS-5 was used (see Table 1).

TABLE 4. Distribution of SCC*mec* types among colonization isolates[a] with various STs

ST	No. (%) of colonization isolates of SCC*mec* type:				Total no. (%) of isolates
	II	III	IV	V_T	
59	0	0	26 (76.4)	5 (14.7)	31 (91.2)
508	0	0	1 (2.9)	0	1 (2.9)
89	1 (2.9)	0	0	0	1 (2.9)
239	0	1 (2.9)	0	0	1 (2.9)
Total	1 (2.9)	1 (2.9)	27 (81.7)	5 (14.7)	34 (100)

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

isolates that were from subjects that lacked an MRSA risk factor, a majority (27 of 34 [79.4%]) harbored SCC*mec* IV. SCC*mec* 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 SCC*mec* IV-containing colonization isolates were from subjects who had an MRSA risk factor.

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

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 SCC*mec*

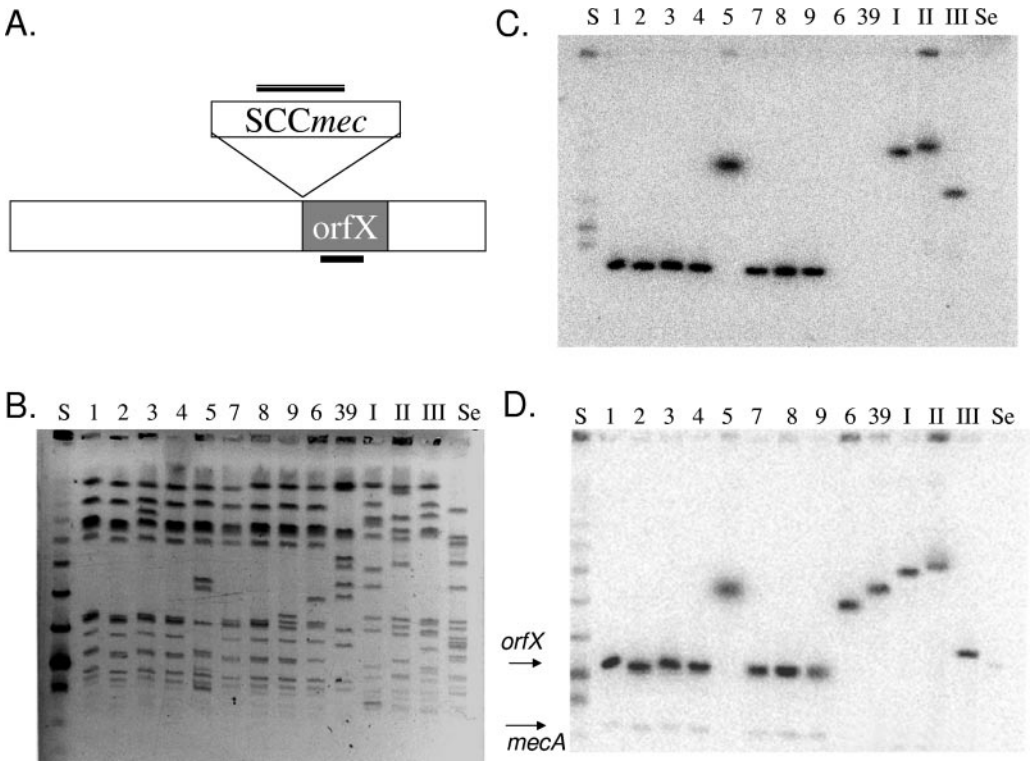

FIG. 2. Southern blotting analysis of ST 59 CAMRSA isolates containing either SCC*mec* V_T or SCC*mec* IV. (A) Map illustrating the hybridization probes (black bars above and below the map), *mecA* and *orfX*. (B) Pulsed-field gel containing SmaI-digested DNA from ST 59 isolates containing SCC*mec* IV (lane 5, strain TSGH 5) or SCC*mec* V_T. 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 SCC*mec* 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*.

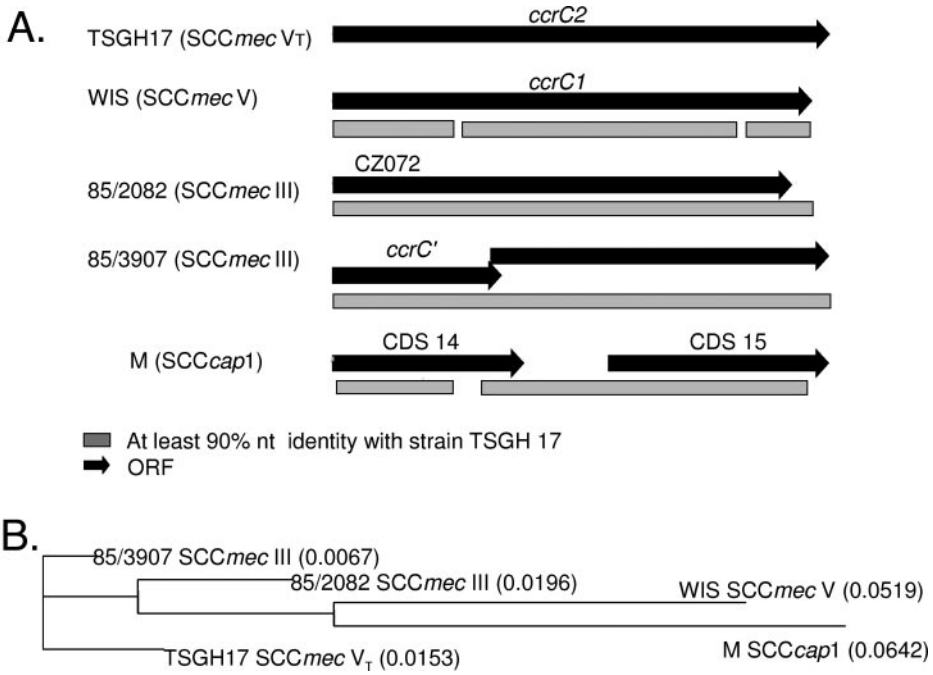

FIG. 3. Comparisons of *ccrC* genes. (A) ORF architecture of the *ccrC* homologues from SCC*mec* V_T (strain TSGH 17), SCC*mec* V (strain WIS), SCC*mec* III (strain 85/2082), SCC*mec* III (strain 85/3907), and SCC*cap*1 (strain M). (B) Sequence alignment tree built from a CLUSTAL W alignment between the 1,677-bp *ccrC* ORF from strain TSGH 17 and the corresponding ~1,680-bp region encompassing the *ccrC* ORFs from SCC*mec* types III, V, and SCC*cap*1 (same sequences as those used in panel A) using the neighbor-joining method of Saitou and Nei (35) using AlignX program in the Vector NTI software suite. Calculated distance based on distances between all pairs of sequence values are shown in parenthesis following the molecule name. (C) Sequence alignment of the 3′ region of *ccrC* between strains TSGH 17 and WIS and the consensus sequence for the corresponding region from 18 other SCC*mec* V_T isolates (starting at position 1249 of TSGH 17 *ccrC* to the end of the ORF). The accession numbers used in the above alignments were: SCC*mec* V (strain WIS, accession no. AB121219, nucleotides 16132 to 17813), SCC*mec* III (strain 85/2082, accession no. AB037671, nucleotides 7232 to 8913, reverse complement), SCC*mec* III (strain 85/3907, accession no. AB047089, nucleotides 7237 to 8917, reverse complement), and SCC*cap*1 (strain M, accession no. U10927, nucleotides 6864 to 8546). A "^" symbol beneath the aligned sequences indicates a nucleotide difference between SCC*mec*V_T and SCC*mec*V. The alignment was performed using default settings with public domain software (http://align.genome.jp) and the Fast/Approximate algorithm. (D) Unrooted dendrogram calculated from a CLUSTAL W multiple sequence alignment between *ccrA1*, *ccrA2*, *ccrA3*, *ccrB1*, *ccrB2*, *ccrB3*, and *ccrC1* (from SCC*mec*V); *ccrC2* (from SCC*mec* V_T); *ccrC3* from SCC*mec* III; and *ccrC4* from SCC*cap*1.

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 SCC*mec* 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 SCC*mec* (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 SCC*mec* 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 SCC*mec* element was not inserted into the *attB* insertion site in the *orfX* gene, as are all other known SCC*mec* 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 SCC*mec* element on a plasmid.

Characterization of the *ccrC* complex and *mec* complex of a new subtype of SCC*mec* V (SCC*mec* V_T). To determine the SCC*mec* type of the nontypeable isolates, the DNA sequences of the *ccr* and *mecA* 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) SCC*mec* III (strains 85/3907 and 85/2082), (ii) SCC*mec* V (strain WIS), and (iii) SCC*cap*1 (strain M). The last element does not contain *mecA* but does contain a *ccrC* homologue and encodes the type

C.

```
                1149                                                                                                1248
consensus SCCmecV_T  AGAAGAACTGAAACAAAAGCATAGTAAACAAACAGAAATAAAATATGATATTGATAGTCTAGAAAAACAAAAAGCAAAAGTTAAAACACAACAAGAACGA
     T17  SCCmecV_T  AGAAGAACTGAAACAAAAGCATAGTAAACAAACAGAAATAAAATATGATATTGATAGTCTAGAAAAACAAAAAGCAAAAGTTAAAACACAACAAGAACGA
     WIS  SCCmecV    AGAAGAACTGAAACAAAAGCATAGTAAACAAACAGAAATAAAATATGATATTGATAGTCTAGAAAAACAAAAAGCAAAAGTTAAAACACAACAAGAACGA

                1249                                                                                                1348
consensus SCCmecV_T  TTATTGGAATTGTTCTTAGATGATGAAATGGATAGCGAAATGTTAAAAGCTAAACAAAGTGAAATGAATCAACAGTTAGAAGTATTAGACCAACAAATTA
     T17  SCCmecV_T  TTATTGGAATTGTTCTTAGATGATGAAATGGATAGCGAAATGTTAAAAGCTAAACAAAGTGAAATGAATCAACAGTTAGAAGTATTAGACCAACAAATTA
     WIS  SCCmecV    TTATTGGAATTGTTCTTAGATGATGAAATGGACAGCGAAATGTTAAAAGCAAAACAAAGTCAGATGAATGAACAACTAGAGATGCTAGATAAGCAAATTA
                                                 ^                                                  ^   ^^   ^^ ^^   ^ ^

                1349                                                                                                1448
consensus SCCmecV_T  AAGAAGCAAAACAAGCAAATCAATCACAGGATGATATACCCAATTTTGATAAG-TTAAAAGCACGACTCATTTTGATGATAACACGATTCAGTGTGTACT
     T17  SCCmecV_T  AAGAAGCAAAACAAGCAAATCAATCACAGGATGATATACCCAATTTTGATAAG-TTAAAAGCACGACTCATTTTGATGATAACACGATTCAGTGTGTACT
     WIS  SCCmecV    AAGAAGCACAACAAGCTATGGAATCACAAGCTGGAGTATCAAATTATGATAAACTTAAGAGC-CGCTTAACCATGATGATTAGCAGATTTAGTGTCTACT
                ^      ^ ^^^      ^ ^  ^^^  ^ ^    ^      ^^    ^   ^ ^^ ^  ^^^       ^ ^^^    ^     ^

                1449                                                                                                1548
consensus SCCmecV_T  TAAGAAAGGCTACACCCGAAGCTAAAAATCAACTTATGAAAATGTTAATTGATTCAATTGAAATTACGACAGATAAACAAGTAAAACTTGTAAGGTATAA
     T17  SCCmecV_T  TAAGAAAGGCTACACCCGAAGCTAAAAATCAACTTATGAAAATGTTAATTGATTCAATTGAAATTACGACAGATAAACAAGTAAAACTTGTAAGGTATAA
     WIS  SCCmecV    TAAGAGAAGCTACACCAGAAGCAAAAAATCAACTTATGAAGATGTTGATTGATTCTATTGAAATTACTACAGATAAACAAGTGAAACTTGTAAGGTATAA
          ^         ^     ^                ^     ^        ^              ^              ^

                1549                                                                                                1648
consensus SCCmecV_T  AATTGATGAAAGTCTTATCCCTCAATCTTTGAAAAAAGATTGGGGGTCTTTTTTTATACCTAAATTTA-ACTTTGTGATAAATGTCACAAAGAAAAATAG
     T17  SCCmecV_T  AATTGATGAAAGTCTTATCCCTCAATCTTTGAAAAAAGATTGGGGGTCTTTTTTTATACCTAAATTTA-ACTTTGTGATAAATGTCACAAAGAAAAATAG
     WIS  SCCmecV    AATTGACGAAAGTCTTATCCCTCAATCTTTGAAAAAAGATTGGGGGTCTTTTTTTATGCCCAAATTCCCAATTTGAAATAGACGGTCGAAACGATTATTT
          ^                                                                  ^  ^     ^^^ ^   ^^   ^ ^ ^^^^   ^^ ^^  ^^

                1649                1686
consensus SCCmecV_T  GATTGA-AAATTTATCACTTTTACCACTT---TTTTAG
     T17  SCCmecV_T  GATTGA-AAATTTATCACTTTTACCACTT---TTTTAG
     WIS  SCCmecV    TATCGACAAAATTACCACTTTTACCACTTAGCTTTTAG
                     ^  ^  ^   ^  ^             ^^^
```

D.

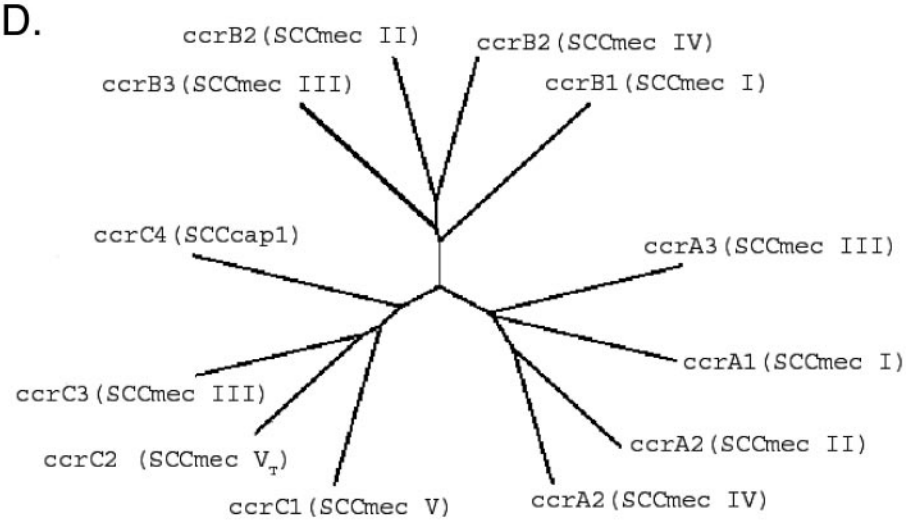

FIG. 3—*Continued.*

1 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% (SCC*mec* III, 85/3907), 94.8% (SCC*mec* III, 85/2082), 90.3% (SCC*mec* V, WIS), and 88.6% (SCC*cap*1, 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 SCC*mec* 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-

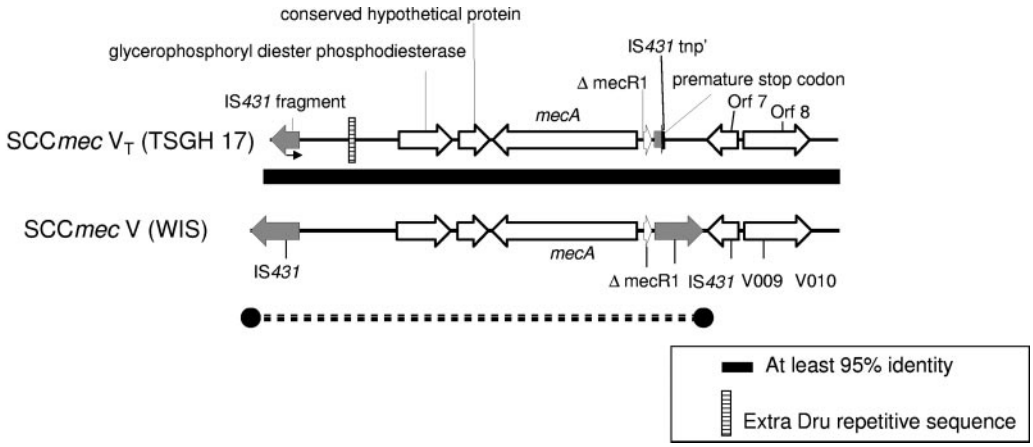

FIG. 4. Comparison of *mec* complexes in SCC*mec* V_T and SCC*mec* V. The map depicts the *mec* C2 complex (indicated by a dashed line beneath the diagram) of SCC*mec* V_T from strain TSGH 17 (top) and the *mec* C2 complex of SCC*mec* V from strain WIS (accession no. AB121219) (bottom). The SCC*mec* V_T signature premature stop codon forming a truncated transposase (tnp′) in IS*431* in strain TSGH 17 is not present in SCC*mec* V. The horizontal bar between the two SCC*mec* elements depicts >95% nucleotide sequence identity. The vertical striped bar indicates the presence of an extra dru in the intergenic hypervariable region in SCC*mec* V_T in strain TSGH 17 compared to strain WIS.

quence alignment tree (Fig. 3B) illustrates that the *ccrC* ORF from SCC*mec* V from strain WIS is most closely related to that of SCC*cap*l and more distantly related to SCC*mec* V_T.

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 *mecA*Δ*mecRI* flanked by IS*431* 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 SCC*mec* 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 IS*431* transposase gene upstream of *mecA* (C_{144} in strain WIS is G_{144} 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 SCC*mec* elements (34). There are four such dru's in strain TSGH 17 and three in strain WIS.

Thus, the SCC*mec* 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 SCC*mec* 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 SCC*mec* V, which we call SCC*mec* V_T.

Determination of the SCC*mec* type in other SCC*mec* nontypeable isolates from Taiwan. To determine whether the remaining SCC*mec* nontypeable isolates contained SCC*mec* V_T, primers described by Ito et al. (16) to characterize SCC*mec* 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 SCC*mec* types I to IV gave a product using these primers, validating the use of γF/CDS15-R as SCC*mec* V screening primers. In addition, no product was detected from the ST 59 strains that contained SCC*mec* 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 SCC*mec* V_T.

To screen for the unique architecture of *mec* complex C2 (17), which is flanked by two complementary inverted repeats of IS*431*, we developed a PCR assay using a single IS*431*-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 SCC*mec* V prototype strain (Table 3). This primer did not amplify a product from the prototype strains containing SCC*mec* 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 (Ta-

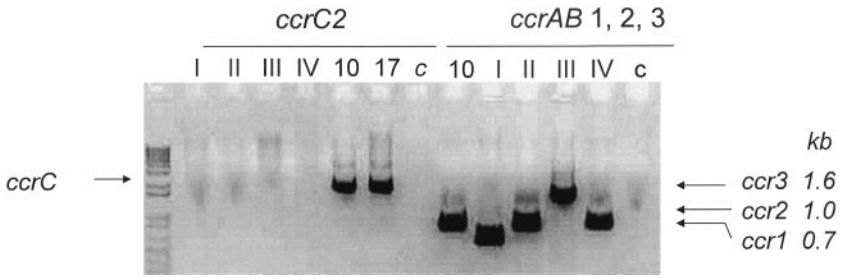

FIG. 5. PCR assay demonstrating the presence of both *ccrAB* type 2 and *ccrC2* in strain TSGH 10. PCR products were resolved by agarose gel electrophoresis stained with ethidium bromide. Lanes contain PCR products produced with either the primer pair γF and CDS15-R to detect *ccrC* (*ccrC*) or the multiplex primers β2, α2, α3, and α4 for detecting *ccrAB* 1, 2, and 3. Lanes contain reactions from either SSTI isolates TSGH 17 (lane 17) or TSGH 10 (lane 10). DNA from control strains containing SCC*mec* I, II, III, and IV are in lanes labeled I (NCTC 10442/SCC*mec* I), II (strain N315, SCC*mec* II), III (strain 85/2082, SCC*mec* III), and IV (strain MW2, SCC*mec* IV) and contain *ccrAB* types 1, 2, 3, and 2, respectively. Lanes c, negative control lanes containing water instead of a DNA template.

ble 3). This is in contrast to *mec* complex class B that contains the *mecRI* (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 TSGH 17 genomic DNA. No product was formed from the prototype strain WIS containing SCC*mec* V (Table 3). *ccrC*-FR also produced a 4.0-kb product from all of the nontypeable strains but not from any of the SCC*mec* 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 SCC*mec* V_T. Therefore, 5 (14.7%) of the 34 colonization isolates contained SCC*mec* V_T and 26 (76.4%) contained SCC*mec* IV (Table 4). In contrast, all 13 nontypeable SSTI isolates studied had SCC*mec* V_T (81.3%) and 2 SSTI isolates (12.5%) had SCC*mec* IV.

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

Evidence for a novel SCC*mec* type IV/type V_T composite element in TSGH 10. TSGH 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 TSGH 10 was determined and is identical to that of TSGH 17. These data suggest that TSGH 10 contains SCC*mec* IV (*ccrAB2* complex and *mec* class B), but the detection of the *ccrC* gene from SCC*mec* 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 SCC*mec* 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 SCC*mec* 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 TSGH 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 SCC*mec* element with a few harboring SCC*mec* 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 SCC*mec* V_T, a variant of the SCC*mec* V element described recently (16). We have also identified SCC*mec* 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 SCC*mec* element other than SCC*mec* IV among a group of CAMRSA isolates (41).

The colonization isolates that carried SCC*mec* V_T were more likely to carry the PVL locus than those carrying SCC*mec* IV. This is also a departure from the situation elsewhere in which the PVL locus has been found exclusively in SCC*mec* IV-containing isolates (41) or in MSSA isolates. Among the SSTI isolates from Taipei, the PVL locus was uniformly present among both SCC*mec* 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

TABLE 5. Distribution of PVL locus carriage and SCC*mec* types among colonization and SSTI isolates[a]

SCC*mec* type	No. of isolates			
	Colonization ($n = 34$)		SSTI ($n = 16$)	
	PVL	Total	PVL	Total
II	0	1	0	0
III	0	1	0	0
IV	1	27[b]	2	2
V_T	4	5	14	14

[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 SCC*mec* IV and a *ccrC2* gene.

associated with nosocomial MRSA or MSSA infections. In the present study, a majority of CAMRSA isolates asymptomatically 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 SCC*mec* V element. SCC*mec* 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 ST1 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 SCC*mec* IV (3, 8). Sporadic ST 59 isolates with SCC*mec* II (3), SCC*mec* III (32) or a nontypeable SCC*mec* 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 SCC*mec* 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 SCC*mec* V-like element has been introduced (but the first in which SCC*mec* V_T has been found).

SCC*mec* V is characterized by the presence of a *ccrC* recombinase complex and *mec* complex type C2 (16). The SCC*mec* V_T variant we identified contains signature features in both its *mec* and *ccr* complexes. The nucleotide polymorphisms we found in the *ccr* region of SCC*mec* V_T 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* (SCC*mec* V), *ccrC3* (SCC*mec* III), and *ccrC4* (SCC*cap*1). 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 SCC*mec* III than to that in the prototype strain SCC*mec* V. Also, the *ccrC1* gene in SCC*mec* V is most similar to that in SCC*cap*1. These observations suggest that SCC*mec* V_T and SCC*mec* V were formed by independent recombination events with *ccrC* 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 SCC*mec* element that contains all of the features of SCC*mec* IV but also contains a *ccrC2* homologue. Whether the origins of SCC*mec* V will be found in that isolate is the subject of ongoing investigation.

SCC*mec* type IV was the fourth allotype of the integrated genomic island (SCC*mec*) 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 SCC*mec* allotypes (21). Since SCC*mec* IV is usually found among CAMRSA isolates, it is interesting that approximately one-third of the SCC*mec* IV-containing colonization isolates were associated with an MRSA risk factor.

The SCC*mec* V element described from strain WIS was similar to SCC*mec* 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 SCC*mec* type V_T strain, was one of the CAMRSA isolates resistant to four non-β-lactam agents (ERY, CLI, TET, and CHL). Completion of the sequence of SCC*mec* V_T 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 SCC*mec* V_T-containing MRSA isolates were among the colonization isolates indicates that the SCC*mec* V_T-containing clone was still circulating in 2003. The differences found in SCC*mec* V_T 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 SCC*mec* IV or SCC*mec* V_T. 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.

ACKNOWLEDGMENTS

This study was supported by grants from the CDC (R01 CCR523379), the National Institutes of Health (R01 AI40481-01A1), the TSGH (TSGH-C92-77), and The Grant Healthcare Foundation (Lake Forest, IL).

We are grateful to Linda McDougal from the CDC (Atlanta, GA) for comparing the pulsed-field types of the isolates described herein with those in the national database. We are grateful to Daniel Kim for providing assistance with and maintaining the MRSA database.

REFERENCES

1. **Adem, P. V., C. P. Montgomery, A. N. Husain, T. K. Koogler, V. Arangelovich, M. Humilier, and R. S. Daum.** Novel association of Waterhouse-Friderichsen syndrome with severe *Staphylococcus aureus* sepsis in children. N. Engl. J. Med., in press.
2. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1994. Current protocols in molecular biology. John Wiley & Sons, Inc., Boston, Mass.
3. **Carleton, H. A., B. A. Diep, E. D. Charlebois, G. F. Sensabaugh, and F. Perdreau-Remington.** 2004. Community-adapted methicillin-resistant *Staphylococcus aureus* (MRSA): population dynamics of an expanding community reservoir of MRSA. J. Infect. Dis. **190:**1730–1738.
4. **CDC.** 1999. Four pediatric deaths from community-acquired methicillin-resistant *Staphylococcus aureus*—Minnesota and North Dakota, 1997–1999. Morb. Mortal. Wkly. Rep. **48:**707–710.
5. **Chambers, H. F.** 2005. Community-associated MRSA: resistance and virulence converge. N. Engl. J. Med. **352:**1485–1487.
6. **Coombs, G. W., G. R. Nimmo, J. M. Bell, F. Huygens, F. G. O'Brien, M. J. Malkowski, J. C. Pearson, A. J. Stephens, and P. M. Giffard.** 2004. Genetic diversity among community methicillin-resistant *Staphylococcus aureus* strains causing outpatient infections in Australia. J. Clin. Microbiol. **42:**4735–4743.
7. **Daum, R. S., T. Ito, K. Hiramatsu, F. Hussain, K. Mongkolrattanothai, M. Jamklang, and S. Boyle-Vavra.** 2002. A novel methicillin-resistance cassette in community-acquired methicillin-resistant *Staphylococcus aureus* isolates of diverse genetic backgrounds. J. Infect. Dis. **186:**1344–1347.
8. **Diep, B. A., F. Perdreau-Remington, and G. F. Sensabaugh.** 2003. Clonal characterization of *Staphylococcus aureus* by multilocus restriction fragment typing, a rapid screening approach for molecular epidemiology. J. Clin. Microbiol. **41:**4559–4564.
9. **Dufour, P., Y. Gillet, M. Bes, G. Lina, F. Vandenesch, D. Floret, J. Etienne, and H. Richet.** 2002. Community-acquired methicillin-resistant *Staphylococcus aureus* infections in France: emergence of a single clone that produces Panton-Valentine leukocidin. Clin. Infect. Dis. **35:**819–824.
10. **Ellis, M. W., D. R. Hospenthal, D. P. Dooley, P. J. Gray, and C. K. Murray.** 2004. Natural history of community-acquired methicillin-resistant *Staphylococcus aureus* colonization and infection in soldiers. Clin. Infect. Dis. **39:**971–979.
11. **Enright, M. C., N. P. Day, C. E. Davies, S. J. Peacock, and B. G. Spratt.** 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus.* J. Clin. Microbiol. **38:**1008–1015.
12. **Gillet, Y., B. Issartel, P. Vanhems, J. C. Fournet, G. Lina, M. Bes, F. Vandenesch, Y. Piemont, N. Brousse, D. Floret, and J. Etienne.** 2002. Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. Lancet **359:**753–759.
13. **Herold, B. C., L. C. Immergluck, M. Maranan, D. S. Lauderdale, R. E. Gaskin, S. Boyle-Vavra, C. D. Leitch, and R. S. Daum.** 1997. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no predisposing risk. JAMA **279:**593–598.
14. **Hussain, F. M., S. Boyle-Vavra, and R. S. Daum.** 2001. Community-acquired methicillin-resistant *Staphylococcus aureus* colonization in healthy children attending an outpatient pediatric clinic. Pediatr. Infect. Dis. J. **20:**763–767.
15. **Ito, T., Y. Katayama, K. Asada, N. Mori, K. Tsutsumimoto, C. Tiensasitorn, and K. Hiramatsu.** 2001. Structural comparison of three types of staphylococcal cassette chromosome *mec* in the chromosome of methicillin-resistant *Staphylococcus aureus.* Antimicrob. Agents Chemother. **45:**1323–1336.
16. **Ito, T., X. X. Ma, F. Takeuchi, K. Okuma, H. Yuzawa, and K. Hiramatsu.** 2004. Novel type V staphylococcal cassette chromosome *mec* driven by a novel cassette chromosome recombinase, *ccrC*. Antimicrob. Agents Chemother. **48:**2637–2651.
17. **Katayama, Y., T. Ito, and K. Hiramatsu.** 2001. Genetic organization of the chromosome region surrounding *mecA* in clinical staphylococcal strains: role of IS*431*-mediated *mecI* deletion in expression of resistance in *mecA*-carrying, low-level methicillin-resistant *Staphylococcus haemolyticus.* Antimicrob. Agents Chemother. **45:**1955–1963.
18. **Lina, G., Y. Piemont, F. Godail-Gamot, M. Bes, M. O. Peter, V. Gauduchon, F. Vandenesch, and J. Etienne.** 1999. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin. Infect. Dis. **29:**1128–1132.
19. **Lu, P. L., L. C. Chin, C. F. Peng, Y. H. Chiang, T. P. Chen, L. Ma, and L. K. Siu.** 2005. Risk factors and molecular analysis of community methicillin-resistant *Staphylococcus aureus* carriage. J. Clin. Microbiol. **43:**132–139.
20. **Luong, T. T., S. Ouyang, K. Bush, and C. Y. Lee.** 2002. Type 1 capsule gene of *Staphylococcus aureus* are carried in a staphylococcal cassette chromosome genetic element. J. Bacteriol. **184:**3623–3629.
21. **Ma, X. X., T. Ito, C. Tiensasitorn, M. Jamklang, P. Chongtrakool, S. Boyle-Vavra, R. S. Daum, and K. Hiramatsu.** 2002. A novel type of staphylococcal cassette chromosome *mec* (SCC*mec*) identified in community-acquired methicillin-resistant *Staphylococcus aureus* strains. Antimicrob. Agents Chemother. **46:**1147–1152.
22. **Maranan, M. C., B. Moreira, S. Boyle-Vavra, and R. S. Daum.** 1997. Antimicrobial resistance in staphylococci. Infect. Dis. Clin. N. Am. **11:**813–849.
23. **McDougal, L. K., C. D. Steward, G. E. Killgore, J. M. Chaitram, S. K. McAllister, and F. C. Tenover.** 2003. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. J. Clin. Microbiol. **41:**5113–5120.
24. **Miller, L. G., F. Perdreau-Remington, G. Rieg, S. Mehdi, J. Perlroth, A. S. Bayer, A. W. Tang, T. O. Phung, and B. Spellberg.** 2005. Necrotizing fasciitis caused by community-associated methicillin-resistant *Staphylococcus aureus* in Los Angeles. N. Engl. J. Med. **352:**1445–1453.
25. **Mongkolrattanothai, K., S. Boyle, M. D. Kahana, and R. S. Daum.** 2003. Severe *Staphylococcus aureus* infections caused by clonally related community-acquired methicillin-susceptible and methicillin-resistant isolates. Clin. Infect. Dis. **37:**1050–1058.
26. **Mongkolrattanothai, K., S. Boyle, T. V. Murphy, and R. S. Daum.** 2004. Novel non-*mecA*-containing staphylococcal chromosomal cassette composite island containing *pbp4* and *tagF* genes in a commensal staphylococcal species: a possible reservoir for antibiotic resistance islands in *Staphylococcus aureus.* Antimicrob. Agents Chemother. **48:**1823–1836.
27. **Moreno, F., C. Crisp, J. H. Jorgensen, and J. E. Patterson.** 1995. Methicillin-resistant *Staphylococcus aureus* as a community organism. Clin. Infect. Dis. **21:**1308–1312.
28. **Naimi, T. S., K. H. LeDell, D. J. Boxrud, A. V. Groom, C. D. Steward, A. K. Johnson, J. M. Besser, C. O'Boyle, R. N. Danila, J. E. Cheek, M. T. Osterholm, K. A. Moore, and K. E. Smith.** 2001. Epidemiology and clonality of community-acquired methicillin-resistant *Staphylococcus aureus* in Minnesota, 1996–1998. Clin. Infect. Dis. **33:**990–996.
29. **Naimi, T. S., K. H. LeDell, K. Como-Sabetti, S. M. Borchardt, D. J. Boxrud, J. Etienne, S. K. Johnson, F. Vandenesch, S. Fridkin, C. O'Boyle, R. N. Danila, and R. Lynfield.** 2003. Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. JAMA **290:**2976–2984.
30. **NCCLS.** 2004. Performance standards for antimicrobial susceptibility testing. Fourteenth informational supplement M100-S14, vol. 24. NCCLS, Wayne, Pa.
31. **O'Donoghue, M. M., and M. V. Boost.** 2004. The prevalence and source of methicillin-resistant *Staphylococcus aureus* (MRSA) in the community in Hong Kong. Epidemiol. Infect. **132:**1091–1097.
32. **Pan, E. S., B. A. Diep, H. A. Carleton, E. D. Charlebois, G. F. Sensabaugh, B. L. Haller, and F. Perdreau-Remington.** 2003. Increasing prevalence of methicillin-resistant *Staphylococcus aureus* infection in California jails. Clin. Infect. Dis. **37:**1384–1388.
33. **Peleg, A. Y., and W. J. Munckhof.** 2004. Fatal necrotising pneumonia due to community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA). Med. J. Aust. **181:**228–229.
34. **Ryffel, C., R. Bucher, F. H. Kayser, and B. Berger-Bachi.** 1991. The *Staphylococcus aureus mec* determinant comprises an unusual cluster of direct repeats and codes for a gene product similar to the *Escherichia coli sn*-glycerophosphoryl diester phosphodiesterase. J. Bacteriol. **173:**7416–7422.
35. **Saitou, N., and M. Nei.** 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4:**406–425.
36. **Salgado, C. D., B. M. Farr, and D. P. Calfee.** 2003. Community-acquired methicillin-resistant *Staphylococcus aureus*: a meta-analysis of prevalence and risk factors. Clin. Infect. Dis. **36:**131–139.
37. **Suggs, A. H., M. C. Maranan, S. Boyle-Vavra, and R. S. Daum.** 1999. Methicillin-resistant and borderline methicillin-resistant asymptomatic *Staphylococcus aureus* colonization in children without identifiable risk factors. Pediatr. Infect. Dis. J. **18:**410–414.
38. **Suzuki, E., K. Kuwahara-Arai, J. F. Richardson, and K. Hiramatsu.** 1993. Distribution of mec regulator genes in methicillin-resistant *Staphylococcus* clinical strains. Antimicrob. Agents Chemother. **37:**1219–1226.
39. **Tenover, F. C., M. V. Lancaster, B. C. Hill, C. D. Steward, S. A. Stocker, G. A. Hancock, C. M. O'Hara, N. C. Clark, and K. Hiramatsu.** 1998. Char-

acterization of staphylococci with reduced susceptibilities to vancomycin and other glycopeptides. J. Clin. Microbiol. **36:**1020–1027.
40. **Tenover, F. C., L. M. Weigel, P. C. Appelbaum, L. K. McDougal, J. Chaitram, S. McAllister, N. Clark, G. Killgore, C. M. O'Hara, L. Jevitt, J. B. Patel, and B. Bozdogan.** 2004. Vancomycin-resistant *Staphylococcus aureus* isolate from a patient in Pennsylvania. Antimicrob. Agents Chemother. **48:**275–280.
41. **Vandenesch, F., T. Naimi, M. C. Enright, G. Lina, G. R. Nimmo, H. Heffernan, N. Liassine, M. Bes, T. Greenland, M. E. Reverdy, and J. Etienne.** 2003. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg. Infect. Dis. **9:**978–984.
42. **Wang, C. C., W. T. Lo, M. L. Chu, and L. K. Siu.** 2004. Epidemiological typing of community-acquired methicillin-resistant *Staphylococcus aureus* isolates from children in Taiwan. Clin. Infect. Dis. **39:**481–487.

JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2005, p. 6223 Vol. 43, No. 12
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.12.6223.2005

ERRATUM

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

Susan Boyle-Vavra, Ben Ereshefsky, Chih-Chien Wang, and Robert S. Daum

Department of Pediatrics, Section of Infectious Diseases, University of Chicago, Chicago, Illinois, and Department of Pediatrics, Tri-Services General Hospital, National Defense Medical Center, Taipei, Taiwan

Volume 43, no. 9, p. 4719–4730, 2005. Page 4720, Table 1, footnote *d*: "15431" should read "IS*431*."
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)."