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A Clonal Complex 12 Methicillin-Resistant *Staphylococcus aureus* Strain, West Australian MRSA-59, Harbors a Novel Pseudo-SCC*mec* Element

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A West Australian methicillin-resistant *Staphylococcus aureus* strain (WA MRSA-59) was characterized by microarray and sequencing. Its pseudo-staphylococcal cassette chromosome *mec* (SCC*mec*) element comprised *dcs*, Q9XB68-*dcs*, *mvaS*-SCC, Q5HJW6, *dru*, *ugpQ*, *ydeM*, *mecA*-*mecR*-*mecI*, txbi *mecI*, *tnp* IS*431*, *copA2*-*mco* (copper resistance), *ydhK*, *arsC*-*arsB*-*arsR* (arsenic resistance), open reading frame PT43, and *per-2*. Recombinase genes, *xylR* (*mecR2*), and PSM-*mec* (phenol-soluble modulin) were absent. We suggest that *mec* complex A should be split into two subtypes. One harbors PSM-*mec* and *xylR* (*mecR2*). It is found in SCC*mec* types II, III, and VIII. The second subtype, described herein, is present in WA MRSA-59 and some coagulase-negative staphylococci.

Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates harbor *mecA* or *mecC* genes, which encode modified penicillin-binding proteins that confer resistance toward most beta-lactam compounds (1–3). The *mec* genes are located on staphylococcal cassette chromosome *mec* (SCC*mec*) elements that facilitate horizontal gene transfer in staphylococci. They contain a *mec* complex which, in addition to *mecA* or *mecC*, consists of various combinations of regulatory genes and insertion sequences (4). Furthermore, they harbor a recombinase gene (*ccr*) complex and the so-called J-regions ("joining" or "junkyard" regions). J-regions include various genes, including additional resistance or virulence determinants. Based on the combination of different *mec* and *ccr* complexes, 11 types of SCC*mec* elements have been described (4) (http://www.sccmec.org/Pages/SCC_TypesEN.html), and subtypes are differentiated based on variations within the J-regions. Truncated SCC*mec* elements lacking *ccr* recombinase genes are known as pseudo-SCC*mec* elements (5). In addition to SCC*mec* elements, a variety of different SCC elements have been described that lack *mecA* but harbor other markers, such as *fusC*, which encodes fusidic acid resistance (5, 6).

In Western Australia (WA), a state-wide MRSA management policy has prevented the transmission of health care-associated MRSA in acute care hospitals (7). However, community-associated MRSA is endemic in the region (8). To distinguish between health care- and community-associated MRSA, all isolates from WA are referred to the Australian Collaborating Center for *Enterococcus* and *Staphylococcus* Species in Perth.

One of the isolates, 07-16590, designated Western Australian MRSA-59 (WA MRSA-59), was found to carry a novel SCC*mec* element. It was isolated in 2007 from sputum of a 56-year-old female patient treated for a chronic pulmonary *Mycobacterium intracellulare* infection. Isolate 07-16590 was assigned to multilocus sequence type 12 and Ridom *spa* type t160. An additional isolate was recovered during a follow-up examination of the index patient in 2008. Two other WA MRSA-59 isolates were recovered in 2008 and 2013, but there were no known epidemiological links.

DNA microarray analyses were performed on these four isolates as previously described (9). In short, *S. aureus* was grown on blood agar and enzymatically lysed. Purified DNA was subjected to a multiplex linear primer elongation that was used for amplification and labeling of virulence- and resistance-associated genes, various typing markers, and species-specific controls (9). Resulting biotin-labeled amplicons were hybridized to arrays with spotted specific oligonucleotides. Hybridizations were visualized by use of a streptavidin-horseradish peroxidase conjugate that triggered a local dye precipitation. Array images were automatically analyzed with regard to the absence or presence of the target genes as well as to strain/clonal complex (CC) assignment.

Like all CC12 isolates, WA MRSA-59 belongs to *agr* group II and capsule type 8. Isolates harbor the enterotoxin homologue open reading frame (ORF) CM14 and *cna* gene (for collagen-binding adhesin). The *sak* gene (for staphylokinase), *scn* (for staphylococcal complement inhibitor), and the enterotoxin genes *sea*-N315 (*sep*) were detected; according to the sequence analysis, they were localized on a hemolysin beta-integrating phage. The enterotoxin B gene (*seb*) was variably present. The isolates lacked *tst-1* (toxic shock syndrome toxin gene) and the genes encoding exfoliative toxins and the Panton-Valentine leukocidin. The carriage of *blaZ* (beta-lactamase) and of *erm*(C) (erythromycin/clindamycin resistance) genes was variable.

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TABLE 1 Primers used for amplification and sequencing

Primer designation	Sequence (5′–3′)	Aim(s)[a]
mecI_02	TCA ATT CAC TTG TCT TAA ACT TTG TAG A	A, S
copA2_02	GAT GAT GTG CAT GGC CAC T	A, S
SaCC12-2	TAT CAT GTC AGT GTT CGC	S
SaCC12-3	TCA TTA CCA ACA CAA GTC	S
SaCC12-5	GCT TTA ATT ACT TTA GCC	S
SaCC12-6	ATT CTA CGC CAC AAT AGC	S

[a] A, amplification; S, sequencing.

Sequencing of isolate 07-16590 was undertaken by Geneservice Source BioScience PLC (Nottingham, United Kingdom) and by GATC Biotech (Constance, Germany) using the Illumina genome analyzer system. Reads were assembled into contigs by using the Velvet *de novo* genome assembler. Contigs were analyzed using various in-house scripts. Illumina sequencing left a gap of approximately 1,000 bp that bisected the SCC sequence, which was most likely caused by difficulties for the assembler in coping with multicopy sequences, such as IS*431*. The gap was closed by conventional sequencing using the primers listed in Table 1. The relevant region was amplified with primers mecI_02 and copA2_02, resulting in an approximately 1,500-bp amplicon. After initial denaturation (60 s at 96°C), 35 cycles of denaturation (15 s at 96°C), annealing (60 s at 50°C), and elongation (90 s at 72°C) were used. The PCR was finished with another elongation step (60 s at 72°C). DNA sequencing was carried out using the primers given in Table 1, the BigDye Terminator v1.1 cycle sequencing kit, and an ABI Prism 3130 genetic analyzer (both from Applied Biosystems, Darmstadt, Germany).

The SCC*mec* element of WA MRSA-59 isolate 07-16590 (Fig. 1; Table 2) consisted of *dcs* (downstream constant segment, locus 1), Q9XB68-*dcs* (truncated putative protein), *tnp* IS*431* (transposase for IS*431*), *mvaS*-SCC (truncated 3-hydroxy-3-methylglutaryl coenzyme [CoA] synthase), Q5HJW6 (putative protein), *dru* (direct repeat units, type dt8b), *ugpQ* (glycerophosphoryl diester phosphodiesterase), *ydeM* (putative dehydratase), txbi *mecA* (bidirectional rho-independent terminator of *mecA*), *mecA* (modified penicillin-binding protein 2a [PBP2a]), *mecR1* (signal transducer protein MecR1), *mecI* (methicillin resistance regulatory protein), txbi *mecI* (bidirectional rho-independent terminator of *mecI*, with an insertion of an inverted repeat for IS*431*, IR-IS*431*, at its downstream end), *tnp* IS*431*, *copA*2 (copper-exporting ATPase), *mco* (multicopper oxidase), *ydhK* (putative lipoprotein; GenBank accession number A8YZ03), *arsC-arsB-arsR* (arsenic resistance gene cluster), ORF PT43 (putative protein associated with arsenic resistance operon from SCC*mec* IX of *S. aureus* JCSC6943; GenBank accession number AB505628.1), and *per*-2 (plasmidic permease).

SCC*mec*-associated recombinase genes *ccrA-ccrB* and *ccrC*, *xylR* (*mecR2*) (homologue of xylose repressor), and the gene encoding PSM-*mec* (SCC-associated phenol-soluble modulin) were not detected.

Previously characterized SCC*mec* elements with *mec* gene complex A (SCC*mec* types II, III, and VIII) harbor genes *mecA*, *mecI*, *mecR1*, PSM-*mec*, and *xylR* (*mecR2*). Based on the observations described herein, we suggest that a second subtype of the *mec* complex A should be recognized. It is characterized by absence of PSM-*mec* and *xylR* (*mecR2*) as well as by insertion of IR-IS*431* into the downstream end of txbi *mecI*. A search of the WGS section of GenBank uncovered that the *mec* complex identified in isolate 07-16590 is also present in *Staphylococcus hominis* M0480 (KK013382.1/JCGQ), *S. hominis* ZBW5 (AKGC), and *Staphylococcus epidermidis* VCU120 (AHLC), suggesting that horizontal gene transfer between different species might have occurred.

A part of the SCC*mec* element of *S. epidermidis* VCU120 is very similar to that of WA MRSA-59, as it harbors the same *mec* gene complex and also copper and arsenic resistance operons in a comparable configuration. However, VCU120 also harbors a *ccrB4* recombinase gene and the ACME 1 element (*opp3B-opp3C*, *arcA-arcB-arcC-arcD*). Since the SCC-associated genes in VCU120 are spread across several contigs, their relative locations have not been elucidated. The other two sequences differ with respect to the presence of *czrC* (*copA*; zinc/copper resistance), of multiple *ccr* genes, and (in M0480 only) of *fusC* accompanied by *tirS*.

The pseudo-SCC*mec* element of 07-16590 and other SCC*mec* elements harbor arsenic and copper resistance factors. The evolutionary benefit of heavy metal resistance operons in staphylococci warrants further investigation. These operons are very common, not only in their core genomes but also in SCC elements, and many SCC*mec* elements comprise multiple, and redundant, heavy metal resistance genes. Selective pressures favoring acquisition and maintenance of heavy metal resistance genes may include environmental exposure, past medical use of heavy metals, the use of heavy metals as growth promoters in veterinary medicine, or a

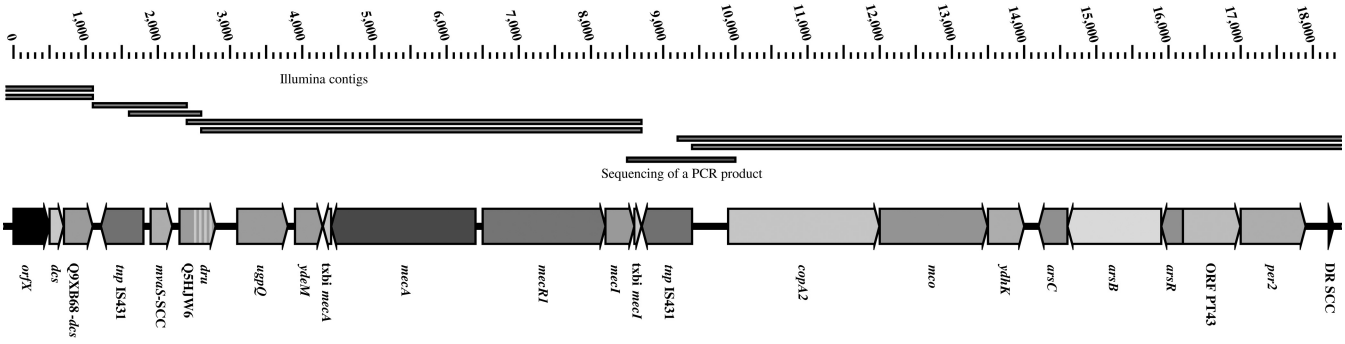

FIG 1 Schematic representation of the pseudo-SCC*mec* element of WA MRSA-59 isolate 07-16590 (for clarity, direct and inverted repeats have been omitted from the drawing, except for the DR-SCC at the downstream end of the element).

TABLE 2 Genes identified in the pseudo-SCC*mec* element of WA MRSA-59 isolate 07-16590

Gene or genetic element	Description and/or gene product	Position within pseudo-SCC*mec* element (starting with *orfX*)	Orientation	Length (bp)	Best match
orfX	23S rRNA methyltransferase/ORF X	1–480	+	480	BA000018.3 (33692:34171; 7 mismatches)
DR-SCC	Direct repeat of SCC	462–480	+	19	
dcs	Downstream constant segment, locus 1	481–762	+	282	BA000018.3 (34172:34453)
DR-SCC	Direct repeat of SCC	564–582	+	19	
Q9XB68-*dcs* trnc.	Putative protein	763–1093	+	331	BA000018.3 (34454:35749 truncated)
IR-IS*431*	Inverted repeat for IS*431*	1094–1109	+	16	
tnp IS*431*	Transposase for IS*431*	1153–1827	−	675	BA000018.3 (36435:37109)
IR-IS*431*	Inverted repeat for IS*431*	1868–1883	−	16	
mvaS trnc.	Truncated 3-hydroxy-3-methylglutaryl CoA synthase	1900–2252	+	353	BA000018.3 (42528:42880)
Q5HJW6	Putative protein (Q9XB76)	2350–2580	+	231	BA000018.3 (42978:43208)
dru	Direct repeat units	2509–2828	+	320	dt8b (5a-2d-2d-4a-0-2g-3b-4e)
ugpQ	Glycerophosphoryl diester phosphodiesterase	3049–3792	+	744	BA000018.3 (43717:44460)
ydeM	Putative dehydratase	3889–4317	+	429	BA000018.3 (44557:44985)
txbi *mecA*	Bidirectional rho-independent terminator of *mecA*	4309–4372	−	65	BA000018.3 (44976:45040)
mecA	Modified PBP2a, conferring methicillin resistance	4363–6369	−	2,007	BA000033.2 (39602:41608), BA000018.3 (45031:47037; 1 mismatch in position 1933)
mecR1	Signal transducer protein MecR1	6469–8226	+	1,758	BA000018.3 (47137:48894)
mecI	Methicillin resistance regulatory protein	8226–8597	+	372	BA000018.3 (48894:49265)
txbi *mecI*	Bidirectional rho-independent terminator of *mecI*	8614–8680	+	66	AHLC01000035.1 (13384:13451)
IR-IS*431*	Inverted repeat for IS*431*	8661–8676 (overlap)	+	16	
tnp IS*431*	Transposase for IS*431*	8720–9394	−	675	BA000018.3 (36435:37109; 5 mismatches)
IR-IS*431*	Inverted repeat for IS*431*	9435–9450	−	16	
copA2	Copper-exporting ATPase	9961–12024	+	2,064	AECP01000057.1 (24361:26424)
mco	Multicopper oxidase	12039–13472	+	1,434	AHKX01000087.1 (24275:25708)
ydhK	Putative lipoprotein (A8YZ03)	13499–13974	+	476	AHKX01000087.1 (25735:26210)
arsC	Arsenate reductase	14180–14575	−	396	AHLC01000035.1 (7490:7885)
arsB	Arsenic pump membrane protein	14592–15881	−	1,290	AHLC01000035.1 (6184:7473)
arsR	Repressor of arsenic resistance gene cluster	15881–16198	−	318	AHLC01000035.1 (5867:6184)
ORF PT43	Putative protein	16221–17037	+	817	AB505628.1 [42213–43024]
per-2	Plasmidic permease	17043–17927	+	885	AHLC01000035.1 (4138:5022)
DR-SCC	Direct repeat of SCC	18250–18268	+	19	BA000018.3 (34255:34273; 3 mismatches)

coselection when localized on mobile genetic elements together with genes encoding antibiotic resistance.

In conclusion, we have described a novel pseudo-SCC*mec* element in *S. aureus* and we suggest that *mec* complex A should be split into two subtypes, based on the mutually exclusive presence of (i) PSM-*mec* and *xylR* (*mecR2*) or (ii) the insertion of IR-IS*431* at the downstream end of txbi *mecI*.

Nucleotide sequence accession number. The sequence of the SCC*mec* element and the adjacent downstream region was submitted to GenBank (accession number KT316803).

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