Novel Staphylococcal Cassette Chromosome *mec* Type, Tentatively Designated Type VIII, Harboring Class A *mec* and Type 4 *ccr* Gene Complexes in a Canadian Epidemic Strain of Methicillin-Resistant *Staphylococcus aureus*[▽]

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Received 20 August 2008/Returned for modification 2 November 2008/Accepted 30 November 2008

Staphylococcal cassette chromosome *mec* (SCC*mec*) is a mobile genetic element characterized by flanking terminal direct and, in most cases, inverted repeat sequences, the *mec* and *ccr* gene complexes, and their surrounding DNA regions. Unique combinations of the *mec* and *ccr* gene complexes generate various SCC*mec* types. Six SCC*mec* types have been reported to date. We describe here a novel SCC*mec* type identified in a Canadian methicillin-resistant *Staphylococcus aureus* (MRSA) epidemic strain. MRSA clinical isolates were screened for known SCC*mec* types by multiplex and conventional PCR methods. Three phenotypically and genotypically identical MRSA clinical isolates with a pulsotype identical to CMRSA9 were identified locally and found to be nontypeable by available SCC*mec* typing schemes. Complete sequencing of the SCC*mec* element revealed a nucleotide fragment of 32,168 bp integrated at an identical chromosomal integration site (*attB_{SCC}*) at the 3' end of the *orfX* gene. The nucleotide sequences at the chromosome-SCC*mec* junction regions were typical of other SCC*mec* types, but the element harbored a unique combination of class A *mec* and type 4 *ccr* gene complexes. Sequence recombination analysis suggested that this unique SCC*mec* type may be derived from homologous recombination between the previously described SCC_{RP62A} of *S. epidermidis* strain RP62A and SCC composite island of *S. epidermidis* ATCC 12228, respectively, or via recombination of other staphylococcal strains that carry the same or similar mobile cassettes. We identified a previously undescribed type of SCC*mec* from isolate C10682, tentatively designated type VIII, and we provide compelling evidence supporting the ability of SCC elements to transfer horizontally or undergo recombination to generate new SCC*mec* types.

Methicillin resistance in *Staphylococcus* spp. results from acquisition of *mecA*, which encodes an additional altered low-affinity penicillin-binding protein (PBP2a), conferring broad resistance to almost all penicillin-related compounds (9). Methicillin-resistant *Staphylococcus aureus* (MRSA) strains have acquired and integrated into their genome a 21- to 67-kb mobile genetic element, termed the staphylococcal cassette chromosome *mec* (SCC*mec*), which harbors the methicillin resistance gene (*mecA*) and other antibiotic resistance determinants (13, 15, 20).

The SCC*mec* element integrates into the *Staphylococcus aureus* chromosome at a unique site (*attB_{SCC}*) located downstream of an open reading frame (ORF) of unknown function known as *orfX* that is near the origin of replication (14). These elements are characterized by the presence of flanking terminal direct and, in most cases, inverted repeats, two essential genetic components (the *mec* gene complex and the *ccr* gene complex), and three joining (J; previously known as junkyard)

regions (13, 15, 18, 20). The *mec* gene complex is composed of IS431 and/or IS1272, *mecA*, and intact or truncated sets of regulatory genes *mecR1* (encoding the signal transducer protein MecR1) and *mecI* (encoding the repressor protein MecI) (13, 15, 18, 20). The *ccr* gene complex (*ccrA*, *ccrB*, and *ccrC*) encodes recombinases of the invertase/resolvase family, which mediate the integration of SCC*mec* into and its excision from the recipient chromosome and are responsible for the mobility of this element. The rest of the SCC*mec* element is comprised of J regions (J1, J2, and J3) that are located between and around the *mec* and *ccr* gene complexes and contain various genes or pseudogenes, including plasmid- or transposon-mediated resistance genes for non-β-lactam antibiotics or heavy metals (16). To date, there are five classes (A, B, C1, C2, and D) of *mec* gene complexes and five allotypes (types 1, 2, 3, 4, and 5) of *ccr* gene complexes which have been described (11, 15). Different combinations of these complex classes and allotypes generate various SCC*mec* types. SCC*mec* elements are currently classified into types I (combination of the type 1 *ccr* and the class B *mec* gene complex; 1B), II (2A), III (3A), IV (2B), V (5C2), and VI (4B), based on the nature of the *mec* and *ccr* gene complexes, and are further classified into subtypes according to differences in their J region DNA (13, 15, 20, 26).

A new type (5C1; tentatively designed type VII) has re-

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[▽] Published ahead of print on 8 December 2008.

TABLE 1. Primers used for characterization of the SCCmecC10682 element in this study

Primer	Primer no.	Sequence (5' to 3')	Spanning position (bp) ^a	Product size (bp) ^a	Reference
ccr-F		GGMGAACAAGTCARAAATGG	25060–26828	1,769	This study
ccr-R		TTGCCTTGATAATAGCCTTC			This study
mecI-F		CCCTTTTATACAATCTCGTT	5955–9589	3,635	32
mecA147-R		ATGCGCTATAGATTGAAAGGAT			32
J3-F20	1	CTTCCTGTATTTCTGTCTATGC	835–1031	197	This study
J3-1R	2	TTTAAAAAATGATTATCCATCAGG			This study
J3-2F	3	CAACATCTAACTCCAACCAG	2451–4360	1,910	This study
J3-3R	4	TGTCTTTCTTACTTTAATATGACGG			This study
J3-1F	5	CCTTTAAATCTACTTTGTCTGC	5090–5234	145	This study
J3-R20	6	AATACGATGATTTTATAGTAGGAG			This study
mecA1	7	GTAGAAATGACTGAACGTCGGATAA	7080–7389	310	28
mecA2	8	CCAATTCCACATGTTTCGGTCTAA			28
mecI-F	9	CCCTTTTATACAATCTCGTT	9444–9589	146	32
mecI-R	10	ATATCATCTGCAGAATGGG			32
J2-F20	11	AGAAGTCTTACACACTCCAGGC	12144–12527	384	This study
J2-3R	12	TGATTGAGTGTAAAGTTTCG			This study
J2-3F	13	GATGATAAATGCTCCACCTG	13024–14840	1,817	This study
J2-4R	14	CTTTATATCTTGATCGATTGC			This study
tnpB-F	15	AACGAGAGTGGAAATTGAAGC	17462–19803	2,342	This study
aad9-R	16	ACTTATCATCACACTCTCCCG			This study
J2-F9	17	CACACAGAAGCCATATTTAGCG	21063–22618	1,556	This study
tnpA-R	18	CTAAGCGATAACACTTCACCG			This study
J2-F6	19	GATTTGTAAATATTGTCGAGG	22420–25261	2,842	This study
J2-R9	20	GATTTACAAGATATACAGCACCG			This study
ccr4-F	21	ATCGCTCATTATGGATACTGC	25079–26828	1,750	This study
ccr4B-R	22	CAATAGCACGTTGTCTTTGGC			This study
comp-F5	23	GAATTTTTGAATGATACTGGC	28303–30163	1,861	This study
J1-R2	24	AATGGATTGGAATAATACTAAGCC			This study

^a Each primer size and spanning position apply to the forward primer and subsequently listed reverse primer.

cently been identified in a community-associated MRSA (CA-MRSA) isolate from Sweden (2). We describe here a novel SCCmec type harboring a previously uncharacterized and unique combination of class A *mec* and type 4 *ccr* gene complexes (4A), tentatively designated type VIII, identified in a Canadian MRSA (CMRSA) epidemic strain, CMRSA9, which is a typical hospital-associated MRSA (HA-MRSA) epidemic strain commonly found in Quebec and other regions in Canada (4).

(This work was presented in part at the 13th International Symposium on Staphylococci and Staphylococcal Infections, Cairns, Queensland, Australia, 7 to 10 September 2008 [34a]).

MATERIALS AND METHODS

Bacterial strains and isolates. The MRSA clinical isolates under study were recovered from patients in local hospitals in Calgary, Alberta, Canada. Strain C10682 was isolated in 2003 from a percutaneous endoscopic gastrostomy site subcutaneous abscess in a 58-year-old male patient, C4631 was isolated in 2004 from an abdominal wound of a 60-year-old male patient, and C5073 was recovered in 2005 from a screening nasal swab from an 80-year-old female patient.

The SCCmec type prototypic MRSA reference strains, including type I (NCTC10442), type II (N315), type III (85/2082), type IVc (MR108), type IVd (JCSC4469), and type V (WIS [WBG8318]-JCSC3624) strains, were kindly provided by K. Hiramatsu and T. Ito from Juntendo University, Tokyo, Japan (13, 15, 20, 24). The SCCmec type IVa and type IVb strains CA05 and 8/6-3P, respectively, were obtained from R. Daum, University of Chicago, Chicago, IL (20). The SCCmec type VI control strain HDE288 was obtained from H. de Lencastre, Rockefeller University, New York, NY (26). The Canadian epidemic MRSA reference strains CMRSA1 to CMRSA10 were provided by National Microbiology Laboratory, Health Canada, Winnipeg, Manitoba, Canada. The U.S. epidemic MRSA reference strains USA100 to USA800 (NRS382, NRS383, NRS384, NRS123, NRS385, NRS22, NRS386, and NRS387, respectively) were

obtained through the Network on Antimicrobial Resistance in *Staphylococcus aureus* program supported under NIAID/NIH contract no. N01-AI-95359.

Identification, SCCmec typing, and molecular characterization of isolates. Staphylococcal isolates were identified morphologically and biochemically by standard laboratory procedures as previously described (34). Clinical MRSA isolates were screened for known SCCmec types by multiplex (32) and conventional (13, 18) PCR methods as previously described, as well as with the use of our new type 4 *ccr* complex primer pair *ccr4-F* (5'-ATCGCTCATTATG-GATACTGC-3') and *ccr4-R* (5'-CAAATGACCCTTGCTCTATAACG-3'). Isolates were typed using pulsed-field gel electrophoresis following a standardized protocol (23). Isolates were tested for the presence of the Pantone-Valentine leukocidin (*lukS-PV* and *lukF-PV*) genes with a triplex PCR assay (21). Staphylococcal protein A gene (*spa*) typing (10, 29), multilocus sequence typing (MLST) (7), and accessory gene regulator (*agr*) typing (27) were conducted as described elsewhere. Profiles based on 35 common *S. aureus* virulence genes comprising 20 exotoxin genes, 12 adhesin genes, and 3 exoenzyme genes were determined by PCR amplification using previously described primer pairs (33).

Long-range PCR and random primer sequencing walking strategy. Frozen bacteria were subcultured twice onto tryptic soy agar plates (Becton Dickinson, Sparks, MD) prior to DNA extraction. Genomic DNA was isolated from 5 ml of bacterial culture by using the QIAamp DNA minikit following protocol D (Qiagen Inc., Mississauga, Ontario, Canada). Two methods were employed for DNA sequencing. Long-range PCR amplification was used to amplify the chromosomal junction-mec complex region as well as the *mec-ccr* complex region. Briefly, 1× PCR XL-Buffer II, 1.2 mM Mg(OAc)₂, 800 μM deoxynucleoside triphosphate (dNTP) blend (200 μM each of dATP, dGTP, dCTP, and dTTP), 1.2 units of *rTth* DNA polymerase (Applied Biosystems, Foster City, CA), 200 nmol of each primer, and 4 μl of template DNA were combined into a 50-μl reaction mixture. Cycling conditions were as follows: an initial denaturation at 93°C for 2 min was followed by 10 cycles of 93°C for 15 s, 52°C for 30 s, and 68°C for 10 min. A further 20 cycles of 93°C for 15 s, 52°C for 30 s, and 68°C for 10 min (Auto X = +20 s per cycle) were done. Long-range PCR products were gel purified with the MinElute gel extraction kit (Qiagen Inc., Mississauga, Ontario, Canada) and sequenced by primer extension using the Applied Biosystems Big-Dye terminator sequencing kit and ABI 3730S automated DNA sequencer.

FIG. 1. A unique combination of class A *mec* and type 4 *ccr* gene complexes identified in a clinical isolate C10682 of a CMRSA epidemic strain. (A) The 32-kb element with 36 predicted ORFs (ORFs CA001 to *cop4*) could be divided into five distinct regions, including the *mec* and the *ccr* gene complexes and their surrounding DNA regions, J1, J2, and J3. Typical direct repeats (DR_{SCC-R} and DR_{SCC-L}), as well as hitherto unreported dyad repeats (DyadR-R and DyadR-L) present at both right and left extremities of the chromosome-SCC*mec* junctions, are shown, but no discernible inverted repeats were found. Primer locations for the 12 PCR targets chosen to characterize the other identical MRSA strains are indicated with solid arrows and numbers corresponding to primer numbers shown in Table 1. Refer to Table 1 for details of each primer. (B) Schematic diagram of the structure of three cassettes of SCC*mec*C10682, SCC_{RP62A}, and SCC-CI showing that SCC*mec*C10682 may be derived from homologous recombination between previously described SCC_{RP62A} and SCC-CI cassettes of *S. epidermidis* RP62A (CP000029) and ATCC 12228 (AE015929; BK001539) strains, respectively. Sequences derived from SCC_{RP62A} are shaded in black, those from SCC-CI are shaded in light gray, and the sequences of the shared homologous region have a mixed pattern of shading.

Due to the inability to amplify the J1 (*ccr*-chromosomal junction) region, a modified random primer sequencing walking strategy was employed using the nested random primers arb1 (5'-GGCCACGCGTCGACTAGTACNNNNNNNNNNGA TAT-3') and arb2 (5'-GGCCACGCGTCGACTAGTAC-3') (3). Round 1 of the random PCR was done with a known primer targeting a region approximately 700 bp from the end of the already sequenced *SCCmec* element and primer arb1. Two microliters of the extracted genomic DNA was added to 48 μ l of PCR mix containing 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 2 mM $MgCl_2$, 200 μ M of each dNTP (dATP, dTTP, dGTP, and dCTP) (Invitrogen Inc., Carlsbad, CA), 0.4 μ M known primer, 0.2 μ M primer arb1, and 2.5 units of *Taq* DNA polymerase (Invitrogen Inc., Carlsbad, CA). Cycling conditions included a 5-min incubation at 96°C and then five cycles of 94°C for 30 s, 30°C for 30 s, and 72°C for 90 s, followed by 30 cycles of 94°C for 30 s, 45°C for 30 s, and 72°C for 2 min. A final incubation at 72°C for 5 min was followed by a hold at 4°C. Round two of the random PCR was done with a second known primer designed more proximal to the end of the previously sequenced element. Two microliters of the unpurified round 1 product was added to a 50- μ l PCR mix containing 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 3 mM $MgCl_2$, 200 μ M of each dNTP (dATP, dTTP, dGTP, and dCTP) (Invitrogen Inc., Carlsbad, CA), 0.4 μ M known primer, 0.2 μ M primer arb2, and 2.5 units of *Taq* DNA polymerase (Invitrogen Inc., Carlsbad, CA), with PCR conditions consisting of 29 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 90 s, was followed by an incubation at 72°C for 5 min and a hold at 4°C. All random PCR products larger than 1 kb were gel purified with the Qiagen MinElute gel extraction kit and sequenced with primer extension as described above.

Sequence and recombination analyses. Sequence homology and identity comparison to other sequences was performed with the NCBI BLAST sequence search (<http://www.ncbi.nlm.nih.gov>). ORF matching/annotation and sequence translation

were accomplished by analysis of the sequence deposited in the GenBank database, using the Vector NTI software program (Invitrogen). The DNASTAR Lasergen version 7.2 software package program (DNASar Inc.) was used to align the sequences and determine similarity and divergence among the sequences (this analysis also included the ClustalW method). Phylogenetic and bootstrap analyses were conducted using MEGA version 4 (30). For the sequence recombination analyses, the sequence was first subjected to scanning for recombination with the NCBI Genotyping Recombinant Sequence Analysis Program (<http://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi>), using all reported types of *SCC_{mec}* sequences from GenBank. Creation of a distance plot and bootscanning were performed using the SimPlot program to identify informative sites and to select crossover breakpoints (19). These breakpoints were used to divide the alignment into segments for phylogenetic tree construction, and the recombination event was further confirmed by bootstrap testing as described above.

Confirmation tests of other clinical isolates/strains harboring SCCmec C10682. MRSA isolates harboring the strain C10682 SCCmec element (SCCmec C10682) were further screened from our collection of local MRSA clinical isolates (May 1999 to December 2006) by using the class A *mec* and type 4 *ccr* gene complex-specific primers *mecI*-F/*mecA*147-R (Table 1) and *ccr4*-F/*ccr4*-R, respectively. The PCR products of strains positive for class A *mec* and type 4 *ccr* genes were subjected to full-length sequencing with primer sets *mecI*-F/*mecA*147-R and *ccr*-F/*ccr*-R (Table 1). For further confirmation, PCR amplification targeting various regions spanning the whole SCCmecC10682 element was performed. The detailed sequences and locations of these 12 primers pairs are listed in Table 1, and a schematic is shown in Fig. 1A.

Nucleotide sequence accession number. The complete sequence of the SCCmec C10682 of strain C10682 has been assigned GenBank accession no. FJ390057.

RESULTS

A unique combination of class A *mec* and type 4 *ccr* gene complexes identified in a CMRSA epidemic clinical strain. Upon initial SCC*mec* type screening of our collection of local MRSA clinical isolates, we discovered a previously uncharacterized combination of *mec* and *ccr* gene complexes in a clinical MRSA isolate designated C10682. Using *mec* and *ccr* gene complex-specific primers (Table 1), we showed by PCR amplification that this isolate contained a class A *mec* gene complex and a type 4 *ccr* gene complex. The DNA fragment encompassing the entire SCC*mec* element of this strain was amplified by long-range PCR, along with nucleotide sequence determination by primer extension. Complete sequencing of the SCC*mec* element revealed the presence of a nucleotide fragment insert of 32,168 bp in an identical chromosomal integration site (*attB*_{SCC}) located at the 3' end of the *orfX* gene but harboring a unique combination of class A *mec* and type 4 *ccr* gene complexes (Fig. 1A).

The complete element, similar to other SCC*mec* types, could be divided into five distinct regions, including the *mec* and the *ccr* gene complexes and their surrounding DNA regions, J1, J2, and J3 (Fig. 1A). The 7,545-bp *mec* complex fragment was shown to possess a typical class A *mec* complex arrangement, including the upstream insertion sequence *IS431* (harboring complementary 17-bp right and left invert repeat sequences [IR-R and IR-L, respectively] at each end), followed by a region that contains four ORFs (Table 2), *mecA* (2,007 bp, resulting in 668 amino acids [aa]) and its complete regulatory genes *mecR1* (1,758 bp; 585 aa) and *mecI* (372 bp; 123 aa), i.e., *IS431-mecA-mecR1-mecI* (Fig. 1A). The entire *mec* gene complex is 100% identical to the prototypic class A *mec* gene complex described for the typical SCC*mec* type II strain of *S. aureus* N315, as well as *S. epidermidis* RP62A, but different from the other classes of *mec* gene complexes (data not shown).

The *ccr* genes contain a total of 2,987 nucleotides which encodes *ccrA* (1,362 bp; 453 aa) and *ccrB* (1,629 bp; 542 aa), with 4 nucleotides overlaying each other between the end of *ccrB* and the beginning of *ccrA* (Table 2). Our *ccrA* showed 100% nucleotide sequence identity with *ccrA4* in the SCC composite island (SCC-CI) of *S. epidermidis* ATCC 12228 while sharing only 89.6% homology with *ccrA4* in the type VI SCC*mec* of HDE288.

Our *ccrB*, on the other hand, was 99.9% homologous to that in the SCC-CI and contained a single nucleotide change at position 926 (A→C), resulting in a glutamic acid-to-alanine substitution at amino acid position 309 (E309A). The gene was 94.5% homologous to the *ccrB4* of the type VI SCC*mec* of HDE288. Phylogenetic and bootstrap analyses of the representative *ccrA*, *ccrB*, and *ccrC* gene complexes confirmed that it belonged to type 4 *ccr* gene complexes (data not shown).

To our knowledge, the combination of a class A *mec* gene complex and a type 4 *ccr* gene complex has never been previously described. A new SCC*mec* type element (SCC*mec* C10682) based on identification of this combination of *mec* and *ccr* gene complexes in our strain is, therefore, purported.

SCC*mec*C10682 may be derived from homologous recombination between previously described SCC_{RP62A} and SCC-CI cassettes of *S. epidermidis* RP62A and ATCC 12228 strains, respectively. Given the great extent of homology between the

present SCC*mec*C10682 ORFs (sequences) and the previously described SCC cassette of *S. epidermidis* RP62A (SCC_{RP62A}) and SCC-CI cassette of *S. epidermidis* ATCC 12228, SCC*mec* recombination in the present SCC*mec*C10682 sequence was examined. The complete SCC*mec*C10682 sequence was first subjected to scanning for recombination with the NCBI Genotyping Recombinant Sequence Analysis Program, using all reported types of SCC*mec* and SCC sequences as the references, and further confirmed by bootscanning and bootstrap testing. The NCBI program detected a potential recombination event leading to the construction of the SCC*mec*C10682 element. Such recombination was found to occur between SCC_{RP62A} and the SCC-CI, with the J3, the *mec* gene complex, and the majority of the J2 region derived from the former and the other sequences (*ccr* gene complex and J1 region) derived from the latter (Fig. 1B). To estimate the location of the apparent crossover site, bootscanning (19) was performed using the complete sequences of the SCC_{RP62A} and the SCC-CI, and the representative type V SCC*mec* of strain WIS was used as an outgroup sequence. The most likely breakpoints were located in three sections, i.e., at nucleotide (nt) position 130 to 22375 (22,246 bp) of SCC*mec*C10682 spanning the 5' upstream region beginning from the right direct repeat (DR_{SCC}-R) of the right extremity of SCC*mec*C10682 to the part of ORF CA026 located near the end of J2 region, which was shown to be 100% identical to that of the RP62A strain (nt 564 to 22809 of SCC_{RP62A}) but only 43.8% identical to that of ATCC 12228 (nt 1 to 24570 of the SCC-CI); nt 22376 to 23500 (1,125 bp), representing the last four ORFs located at the end of the J2 region (before the *ccr* gene complex) with 91.3 to 96.3% identity among these three strains (nt 22810 to 23933 of SCC_{RP62A}; nt 24571 to 25694 of the SCC-CI); and nt 23501 to 32305 (8,805 bp) from the *ccr* gene complex to the left extremity of SCC*mec* C10682 (5 bp before the left direct repeat [DR_{SCC}-L]), which was 99.9% identical to that of ATCC 12228 (only 6-bp difference; nt 25695 to 34489 of the SCC-CI) but had only 57.8% homology with that of strain RP62A (nt 23524 to 28612 of SCC_{RP62A}) (Table 2). This recombination event was further confirmed by bootstrap testing by constructing separate phylogenetic trees of the resulting regions with bootstrap support of 100%, 75%, and 100% for the three sections mentioned above, respectively (data not shown).

The boundaries of SCC*mec*C10682. SCC*mec*C10682 was integrated at exactly the same nucleotide position at the 3' end of the *orfX* gene as the type I SCC*mec* of strain NCTC10442, sharing identical characteristic nucleotide sequences at both left and right extremities of the chromosome-SCC*mec* junction regions, as well as identical nucleotide sequences of integrated site *attB*_{SCC}, but differing from SCC_{RP62A} and the SCC-CI of ATCC 12228 and other reported SCC*mec* types (Fig. 2). It contains the same 12-bp (5'-TAT CAT AAG TAA-3') flanking direct repeat (DR_{SCC}-L and DR_{SCC}-R) at both junctions as strain NCTC10442 but lacks any inverted repeats (IR_{SCC}-L and IR_{SCC}-R) in SCC*mec*C10682 (Fig. 2). Instead, it harbors a pair of 15-bp dyad repeats, referred to as DyadR-L (5'-ATC TTG AAA CGA AGT-3') and DyadR-R (5'-TGAAG-CAAAGTTCTA-3'), at its junctions, which are 91 bp away from the DR_{SCC}-R and 255 bp from the DR_{SCC}-L, respectively (Fig. 2 and Table 2).

TABLE 2. ORFs in and around SCCmecC10682

Genetic region	ORF or key structural component ^a	Position (bp)	Size (bp)	Protein size (aa) ^b	% Identity	Homolog(s) (strain[s])	Information ^c
Chromosome	<i>orfX</i>	1–151	151	NA	100	SARCOL0025 (COL)	Conserved hypothetical protein (partial cds)
Repeat region	DR _{SCC} -R	137–148	12	NA			Direct repeat at right extremity of SCCmec
	DyadR-R	240–254	15	NA	100; 100	(COL); (RP62A)	Dyad repeat at right extremity of SCCmec
J3	CA001	329–1624	1,296	431	100	SERP2528 (RP62A)	Conserved hypothetical protein
	CA002	2039–2278	240	79	100	SERP2527 (RP62A)	Conserved hypothetical protein
<i>mec</i> gene complex	IR-R*	2250–2266	17	NA	100	(RP62A)	Right inverted complementary repeat of IS431 element
	<i>tnp</i> *	2310–2984	675	224	100	SERP2526 (RP62A)	Transposase for IS431 element
	IR-L*	3025–3041	17	NA	100	(RP62A)	Left inverted complementary repeat of IS431 element
	CA004	3242–3409	168	55	100	SERP2525 (RP62A)	Truncated probable HMG-CoA synthase
	CA005	3507–3737	231	76	100	SERP2524 (RP62A)	Conserved hypothetical protein
	CA006	4246–4989	744	247	100	SERP2523 (RP62A)	Glycerophosphoryldiester phosphodiesterase homologue
	CA007	5086–5514	429	142	100	SERP2522 (RP62A)	MaoC domain protein dehydratase
	<i>mecA</i>	5560–7566	2,007	668	100	SERP2521 (RP62A)	Penicillin binding protein 2'
	<i>mecR1</i>	7666–9423	1,758	585	100	SERP2520 (RP62A)	Methicillin resistance protein
	<i>mecI</i>	9423–9794	372	123	100	SERP2519 (RP62A)	Methicillin resistance regulatory protein
J2	CA011	9895–10107	213	70	100	SERP2518 (RP62A)	Conserved hypothetical protein
	<i>xylR</i>	10302–11411	1,110	369	100	SERP2517 (RP62A)	Xylose repressor homologue
	CA013	11525–11785	261	86	100	SERP2516 (RP62A)	Conserved hypothetical protein
	CA014	11921–12781	861	286	99	SERP2516 (RP62A)	Conserved hypothetical protein
	CA015	12892–13956	1,065	354	100	SERP2515 (RP62A)	Rhodanese-like domain protein
	CA016	14092–14352	261	86	100	SERP2514 (RP62A)	Conserved hypothetical protein
	CA017	14352–14996	645	214	100	SERP2513 (RP62A)	Conserved hypothetical protein
	CA018**	15335–15946	612	203	100	SERP2512 (RP62A)	Methyltransferase type 12
	CA019**	16356–16556	201	66	100	SERP2511 (RP62A)	Peptide L
	<i>ermA</i> **	16531–17262	732	243	100	SERP2510 (RP62A)	rRNA adenine N-6-methyltransferase
	<i>aad(9)</i> **	17388–18170	783	260	100	SERP2509 (RP62A)	Streptomycin 3'-adenyltransferase
	<i>tnpC</i> **	18321–18698	378	125	100	SERP2508 (RP62A)	Transposase C
	<i>tnpB</i> **	18705–20597	1893	630	100	SERP2507 (RP62A)	Transposase B
	<i>tnpA</i> **	20594–21679	1,086	361	100	SERP2506 (RP62A)	Transposase A
	CA025	21798–22115	318	105	100	SERP2505 (RP62A)	Truncated <i>radC</i> DNA repair protein
	CA026	22136–22642	507	168	96; 87	SERP2504 (RP62A); SE0052 (ATCC 12228)	Hypothetical protein
	CA027	22658–22969	312	103	96; 95	SERP2503 (RP62A); SE0053 (ATCC 12228)	Hypothetical protein
	CA028	22971–23063	93	30	97; 84	SERP2502 (RP62A); SE0054 (ATCC 12228)	Conserved hypothetical protein
	CA029	23056–23406	351	116	90; 98	SERP2501 (RP62A); SE0055 (ATCC 12228)	Conserved hypothetical protein
<i>ccr</i> gene complex	<i>ccrB4</i>	23907–25535	1,629	542	99	SE0056 (ATCC 12228)	Cassette chromosome recombinase B4
	<i>ccrA4</i>	25532–26893	1,362	453	100	SE0057 (ATCC 12228)	Cassette chromosome recombinase A4
J1	CA032	27080–27661	582	193	100	SE0058 (ATCC 12228)	Putative membrane protein
	<i>ccrA</i>	27937–28056	120	39	100	SE0059 (ATCC 12228)	Cassette chromosome recombinase A
	CA034	28168–28527	360	119	100	SE0060 (ATCC 12228)	Hypothetical protein
	CA035	29163–29261	99	32	100	SE0061 (ATCC 12228)	Hypothetical protein
	<i>copA</i>	29381–31315	1,935	644	99	SE0062 (ATCC 12228)	Copper-transporting ATPase CopA
Repeat region	DyadR-L	32040–32054	15	NA			Dyad repeat at left extremity of SCCmec
	DR _{SCC} -L	32310–32321	12	NA	100; 93	(HDE288); (COL)	Direct repeat at left extremity of SCCmec
Chromosome	CA037	32624–32716	93	30	100	SACOL0057 (COL)	Conserved hypothetical protein
	CA038	32863–33371	509	NA	100	SACOL0058 (COL)	Conserved hypothetical protein (partial cds)

^a *, IS431; **, Tn554.^b NA, not applicable.^c cds, coding region; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A.

FIG. 2. The boundaries of SCCmecC10682. SCCmecC10682 was integrated at exactly the same nucleotide position at the 3' end of *orfX* gene (with identical characteristic nucleotide sequences at both left and right extremities of the chromosome-SCCmec junction regions as well as an identical nucleotide sequence of integrated site *attB_{scc}*) as the prototypic type I SCCmec of strain NCTC10442. It differed, though, from SCC_{RP62A} and the SCC-CI of ATCC 12228 and other reported SCCmec types. It contained a pair of hitherto unreported dyad repeats (DyadR-R and DyadR-L) but lacked discernible inverted repeats. Chromosomal sequences are boxed in gray, DR_{scc} sequences are indicated with bold type and a dotted box, while IR_{scc} sequences are indicated with a gray arrow. Consensus *attB_{scc}*, DR_{scc}-R, IR_{scc}-R, DR_{scc}-L, and IR_{scc}-L sequences are indicated above. Dyad repeats are shown below with nucleotide distance from the direct repeat indicated. MSSA, methicillin-susceptible *S. aureus*.

Overall structural comparison of SCCmecC10682 with other reported prototypic SCCmec types. In addition to having unique recombinatory sequences and boundaries, the complete SCCmec_{C10682} element was revealed by sequence analysis to have a G+C content (of the complete 32,168-bp sequence) of 31.7%, which was lower than that for *S. aureus* (32.8 to 32.9%). There were a total of 36 ORFs which were either previously described and/or were larger than 100 bp (Fig. 1A and Table 2).

The J1 region (extending from the left chromosome junction-*ccr* complex) was comprised of five ORFs, including those encoding the copper-transporting ATPase (*copA*), two hypothetical proteins (CA034 and CA035), a putative membrane protein (CA032), and a truncated *ccrA* gene (Fig. 1A and Table 2). With the exception of *copA*, which shows 99% homology, this region is 100% identical to a region between DR-SCC_{pbp4} and IS431, downstream of *ccrAB4* of the SCC-CI element in ATCC 12228. Of note, the truncated *ccrA* gene (120 bp; 39 aa) has been found only in SCC-CI from the genomic sequence of *S. epidermidis* ATCC 12228 (35). It contains a single 120-bp coding region, for a hypothetical 39-aa protein, with high degrees of sequence homology to the outer flanking regions of *ccrA3* (82.1%), *ccrA2* (74.4 to 82.1%), and *ccrA1* (76.9%) but lower degrees of homology to *ccrA4* (41.0 to 46.2%) and *ccrB* (7.7 to 19.4%) genes. The putative membrane protein CA032 also shows 91% homology to a protein adjacent

to the *ccr* gene complex in SCCmec type I and 92% homology to a protein adjacent to the *ccr* gene complex in *S. hominis* SCC₁₂₂₆₃.

Region J2 (extending between the *ccr* and *mec* gene complexes) was large and contained 19 ORFs, 7 of which (from CA018 to *tnpA*) belong to the mobile genetic element Tn554, a carrier of genes for erythromycin resistance (*ermA*) and streptomycin/spectinomycin resistance (*aad9*) (Fig. 1A and Table 2). ORFs CA026 and CA027 represent two highly conserved sequences found in various SCC elements, including SCCmec types I, II, IV, V, and VI, SCC_{RP62A}, SCCmecZH47, SCC₁₂₂₆₃, and SCC-CI, with homologies ranging from 85 to 96% for CA026 and 91 to 96% for CA027. The remainder of the genes in region J2 show high degrees of homology to genes in the SCCmec type II and III and SCC_{RP62A} elements, with sequence identities ranging from 99 to 100%.

The J3 region (extending from the right chromosome junction to the *mec* gene complex) is short, consisting of only two ORFs (CA001 and CA002), which code for conserved hypothetical proteins present in SCC_{RP62A} (100% identical) (Fig. 1A and Table 2) as well as in SCCmec types I, II, and IV (99% homology; data not shown).

The size (32 kb) of the SCCmecC10682 element in this HA-MRSA isolate compared with other HA-MRSA strains is similar to that of the type I SCCmec element of NCTC10442 (34 kb) but smaller than the type II (53 kb) and type III (67 kb;

Phenotypic, genotypic, and molecular characterization of epidemic strain C10682. The clinical MRSA isolate C10682 was isolated in 2003 from a percutaneous endoscopic gastrostomy site subcutaneous abscess in a 58-year-old male patient

from one of our local hospitals. Further screening of our collection of local MRSA clinical isolates by using the class A *mec* and type 4 *ccr* gene complex-specific primers (Table 1) revealed that two additional clinical isolates (C4631 from an abdominal wound in a 60-year-old male patient in 2004 and C5073 from a screening nasal swab in an 80-year-old female patient in 2005), as well as the CMRSA epidemic reference strain CMRSA9, carried the class A *mec* gene complex along with the type 4 *ccr* gene complex. Those isolates were found to be nontypeable by available SCC*mec* typing schemes. The assumption, therefore, was that these strains would also harbor the SCC*mec*C10682 element. In order to confirm this, PCR

amplification targeting various regions spanning the whole *SCCmecC10682* element was performed. As seen in Fig. 1A, 12 PCR targets at locations throughout the element, including one spanning the *SCC_{RP62A}*-*SCC-CI* recombination junction, were chosen (primers 19 and 20; Table 1). PCR results for all genes tested in each of the three strains were positive (data not shown), indicating that the same *SCCmecC10682* element was present in the tested local strains. To further confirm the same unique combination of class A *mec* and type 4 *ccr* gene complexes as in isolate C10682, partial *ccr* and *mec* gene complex sequencing was conducted, and it indicated that the three tested strains carried the same unique combination as the prototypic isolate C10682.

CMRSA9 is a typical HA-MRSA epidemic strain commonly found in Quebec and other regions in Canada (4). These isolates were subjected to further genotypic and phenotypic characterization for comparison with previously reported prototypic *SCCmec* type strains (types I to VI, including subtypes IVa to IVd), as well as the other major epidemic strains in Canada (strains CMRSA1 to CMRSA10) and the United States (strains USA100 to USA800) (Fig. 3). Molecular typing revealed unique genetic profiles for all four of these isolates (all of which harbored the unique *SCCmec* type with the class A *mec*/type 4 *ccr* gene complex), sharing the same CMRSA9 pulsotype, the same MLST type (ST8), *spa* type t008, and *agr* type I, and they lacked Pantone-Valentine leukocidin genes (Fig. 3A). This genomic background is similar to those of the prototypic *SCCmec* type I strains NCTC10442 and COL, but it is different from those of other reference strains (Fig. 3A). We also performed PCR assays to detect 35 of the most common *S. aureus* virulence genes in these isolates. These four isolates possessed identical virulence gene profiles (Fig. 3B). All isolates also exhibited identical antimicrobial susceptibility and resistance phenotypes, including resistance to β -lactams, erythromycin, ciprofloxacin, and mupirocin but susceptibility to gentamicin, rifampin (rifampicin), trimethoprim-sulfamethoxazole, and vancomycin, which is a typical HA-MRSA antibiotic susceptibility/resistance profile. These data suggest that all four of these isolates belong to the same strain, sharing a similar genomic background and carrying the novel *SCCmecC10682* type.

DISCUSSION

The essential and common traits of the *SCCmec* elements include (i) carriage of a *mec* gene complex; (ii) carriage of a *ccr* gene complex; (iii) integration at a specific nucleotide region (*attB_{scc}*), which is located at the 3' end of *orfX*; and (iv) demarcation by characteristic nucleotides, mostly associated with direct repeats and/or inverted repeats (5, 13, 15, 18, 20). Complete sequencing of *SCCmecC10682* reveals that it carries a complete class A *mec* gene complex and a type 4 *ccr* gene complex, integrates in an identical chromosomal integration site (*attB_{scc}*) at the 3' end of the *orfX* gene, and is flanked by nucleotide sequences characteristic of direct and dyad repeats at the chromosome-*SCCmec* junction regions. This new element not only shares all essential and common traits typical of other prototypic *SCCmec* types but also harbors a previously uncharacterized unique combination of the *mec* and *ccr* gene complexes. According to the current criteria for the classifica-

tion of *SCCmec* types (5, 13, 15, 18, 20), the *SCCmecC10682* element not only qualifies as a *SCCmec* element but also represents a novel type of *SCCmec*. After we consulted the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements for the most appropriate name (roman numeral) for this new type of *SCCmec* element, it was suggested to tentatively assign our *SCCmecC10682* (4A) the type *SCCmec* type VIII, since another new type (5C1; tentatively designed VII) identified in a CA-MRSA isolate from Sweden (2) is currently under review. However, there are only six currently recognized types of *SCCmec* to date, and they are types I (1B), II (2A), III (3A), IV (2B), V (5C2), and VI (4B) (13, 15, 20, 26).

Our novel *SCCmecC10682* was identified from Canadian epidemic MRSA strain CMRSA9. CMRSA9 is a typical HA-MRSA epidemic strain commonly found in Quebec and other regions in Canada (4). Previously, CMRSA9 was reported as being of *SCCmec* type II (4), presumably because the entire *SCCmec* element was not sequenced. Instead of sequencing, an older *SCCmec* multiplex PCR typing assay (25) was used to characterize this *SCCmec* element (4), in which two (loci C and D) of the four known loci (B, C, D, and G) for *SCCmec* type II were also found in our *SCCmecC10682*. Although there is no identical reference strain for CMRSA9, its genomic background is quite similar to those of the strains NCTC10442 (the first MRSA isolate from England in 1961) (17) and COL (an early MRSA strain isolated from England in the early 1960s) (6), with a similar pulsed-field gel electrophoresis pattern and the same *spa* type, t008 (Fig. 3A). The CMRSA9 strain has an MLST designation of ST8, whereas NCTC10442 and COL have designation ST250 (Fig. 3A). However, they belong to the same clonal complex, CC8, and the only difference between them (ST8 and ST250) is the sequence at one gene, *yqi* (acetyl coenzyme A acetyltransferase; MLST profile number 3 for CMRSA9 in contrast to 16 for NCTC10442 and COL), while the other six genes are identical (Fig. 3A). They also have similar virulence gene profiles (Fig. 3B). Moreover, they share identical characteristic nucleotide sequences at both left and right extremities of the chromosome-*SCCmec* junction regions, as well as identical nucleotide sequences for integrated site *attB_{scc}*, which differ from those for *SCC_{RP62A}* and *SCC-CI* of *S. epidermidis* strains RP62A and ATCC 12228, respectively, as well as for those of the other reported *SCCmec* types (Fig. 2). Both NCTC10442 (*SCCmec* type I prototypic strain) and COL carry type I *SCCmec*; however, CMRSA9 carries a totally different type of *SCCmec* element. More interestingly, our sequence recombination analyses suggested that this unique *SCCmec* type might be generated from homologous recombination between the previously described *SCC_{RP62A}* and *SCC-CI* of *S. epidermidis* strains RP62A and ATCC 12228, respectively. However, it is not clear how, where, and from which ancient strain(s) this CMRSA9 strain evolved and how it recently became one of the epidemic strains in Canada.

S. epidermidis RP62A (ATCC 35984) is a methicillin-resistant biofilm-producing isolate (12). Its complete genome (~2.6 Mb) has been sequenced and carries a type II *SCCmec* which is 98% identical at the nucleotide level and identical in gene organization (with the exception of the region from *pUB110* flanked by *IS431-mec*) to that of the *S. aureus* type II *SCCmec* of prototypic strain N315 (8). Another *S. epidermidis* strain,

ATCC 12228, is a non-biofilm-forming, non-infection-associated strain (1). Although it is a methicillin-susceptible strain, analysis of its genomic sequence (~2.5 Mb) reveals that it harbors an SCC element named SCC_{pbp4} which lacks the methicillin resistance determinant gene (*mecA*) but contains two pairs of *ccrA* and *ccrB* genes along with multiple insertion sequence elements, a restriction-modification system (*hsdS* and *hsdM*), and genes encoding penicillin binding protein 4 (*pbp4*) and conferring resistance to mercury and cadmium (Fig. 1B) (8, 22, 35). The two strains, although different in pathogenicities and structures of SCC islands, share 85% identity in predicted protein-coding sequences (31). Our sequence recombination analyses revealed that the sequences of the 5' upstream region (carrying the *mec* gene complex) and the 3' downstream region (carrying the *ccr* gene complex) of the new SCC_{mec}C10682 cassette were identical to those of the SCC_{mec} of RP62A (SCC_{RP62A}) and the SCC element of ATCC 12228 (SCC-CI), whereas the middle segment shared 91.3 to 96.3% identity among these strains. These findings suggest that this unique SCC_{mec} type might be derived from homologous recombination between *S. epidermidis* strains RP62A and ATCC 12228. Therefore, we hypothesize that the CMRSA9 strain may be derived from an early ancestral MRSA strain(s) such as NCTC10442 or COL and replaced its original type I SCC_{mec} with the new SCC_{mec}C10682 type through a recombination event with similar *S. epidermidis* or other staphylococcal strains (other coagulase-negative staphylococci, MRSA, or methicillin-susceptible *S. aureus*) that carry the mobile cassette SCC_{RP62A} or SCC-CI, as in the RP62A and ATCC 12228 strains, while the ancestral MRSA strain was evolving under the selective pressures.

Although further detailed studies are needed to support this hypothesis, the present study readily provides compelling evidence supporting the ability of SCC elements to transfer horizontally or undergo recombination to generate new SCC_{mec} types.

ACKNOWLEDGMENTS

We thank T. Ito and K. Hiramatsu for the kind gift of the SCC_{mec} control strains NCTC10442, N315, 85/2082, MR108, JCSC4469, and WIS; R. Daum for strains CA05 and 8/6-3P; and H. de Lencastre for the strain HDE288.

This work was partially supported by the Centre for Antimicrobial Resistance (CAR), Calgary Health Region/Calgary Laboratory Services/University of Calgary.

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