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Mosaic Staphylococcal Cassette Chromosome *mec* Containing Two Recombinase Loci and a New *mec* Complex, B2▽

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A novel staphylococcal cassette chromosome (SCC) *mec* from a clinical methicillin-resistant *Staphylococcus aureus* isolate (ST100/CC5) had a mosaic structure, composed of SCC DNA from several different backgrounds. It harbored two complete *ccr* loci and a new variant of *mec* complex B, with Δ*mecR1* interrupted by the aminoglycoside resistance transposon Tn*4001*.

Methicillin resistance in *Staphylococcus aureus* (MRSA) is facilitated by the acquisition of the staphylococcal cassette chromosome *mec* (SCC*mec*), which integrates site specifically into the staphylococcal genome and carries *mecA*, encoding the alternative penicillin-binding protein PBP2a, a β-lactam-insensitive transpeptidase (6, 11, 13, 22, 26). The precise excision and site- as well as orientation-specific integration of this element depend on the action of cassette chromosome recombinase genes (*ccr*'s) located within the element (13).

Five main types of SCC*mec* have been described so far, each differing in size and composition and characterized according to its type of *ccr* locus and *mec* complex (3, 7, 25). *mec* complexes differ in the extents of insertion sequence (IS)-mediated deletions in the *mecA* regulatory genes *mecR1* and *mecI* and the presence and location of insertion sequence IS*431*, IS*1182*, or IS*1272* (23). Apart from the *ccr* and *mec* complexes, and some common mobile resistance elements, SCC*mec* subtypes harbor variable J (junkyard) regions containing truncated and nonessential genes and genes of unknown functions (8). In addition to the major types, a number of new SCC elements, including non-*mecA*-carrying cassettes, have recently been discovered (4, 5, 9, 14, 15, 17, 19).

An epidemiological study of methicillin-resistant staphylococci from Zurich in 2003 identified several strains which contained multiple *ccr* loci (21). Here, we describe the SCC*mec* of one of these isolates, $MRSA_{ZH47}$.

$MRSA_{ZH47}$ is of multilocus sequence type 100 and belongs to clonal complex 5, a genotype previously identified in Argentina (1, 24). In addition to its β-lactam resistance, it was resistant to aminoglycosides and carried a *blaZ*-encoded penicillinase. Its SCC*mec* type could not be determined by standard multiplex PCR (20), and additional *ccr* typing indicated that it contained both *ccr2* and *ccrC* loci (21).

Southern hybridization of SmaI-digested chromosomal DNA, separated by pulsed-field gel electrophoresis, showed that *mecA*- and *ccr2*-hybridizing sequences were colocated on a separate SmaI fragment from the *ccrC*-hybridizing sequence (data not shown).

Transient overexpression of *ccrAB2*, facilitating the precise excision of all major SCC*mec* types (10, 12, 13, 18), was used to cure $MRSA_{ZH47}$. Southern hybridization showed that the resulting oxacillin-susceptible clone $MRSA_{ZH47}$c had lost *mecA* and both *ccr* loci, indicating that all three were present on a single excisable SCC element containing an internal SmaI restriction site (data not shown). The susceptibility profile of $MRSA_{ZH47}$c showed that aminoglycoside resistance had also been lost.

A cosmid library of $MRSA_{ZH47}$ DNA, consisting of over 600 clones with estimated inserts of about 45 kb, was constructed using a SuperCos1 cosmid vector kit (Stratagene, La Jolla, CA). Screening of the library by colony blot analysis using *ccr2*- or *ccrC*-specific probes identified 11 clones that hybridized to *ccr2*, 7 that hybridized to *ccrC*, and 2 that hybridized to both probes. Cosmids were end sequenced and the sequences compared to the genome sequence of *S. aureus* Mu50, revealing that the two cosmids hybridizing with both probes each contained one end of the SCC*mec* element and together completely covered it (data not shown).

Primers specific for known *orfX*, *ccrC2*, IS*431*, and *mecA* nucleotide sequences were used to synthesize long-range PCR products that were subcloned into either pUC19 or pBluescript SK(+). Inserts were end sequenced and the obtained sequences assembled. The double-stranded nucleotide sequence of the 33.7-kb element was completed by primer walking.

This SCC*mec* proved to be unique, containing elements and properties not previously described. GeneMark.hmm (16) and BLASTX (2) identified 33 open reading frames (ORFs), all of which were identical or highly similar in sequence to previously annotated staphylococcal genes (Table 1 and Fig. 1).

The *orfX* insertion site and the characteristic terminal inverted and direct repeats, generated upon insertion, were almost identical to those of other, previously described SCC*mec* elements (12). However, the left-end (proximal) direct repeat sequence contained a nucleotide transition of an adenine to guanine (Table 1), which has not been found elsewhere and increased the identity between the junctional direct repeats. This mutation did not impede the excision of the element, as demonstrated by the precise curing of $SCCmec_{ZH47}$ from the chromosome of $MRSA_{ZH47}$. It could, however, possibly influence the stability or transfer frequency of the element.

$SCCmec_{ZH47}$ contained a new *mec* complex that we have

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TABLE 1. ORFs encoded on SCC*mec*$_{ZH47}$

Coding sequence no.	Name[a]	Position (bp)	Identity (%)[b]	Homolog(s)[c]	Information[d]
	DR-L	754–771			Integration site sequence of SCC*mec*, A→G substitution at position 16 of left direct repeat
	IR-L	757–764			Integration site sequence of SCC*mec*; left inverted repeat
1	ZH02	1017–1322	98	CZ078 (85/2082)	Hypothetical protein, predicted restriction endonuclease domain (COG 3183)
2	ZH03	1887–2384	100	CZ077 (85/2082)	Conserved hypothetical protein (COG 3680)
3	ZH04	2468–3967	100	CZ076 (85/2082)	Hypothetical protein
4	ZH05	4193–5293	100	CZ075 (85/2082)	Hypothetical protein; DNA polymerase A family domain (Pfam 00476.12)
5	ZH06	5286–5657	94	CZ074 (85/2082)	Hypothetical protein (DUF 1092)
6	ZH07	5654–7273	100	3 half: CG008 (85/3907)	Hypothetical protein; POX_D5 domain associated with viral DNA replication (Pfam 03288.11)
			100	5 half: unnamed ORF (TSGH17)	
7	*ccrC*	7498–9174	94	*ccrC2* (TSGH17)	Cassette chromosome recombinase C
			89	*ccrC3* (85/2082)	
8	ZH09	9280–9618	98	SSP0034 (ATCC 15305)	Hypothetical protein
9	ZH10	9714–10025	90	SSP0032 (ATCC 15305)	Hypothetical protein, contains SmaI restriction site
10	ZH11	10041–10547	90	CZ068 (85/2082)	Conserved in gram-positive and -negative bacteria but of unknown function (COG 4333)
11	IS431	10696–11370	99	IS*431* (SCC*mec* types I–IV)	Insertion sequence IS*431*
12	ZH13	11628–11795	100	ORF CN041 (N315) and in SCC*mec* types I–V	Putative HMG-coenzyme A-synthase (cholesterol biosynthesis)
13	*ugpQ*	12712–13455	100	*ugpQ* (all SCC*mec* types)	Glycerophosphoryl diester phosphodiesterase
14	ZH15	13552–13980	100	SA0037 (N315) and in SCC*mec* types I–V	Hypothetical protein, MaoC-like domain (Pfam 01575.11)
15	*mecA*	14026–16032	100	WIS (SCC*mec* type V) and TSGH17 (SCC*mec* type V_T)	Penicillin-binding protein PBP2a
16	Δ*mecR1′*	16132–16958	100	Δ*mecR1* from *mec* complex B	First 826 bp of Δ*mecR1*, truncated signal transducer MecR1 from *mec* complex B
	DR_{Tn4001}	16951–16958			Repeated region generated by transposon integration
	IR_{Tn4001}	16959–17059			Repeated region generated by transposon integration
17	IS256L	17060–18232	100	Tn*4001*	IS of Tn*4001*
18	*aac*	18235–18681	100	Tn*4001*	Putative *N*-acyltransferase, GNAT family
19	*aac*(2′)–*aph*(6″)	18682–20121	100	Tn*4001*	Aminoglycoside-(2′)-acetyltransferase-aminoglycoside-(6″)-phosphotransferase
20	IS256R	20251–21423	100	Tn*4001*	IS of Tn*4001*
	IR_{Tn4001}	21424–21524			Repeated region generated by transposon integration
	DR_{Tn4001}	21525–21532			Repeated region generated by transposon integration
21	Δ*mecR1″*	21533–21692	100	Δ*mecR1* from *mec* complex B	Remainder of Δ*mecR1*, interrupted by Tn*4001* insertion
22	ZH22	21595–21924	100	Δ*hsdR* (MW2)	Truncated hypothetical protein, similar to type I restriction endonuclease
23	ZH23	21915–23438	100	IS*1272* transposase from *mec* complex B	aa 1–102: transposase and inactivated derivatives (COG 3666)
					aa 185–459: transposase DDE domain
24	ZH24	23574–24083	100	Various type IV SCC*mec*s	Hypothetical protein
25	ZH25	24095–24406	100	Various type IV SCC*mec*s	Hypothetical protein
26	ZH26	24493–24843	100	Various type IV SCC*mec*s	Hypothetical protein
27	*ccr2B*	25365–26993	100	*ccrB* SCC*mec* type IVd (JCSC4469)	Cassette chromosome recombinase B
28	*ccr2A*	27015–28364	100	*ccrA* SCC*mec* type IVe (AR43)	Cassette chromosome recombinase A
29	ZH29	28598–30391	100	R004 (MR108)	aa 1–382: superfamily II helicase and inactivated derivatives (COG 5519)
30	ZH30	30391–30687	100	Different type IV SCC*mec*s	Hypothetical protein
31	ZH31	30880–31926	100	Different type IV SCC*mec*s (ATCC 12228)	Hypothetical protein
32	ZH32	32381–33535	100	CR008 (MR108)	aa 1–122: *abi* alpha protein, predicted transcriptional regulator (COG 2865)
33	ZH33	33565–34365	100	Different type IV SCC*mec*s and SE0042 (ATCC 12228)	Hypothetical protein, abortive phage resistance protein
	IR-R	34423–34430			Integration site sequence of SCC*mec*; right inverted repeat
	DR-R	34432–34449			Integration site sequence of SCC*mec*; right direct repeat

[a] ORFs of known functions are named accordingly; putative and hypothetical ORFs have been assigned ZH numbers.
[b] Amino acid sequence identity.
[c] The name of the homologous gene is indicated, and the strain in which it was found is indicated in parentheses. If homologous ORFs were unnamed, the SCC*mec* type in which they were found is indicated.
[d] Information about the element, e.g., about the encoded protein. Positions and reference numbers of known protein domains are indicated. aa, amino acids; HMG, 3-hydroxy-3-methylglutaryl; COG, cluster of orthologous groups of proteins.

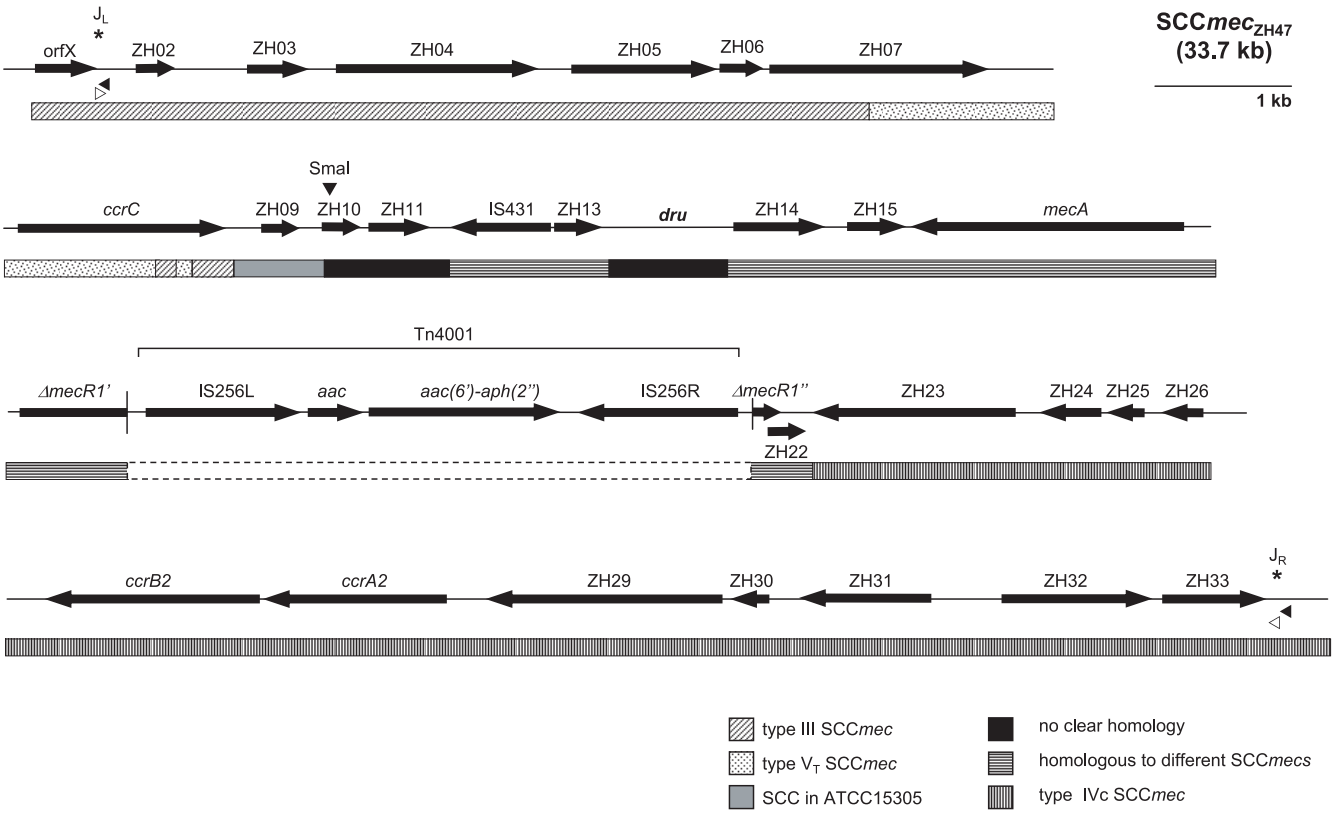

FIG. 1. Genetic map of the mosaic SCC*mec* element from MRSA$_{ZH47}$. Open reading frames are symbolized by arrows, and the bars below them indicate homologies to previously described SCC elements. Integration site sequences for SCC*mec* (*), direct repeats of chromosomal junctions (◀), and inverted repeats (▷) are indicated. J_L denotes the junction proximal and J_R the junction distal to the origin of replication.

named B2 because of its similarity to *mec* complex B. The new B2 complex differed in that the 987-bp Δ*mecR1* fragment was interrupted by insertion of the aminoglycoside resistance transposon Tn*4001* at base pair position 820 (Fig. 1), and while the *mecA* promoter region was identical to that of *mec* complex B, the *mecA* gene sequence was identical to that of SCC*mec* types V and V_T.

In addition to a *ccrAB2* locus at the usual position downstream of *mecA*, the element possessed a *ccrC* locus between *orfX* and the *dru* element (Fig. 1). The *ccrA2* sequence was identical to that of SCC*mec* type IVe, while the *ccrB2* sequence was identical to that of SCC*mec* type IVd. Comparison of the *ccrC* sequence to published variants revealed high levels of similarity to the *ccrC2* and *ccrC3* sequences of SCC*mec* types V_T and III, respectively (Table 1).

The element as a whole appeared mosaic in structure. The presence of both a *ccrAB2* locus and a variant *ccrC* locus and of regions with strong similarity to several different SCC elements, including the typical hospital-acquired MRSA type III SCC*mec*, the community-associated MRSA SCC*mec* types IV and V_T, and SCC*mec* from the non-*S. aureus* species *Staphylococcus saprophyticus*, suggests that SCC*mec*$_{ZH47}$ had been assembled via several recombination events (Fig. 1).

Most of the new SCC and SCC*mec* elements recently discovered, including the SCC*mec*$_{ZH47}$ described here, appear to have acquired regions from other SCC elements, suggesting that significant intra- and interspecies exchange and recombination of SCC DNA occurs.

Nucleotide sequence accession number. The nucleotide sequence newly determined in this study was deposited in the EMBL database under accession number AM292304.

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