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Staphylococcus aureus CC395 harbours a novel composite staphylococcal cassette chromosome *mec* element

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Background: CoNS species are likely reservoirs of the staphylococcal cassette chromosome *mec* (SCC*mec*) in *Staphylococcus aureus*. *S. aureus* CC395 is unique as it is capable of exchanging DNA with CoNS via bacteriophages, which are also known to mediate transfer of SCC*mec*.

Objectives: To analyse the structure and putative origin of the SCC*mec* element in *S. aureus* CC395.

Methods: The only MRSA CC395 strain described in the literature, JS395, was subjected to WGS, and its SCC*mec* element was compared with those found in CoNS species and other *S. aureus* strains.

Results: JS395 was found to carry an unusually large 88 kb composite SCC*mec* element. The 33 kb region downstream of *orfX* harboured a type V SCC*mec* element and a CRISPR locus, which was most similar to those found in the CoNS species *Staphylococcus capitis* and *Staphylococcus schleiferi*. A 55 kb SCC element was identified downstream of the type V SCC*mec* element and contained a mercury resistance region found in the composite SCC element of some *Staphylococcus epidermidis* and *S. aureus* strains, an integrated *S. aureus* plasmid containing genes for the detoxification of cadmium and arsenic, and a stretch of genes that was partially similar to the type IVg SCC*mec* element found in a bovine *S. aureus* strain.

Conclusions: The size and complexity of the SCC*mec* element support the idea that CC395 is highly prone to DNA uptake from CoNS. Thus CC395 may serve as an entry point for SCC*mec* and SCC structures into *S. aureus*.

Introduction

Methicillin resistance in *Staphylococcus aureus* is encoded by the *mecA* gene, which is harboured on so-called staphylococcal cassette chromosome *mec* (SCC*mec*) elements. The existing literature suggests that these SCC*mec* elements have their origin in CoNS.[1] Recent studies have shown that SCC*mec* elements, or parts of them, can be exchanged by bacteriophages between different *S. aureus* strains.[2,3] We have recently described the unusual *S. aureus* CC395 strain,[4,5] which is unable to undergo phage-mediated DNA exchange with other *S. aureus* strains because its wall teichoic acid (WTA), the major staphylococcal phage receptor, is different from those of other *S. aureus* strains. Instead, its WTA resembles that of CoNS and *S. aureus* CC395 is consequently able to exchange DNA with CoNS species.[4] Thus, *S. aureus* CC395 may have an increased capacity for acquiring mobile genetic elements (MGEs), including SCC*mec*, from CoNS. Here, we analyse the structure and putative origin of the SCC*mec* element in *S. aureus* CC395.

Materials and methods

So far, the only MRSA CC395 strain described in the literature was recovered from a patient in Switzerland in 2008[6,7] and was later termed JS395.[4] We performed WGS of JS395 on the Pacific Biosciences RSII system. The nucleotide sequences were *de novo* assembled with Quiver and annotated by the NCBI Prokaryotic Genome Annotation Pipeline. The genome sequences were analysed using BLAST,[8] ISfinder,[9] CRISPRFinder[10] and the direct repeat unit (*dru*) typing web tool.[11] The complete genome sequences of the chromosome and plasmid were deposited in DDBJ/ENA/GenBank under the accession numbers CP012756 and CP012757, respectively.

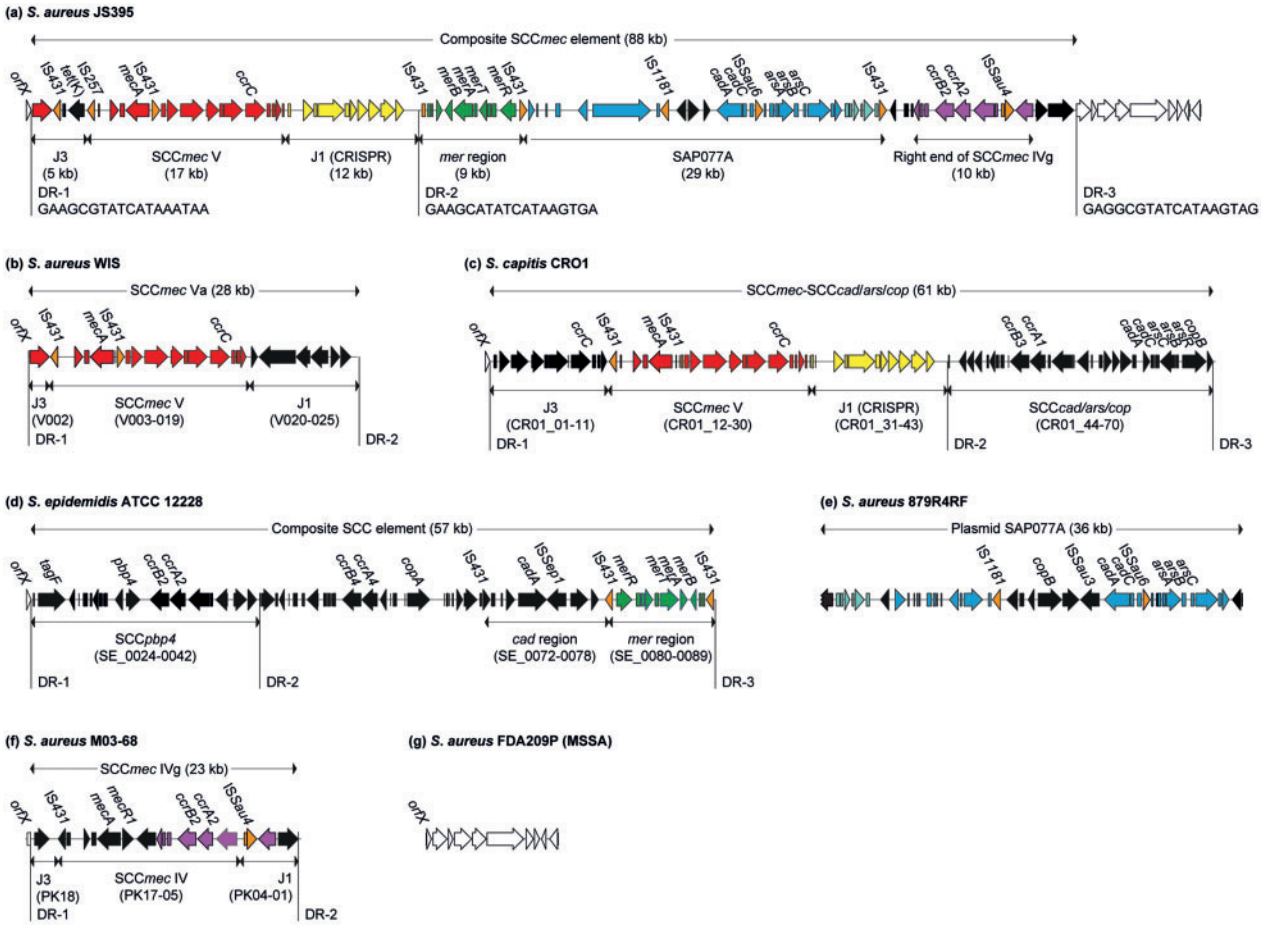

Figure 1. Comparative structure analysis of the composite SCC*mec* element in *S. aureus* JS395 (DDBJ/ENA/GenBank accession number CP012756) (a), the type Va SCC*mec* element in *S. aureus* strain WIS (AB121219) (b), the SCC*mec*-SCC*cad/ars/cop* element in *S. capitis* strain CR01 (KF049201) (c), the composite SCC element in *S. epidermidis* strain ATCC 12228 (AE015929) (d), the *S. aureus* plasmid SAP077A (GQ900428) (e), the type IVg SCC*mec* element in *S. aureus* strain M03-68 (DQ106887) (f) and the region surrounding the ISS in the MSSA strain FDA209P (AP014942) (g). The DR sequences containing the ISSs are shown.

Results and discussion

The complete genome of JS395 consisted of a 2 846 866 bp chromosome and a 42 747 bp plasmid. JS395 belonged to ST1093 (a double-locus variant of ST395) and was positive for *tagN*, an *S. aureus* CC395-specific WTA gene[4,5] and the methicillin resistance gene, *mecA*.

An 88 kb composite SCC*mec* element containing 89 ORFs (ACH32_07170 to ACH32_07610) was found to be inserted into the characteristic 3′ end of the *orfX* gene (Figure 1). We found three direct repeat (DR) sequences containing an insertion site sequence (ISS), which serves as an integration site in the staphylococcal chromosome. Two DRs were identified at the left and right chromosomal junctions, respectively, and one DR was identified 33 kb downstream of *orfX*. Analysis of the left and right chromosomal junctions revealed that the flanking regions had an organization similar to the region surrounding the ISS in the MSSA strain FDA209P.[12]

The 33 kb region identified immediately downstream of *orfX* harboured a type V (5C2) SCC*mec* element and contained 31 ORFs (Figure 1). Detailed analysis showed that the structure of the J1 region and *mec* and *ccr* gene complexes, but not the J3 region, was nearly identical to those found in the SCC*mec* elements of two CoNS species, *Staphylococcus capitis* strain CR01[13] and *Staphylococcus schleiferi* strain TSCC54,[14] and in *S. aureus* strain 08BA02176.[15] In contrast, the J3 region resembled that found in the type V (5C2) SCC*mec* of *S. aureus* strain WIS,[16] apart from the fact that JS395 harboured a tetracycline resistance gene, *tet*(K), on an IS*431*-flanked integrated copy of a truncated pT181-like plasmid (IS*431* is also known as IS*257*).

Of note, the J1 region contained a CRISPR locus encoding an adaptive immune system.[17] We identified six CRISPR spacers in JS395, which were identical to CRISPR spacers in *S. capitis* strain CR01 and *S. schleiferi* strain TSCC54, respectively (Figure 2). CRISPR loci have previously been identified in three *S. aureus* strains [08BA02176 (CC398), MSHR1132 (CC75) and M06/0171 (ST779)].[15,18,19] The last four CRISPR spacers in JS395 were present in *S. aureus* strain 08BA02176 (Figure 2). By contrast, the CRISPR spacers in *S. aureus* strains MSHR1132 and M06/0171 were unique. BLAST searches revealed that the fourth and fifth CRISPR spacers

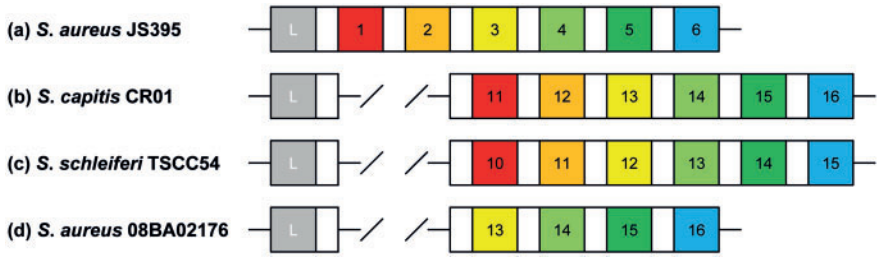

Figure 2. Comparison of the CRISPR arrays in *S. aureus* JS395 (DDBJ/ENA/GenBank accession number CP012756) (a), *S. capitis* strain CR01 (KF049201) (b), *S. schleiferi* strain TSCC54 (AP014944) (c) and *S. aureus* strain 08BA02176 (CP003808) (d). The CRISPR array consists of short DNA repeats (white boxes) separated by equally short spacer sequences (coloured, numbered boxes) and is preceded by a leader sequence (grey boxes).

in JS395 were nearly identical to sequences from an *S. aureus* phage, GRCS, isolated from raw sewage in India,[20] and a plasmid, SAP020A, isolated from a CoNS species (DDBJ/EMBL/GenBank accession number GQ900386), respectively. Together, these findings support horizontal transfer of the CRISPR locus between *S. capitis*, *S. schleiferi*, *S. aureus* CC395 and *S. aureus* CC398.

To further investigate the relationships between the JS395 SCC*mec* element and those of *S. aureus* strains WIS and 08BA02176, *S. capitis* strain CR01 and *S. schleiferi* strain TSCC54, we characterized the *dru* region. The JS395 SCC*mec* element had a unique *dru* type, dt9v (**5a**-**2d**-**4a**-**0**-**2d**-**2 g**-**3b**-**4e**-**3e**), which differed slightly from *dru* types dt11a (**5a**-**2d**-**4a**-**0**-**2d**-5b-3a-**2 g**-**3b**-**4e**-**3e**) found in *S. aureus* strain WIS, dt11ax (**5a**-**2d**-**4a**-**0**-**2d**-6f-3a-**2 g**-**3b**-**4e**-**3e**) found in *S. schleiferi* strain TSCC54, and dt11c (**5a**-**2d**-**4a**-**0**-**2d**-5b-3a-**2 g**-4b-**4e**-**3e**) found in *S. capitis* strain CR01 and *S. aureus* strain 08BA02176 (repeat sequences present in JS395 are in bold and underlined), supporting the idea that the JS395 SCC*mec* element is relatively closely related to the other SCC*mec* elements.

Immediately downstream of the 33 kb SCC*mec* region we identified a second, 55 kb SCC region harbouring 58 ORFs (Figure 1). A comparison of the structure with other sequences identified three regions with similarities to previously described SCC elements and plasmids. The first region was flanked by two copies of IS*431* and encompassed 12 ORFs. This region included genes for the detoxification of mercury (*merR*, *merT*, *merA* and *merB*) and had an organization similar to the *mer* region found in the composite SCC element of *Staphylococcus epidermidis* strain ATCC 12228 and in the type III SCC*mec* elements of *S. aureus* strain 85/2082.[21] The second region encompassed 30 ORFs and was also flanked by two copies of IS*431*. Several of these ORFs, including genes for the detoxification of cadmium (*cadC* and *cadA*) and arsenic (*arsA*, *arsB* and *arsC*), were highly homologous to those found in the *S. aureus* plasmid, SAP077A (DDBJ/EMBL/GenBank accession number GQ900428). The third region, encompassing 16 ORFs, was partially similar to the type IVg (2B) SCC*mec* of the bovine *S. aureus* strain M03-68, including the *ccrA2B2* gene complex and the J1 subtype IVg-specific ORF, PK05.[22]

The SCC*mec* element in JS395 is substantially larger than the archetypal SCC*mec* elements of *S. aureus*, which range from 21–24 kb for the type IV SCC*mec* element found in community-adapted MRSA to 67 kb for the type II SCC*mec* element.[23] This is due to the presence of multiple MGEs, including two SCC elements, a CRISPR locus, two IS*431*-flanked integrated plasmids and an IS*431*-flanked *mer* region, several of which seem to originate from CoNS. These findings are consistent with previous findings that *S. aureus* CC395 is capable of extensive DNA exchange with CoNS.[4] However, some MGEs had their closest counterparts in other *S. aureus* strains, indicating that *S. aureus* CC395 can also exchange DNA with other *S. aureus* strains by mechanisms other than transduction. Thus *S. aureus* CC395 may serve as a hub for the continuous exchange of CRISPR as well as antimicrobial resistance and virulence genes between CoNS and *S. aureus*.

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Transparency declarations

None to declare.

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