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

Jesper Larsen1*, Paal S. Andersen2, Volker Winstel2,3† and Andreas Peschel2,3

1Department of Microbiology and Infection Control, Statens Serum Institut, Copenhagen, Denmark; 2Infection Biology, Interfaculty Institute of Microbiology and Infection Medicine, University of Tübingen, Tübingen, Germany; 3German Center for Infection Research (DZIF), Partner Site Tübingen, Tübingen, Germany

*Corresponding author. Tel: +45-3268-8635; Fax: +45-3268-3231; E-mail: jl@ssi.dk
†Present address: Department of Microbiology, University of Chicago, Chicago, IL, USA.

Received 2 August 2016; returned 5 October 2016; revised 11 October 2016; accepted 21 November 2016

Background: CoNS species are likely reservoirs of the staphylococcal cassette chromosome mec (SCCmec) 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 SCCmec.

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

Methods: The only MRSA CC395 strain described in the literature, JS395, was subjected to WGS, and its SCCmec 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 SCCmec element. The 33 kb region downstream of orfX harboured a type V SCCmec 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 SCCmec 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 IV SCCmec element found in a bovine S. aureus strain.

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

Introduction

Methicillin resistance in Staphylococcus aureus is encoded by the mecA gene, which is harbouring on so-called staphylococcal cassette chromosome mec (SCCmec) elements. The existing literature suggests that these SCCmec elements have their origin in CoNS.1 Recent studies have shown that SCCmec 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 SCCmec, from CoNS. Here, we analyse the structure and putative origin of the SCCmec 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 20086,7 and was later termed JS395.5 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,8 CRISPRFinder9 and the direct repeat unit (dru) typing web tool.10 The complete genome sequences of the chromosome and plasmid were deposited in DDBJ/ENA/GenBank under the accession numbers CP012756 and CP012757, respectively.

© The Author 2017. Published by Oxford University Press on behalf of the British Society for Antimicrobial Chemotherapy. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
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, a Staphylococcus aureus CC395-specific WTA gene4,5 and the methicillin resistance gene, mecA.

An 88 kb composite SCCmec 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 (SC2) SCCmec 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 SCCmec elements of two CoNS species, Staphylococcus capitis strain CR0113 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 (SC2) SCCmec of S. aureus strain WIS,16 apart from the fact that JS395 harboured a tetracycline resistance gene, tet(K), on an IS431-flanked integrated copy of a truncated pT181-like plasmid (IS431 is also known as IS257).

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 (KF049201) and S. schleiferi strain TSCC54,14 and in S. aureus strain 08BA02176.15 In 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 1. Comparative structure analysis of the composite SCCmec element in S. aureus JS395 (DDBJ/ENA/GenBank accession number CP012756) (a), the type Va SCCmec element in S. aureus strain WIS (AB121219) (b), the SCCmec-SCCcad/ars/cap 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 SCCmec 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.

Larsen et al.
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 SCCmec element and those of *S. aureus* strains WIS and O8BA02176, *S. capitis* strain CR01 and *S. schleiferi* strain TSCC54, we characterized the dru region. The JS395 SCCmec 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 O8BA02176 (repeat sequences present in JS395 are in bold and underlined), supporting the idea that the JS395 SCCmec element is relatively closely related to the other SCCmec elements.

Immediately downstream of the 33 kb SCC 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. The first region encompassed 12 ORFs. This region included genes for the de-toxification 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 elements of *S. aureus* strain 85/2082.21 The second region encompassed 30 ORFs and was also flanked by two copies of IS431 and encompassed 12 ORFs. This region included genes for the de-toxification of cadmium (*cadI* 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 similarly partial to the type IV (2B) SCCmec of the bovine *S. aureus* strain M03-68, including the ccrA2B2 gene complex and the J1 subtype IVg-specific ORF, PK05.22

The SCCmec element in JS395 is substantially larger than the archetypical SCCmec elements of *S. aureus*, which range from 21–24 kb for the type IV SCCmec element found in community-adapted MRSA to 67 kb for the type II SCCmec element.23 This is due to the presence of multiple MGEs, including two SCC elements, a CRISPR locus, two IS431-flanked integrated plasmids and an IS431-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.6 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*.

**Acknowledgements**

We thank Jacques Schrenzel and Patrice Francois (University of Geneva, Geneva, Switzerland) for providing the MRSA CC395 strain.

**Funding**

This work was supported by the German Research Council [Transregio 34 (TRR34)] and the German Center for Infection Research [Thematic Translation Unit (TTU) Healthcare-Associated and Antibiotic-Resistant Bacterial Infections (HAARBI)] to A. P.

**Transparency declarations**

None to declare.

**References**


