Characterization of a complex context containing mecA but lacking genes encoding cassette chromosome recombinases in Staphylococcus haemolyticus

Zhiyong Zong1,2

Abstract

Background: Methicillin resistance determinant mecA is generally transferred by SCCmec elements. However, the mecA gene might not be carried by a SCCmec in a Staphylococcus haemolyticus clinical isolate, WCH1, as no cassette chromosome recombinase genes were detected. Therefore, the genetic context of mecA in WCH1 was investigated.

Results: A 40-kb region containing mecA was obtained from WCH1, bounded by orfX at one end and several orfs of S. haemolyticus core chromosome at the other. This 40-kb region was very complex in structure with multiple genetic components that appeared to have different origins. For instance, the 3.7-kb structure adjacent to orfX was almost identical to that on the chromosome of Staphylococcus epidermidis RP62a but was absent from S. haemolyticus JCSC1435. Terminal inverted repeats of SCC were found but no ccr genes could be detected. mecA was bracketed by two copies of IS431, which was flanked by 8-bp direct target repeat sequence (DR).

Conclusions: The presence of 8-bp DR suggests that the two copies of IS431 might have formed a composite transposon for mobilizing mecA. This finding is of significance as multiple copies of IS431 are commonly present in the contexts of mecA, which might have the potential to form various composite transposons that could mediate the mobilization of mecA. This study also provides an explanation for the absence of ccr in some staphylococci isolates carrying mecA.

Background

Methicillin-resistant staphylococci represent a great challenge for treatment and public health. In staphylococci, methicillin resistance is mainly due to the expression of the mecA gene, which specifies penicillin binding protein 2a (PBP2a), a transpeptidase with a low affinity for β-lactams [1,2]. mecA is carried by a mobile genetic element (MGE) termed the staphylococcal cassette chromosome mec (SCCmec) [2,3]. Generally, SCCmec contains two essential components, i.e. the mec gene complex and the ccr gene complex. The mec gene complex consists of mecA, the regulatory genes and associated insertion sequences and has been classified into six different classes, i.e. A, B, C1, C2, D and E. Cassette chromosome recombinase (ccr) genes (ccrC or the pair of ccrA and ccrB) encode recombinases mediating integration and excision of SCCmec into and from the chromosome [2,3]. The ccr gene(s) and surrounding genes form the ccr gene complex.

A Staphylococcus haemolyticus clinical isolate, WCH1, was found carrying mecA but no ccr genes. Although clinical isolates of S. haemolyticus containing mecA but lacking ccr genes have been reported previously [4-6], information about the detailed contexts of mecA is largely absent. The genetic context of mecA in WCH1 was therefore investigated using long-range PCR, PCR mapping, inverse PCR and sequencing as described previously [7].
Results and discussion

The minimum inhibitory concentration (MIC) of cefoxitin against WCH1 was 128 μg/mL. A 40-kb region containing mecA was obtained from WCH1, abutting orfX at one end and seven orfs designated orf39 to orf44 here (Table 1), which were also present in the oriC environ on the chromosome of the completely-sequenced Staphylococcus aureus strain JCSC1435 (GenBank accession no. AP006716) [8], at the other (Figure 1). The partial sequence of orfX obtained was 99% identical to that of S. haemolyticus JCSC1435. orf39 to orf44 were identical to the counterparts of S. haemolyticus JCSC1435 and were not part of any known mobile genetic elements (MGE), confirming that these orfs indeed belonged to the core chromosome of S. haemolyticus.

mecA is bracketed by two copies of IS431 flanking by an 8-bp direct repeat sequence

WCH1 had a class C1 mec gene complex composed of mecA, mecR1A truncated by the insertion of the insertion sequence IS431, several other genes and another copy of IS431 downstream of mecA with the two copies of IS431 at the same orientation (Figure 1). The class C1 mec gene complex is also present in SCCmec types VII and X of Staphylococcus aureus and several unnamed types of SCCmec in coagulase-negative staphylococci (CoNS) [9].

An 8-bp identical sequence (CTTTTTTGCC; Figure 1) was identified flanking the two copies of IS431. The 8-bp DR was part of the spacer sequence between arsR (encoding an arsenical resistance operon repressor) and copA (encoding a copper-exporting ATPase). The presence of a direct repeat (DR) suggested that the two copies of IS431 might have formed a composite transposon with the potential to mediate the mobilization of mecA into different genomic locations. This mecA-carrying IS431-formed composite transposon was designated Tn6191 according to the transposon database (www.ucl.ac.uk/eastman/tn/). Composite transposons formed by IS431 generating 8-bp AT-rich DR on insertion have been seen before, such as Tn6072 carrying ccrC and the aminoglycoside resistance determinant aacA found in a ST239 S. aureus [10]. Two copies of IS431 have also been found to mediate the transposition of plasmids pUB110 encoding bleomycin resistance [11] and pT181 encoding tetracycline and mercury resistance [12]. However, Tn6072 and other IS431-formed composite transposons do not contain mecA. IS431 is widely distributed in Staphylococcus epidermidis, S. haemolyticus and methicillin-resistant S. aureus (MRSA) [13] and appears to play a vital role in generating mosaicism in the genetic contexts of mecA. The insertion of IS431 and homologous recombination between different copies of IS431 can result in acquisition, loss and rearrangements of genetic components [14,15]. Therefore, IS431 apparently serves as the “adapters” allowing genetic components to be linked and clustered together to form complicated genetic contexts of mecA.

In GenBank and literature, e.g. [3], there are many cases in which mecA is bracketed by two copies of IS431, either at the same or opposite orientations, i.e. the class C1 or C2 mec complex. In these cases, two copies of IS431 have the potential to form a composite transposon mediating the mobilization of mecA but no 8-bp DR could be identified flanking the class C1 or C2 mec complexes. This suggests that the two copies of IS431 might have inserted in tandem rather than mobilize together as a unit. Alternatively, IS431 might behave likes IS26 [16], an insertion sequence also of the IS6 family, that could lead to adjacent deletions, leaving no DR.

No ccr genes could be identified in this large region containing mecA. In the 1970s and 1980s, it was found that methicillin resistance could be transferred by phages [17-21] in experimental conditions and could be also carried by a transposon, Tn4291, located on a naturally occurring plasmid, pl524 [21]. However, these studies were carried out before the identification of mecA and no sequence information was available for the phages carrying methicillin resistance, Tn4291 and pl524. It remains unclear whether methicillin resistance in these experiments was due to the expression of mecA. In particular, Tn4291 mediated resistance to methicillin but not to penicillin, raising the possibility that the methicillin resistance determinant carried by Tn4291 was actually not mecA. mecA is usually transferred by SCCmec, but mecA existed in the absence of any known types of ccr genes have been found in both MRSA and CoNS previously. In particular, no known ccr genes were detected for an half of methicillin-resistant S. haemolyticus isolates from a hospital in Tunisia [22], suggesting that elements carrying mecA but lacking ccr genes might be common in S. haemolyticus. However, the detailed genetic context of mecA were not characterized in these cases and therefore the exact reasons for the absence of ccr genes remain unclear [2]. The present study provides a detailed example that mecA was in a context without ccr genes and might be able to be transferred by a MGE other than SCCmec.

A complex SCC-like remnant containing components with various origins

This 40-kb region between orfX and orf39 contained five copies of IS431 (designated IS431-1 to -5 from upstream of to downstream of mecA, respectively) and three terminal inverted repeats (IR) of SCC elements (Figure 1). An IR was in the orfX, designated IR1 here. The second IR another was located between the lipoprotein-encoding gene, lip, and a putative Acyl-CoA acyltransferase-encoding gene, acf, designated IR2 here. The third IR was adjacent
Table 1 Genes and MGE in the genetic context of mecA in WCH1

| Gene or MGE | Position a | Product | Closest match b,c |
|-------------|------------|---------|------------------|
| orfX | 1-316 | Hypothetical protein | 99%, S. haemolyticus JCSC1435 |
| ADP | 445-1431 | ADP-ribosylglycohydrolase | 99%, S. epidermidis RP62a (locus SERP2218) |
| penM | 1450-2784 | Cytosine/uracil, uracil, thiamine, allantoin permease family protein | 99%, S. epidermidis RP62a (locus SERP2217) |
| rbkΔ d | 2781-3719 | Ribokinase | 99%, S. epidermidis RP62a (locus SERP2216) |
| IS431 | 3701-4401 | IS431 | |
| merR | 4888-5235 | Transcriptional regulator of the merR family | 100%, type IX SCCmec of S. aureus JCSC6943 and S. haemolyticus JCSC1435 (locus SH0094) |
| thiJ | 5313-5996 | Thi/PfpI family protein | 100%, type IX SCCmec of S. aureus JCSC6943 and S. haemolyticus JCSC1435 (locus SH0095) |
| orf8 | 6018-6683 | NAD dependent epimerase/dehydratase family protein | 100%, type IX SCCmec of S. aureus JCSC6943, type X SCCmec of S. aureus JCSC6945 and S. haemolyticus JCSC1435 (locus SH0096) |
| orf9 | 6687-7691 | Oxidoreductase, zinc-binding dehydrogenase family protein | 100%, type IX SCCmec of S. aureus JCSC6943 and S. haemolyticus JCSC1435 (locus SH0097) |
| orf10 | 7700-8065 | Hypothetical protein of the COG4270 superfamily, predicted membrane protein | 100%, type IX SCCmec of S. aureus JCSC6943 and S. haemolyticus JCSC1435 (locus SH0098) |
| cadD | 8601-9218 | Cadmium binding protein | 100%, type IX SCCmec of S. aureus JCSC6943 and S. haemolyticus JCSC1435 (locus SH0099) |
| cadX | 9237-9578 | Cadmium resistant accessory protein | 100%, type IX SCCmec of S. aureus JCSC6943 and S. haemolyticus JCSC1435 (locus SH0100) |
| arsC | 9999-9598 | Arsenate reductase | 100%, type IX SCCmec of S. aureus JCSC6943 and S. haemolyticus JCSC1435 (locus SH0101) |
| arsB | 11306-10017 | Arsenical pump membrane protein | 99%, type IX SCCmec of S. aureus JCSC6943 and S. haemolyticus JCSC1435 (locus SH0102) |
| arsR | 11623-11302 | Arsenical resistance operon repressor | 100%, type IX SCCmec of S. aureus JCSC6943, type X SCCmec of S. aureus JCSC6945 and S. haemolyticus JCSC1435 (locus SH0103) |
| IS431 | 11697-12486 | IS431 | |
| mecRΔ | 12503-12487 | Signal transducer protein | |
| mecA | 12603-14609 | Penicillin binding protein 2a | |
| orf19 | 15083-14655 | Hypothetical protein | |
| maoC | 15923-15180 | Putative acyl dehydratase maoc | |
| orf21 | 17208-16840 | Putative HMG-CoA synthase (partial) | |
| IS431 | 17209-17998 | IS431 | |
| copA | 20277-21710 | Copper-transporting atpase | 99%, type X SCCmec of S. aureus JCSC6945. |
| orf24 | 25274-23736 | Sodium/proline symporter (High affinity proline permease) | 78%, S. saprophyticus ATCC 15305 (locus SSP0399) |
| IS431Δ | 26462-27184 | IS431, truncated | |
| FAD | 27261-28382 | FAD-dependent pyridine nucleotide-disulphide oxidoreductase | 66%, a few S. aureus strains, e.g. COL |
to orf39, part of the core chromosome of *S. haemolyticus*, designated IR3 here (Figure 1). This 40-kb region was actually bracketed by two IR, IR1 and IR3, resembling the remnant of a SCC-like element but without *ccr* genes. In light of the presence of an internal IR, IR2, this *ccr*-absent large region was a remnant of a composite SCC element or comprised remnants of multiple SCC elements.

The 3.7-kb region between orfX and the IS431-1 was designated R1 (representing region 1) and contained genes encoding ADP-ribosylglycyohydrolase, permease and ribokinase. R1 was almost identical to the counterpart (loci SERP2216 to SERP2218) of the integrative plasmid vSe1 on the chromosome of *S. epidermidis* RP62a (GenBank accession no. CP000029) but was absent from *S. haemolyticus* JCSC1435, suggesting a foreign origin. Of note, the ribokinase-encoding gene, *rbk*, was truncated at the 3′ end by the insertion of IS431, leaving a 920 bp remnant of the 939 bp gene.

The region between the IS431-1 and IR2 was designated R2. As mentioned above, Tn6191 was inserted into the spacer between *arsR* and *copA* in R2. Besides Tn6191, R2 also contained a few genes, the *cadXD* operon mediating resistance to cadmium and the *ars* operon required for detoxifying arsenate. In R2, the sequence from the IS431-1 to *arsB* was closest (99.9% similarity) to the counterpart in the type IX SCCmec of *S. aureus* strain JCSC6943 (GenBank accession no. AB505628), while that from *arsB* to IR2 excluding Tn6191 was almost identical to the corresponding region in the type X SCCmec of *S. aureus* JCSC6945 (GenBank accession no. AB505630). This suggests that R2 might have resulted from homologous recombination between the *ars* operons of the type IX and

### Table 1 Genes and MGE in the genetic context of meCA in WCH1 (Continued)

| Gene    | Accession/Name | Function                                      | Description | Similarity |
|---------|----------------|-----------------------------------------------|-------------|------------|
| *deoB*  | 28376-29272    | FeoB family ferrous iron transporter          | 68%         |            |
| orf31   | 29337-29717    | Putative transmembrane protein                | 73%         |            |
| IS431Δ  | 30690-20981    | IS431, incomplete due to internal termination | 71%         |            |
| orf32   | 31660-33822    | ABC-type bacteriocin transport family protein  | 100%        |            |
| orf33   | 34541-35809    | DUF867 type protein, putative phage-related   | 71%         |            |
| ccr     | 38832-37669    | Chromate transporter                           | 60%         |            |
| arsC    | 39261-38869    | Arsenate reductase                            | 97%         |            |
| arsB    | 40577-39279    | Arsenical pump membrane protein               | 92%         |            |
| arsR    | 40885-40571    | Arsenical resistance operon repressor         | 91%         |            |
| orf39   | 41223-41771    | DUF576 type protein                           | 100%        |            |
| orf40   | 41768-41935    | Hypothetical protein                          | 100%        |            |
| orf41   | 42126-43013    | Hypothetical protein, similar to mecas         | 100%        |            |
| orf42   | 43522-44046    | DUF3267 type protein                          | 100%        |            |
| orf43   | 44998-44120    | Hypothetical protein, similar to cobalamin    | 100%        |            |
| orf44   | 45625-46248    | Hypothetical protein, similar to Zn-binding   | 100%        |            |

* Positions are according to GenBank accession no. JQ764731.
* GenBank accession no.: *S. aureus* LGA251 (FR821779), *S. aureus* JCSC6943 (AB505628), *S. aureus* JCSC6945 (AB505630), *S. aureus* MSHR1132 (FRB21777), *S. carnosus* TM300 (AMQ25250), *S. epidermidis* ATCC 12228 (AE015929), *S. epidermidis* RP62a (CP000029), *S. haemolyticus* JCSC1435 (AP006716), *S. saprophyticus* ATCC 15305 (AP008934), *Oceanobacillus iheyensis* HTE831 (BA000028), *S. aureus* plasmid SAP099B (GQ900449), *S. aureus* plasmid EDNA (AP003089), *S. epidermidis* plasmid SAP105A (GQ900452), *S. xylosus* plasmid pSX267 (M80565).
* Closest matches of MGE (IS431 and ISS41) and genes belonging to the meca complex are not listed as there are many identical matches.
* Truncated by IS431 with 19 bp of the 3′ end missing and the read frame extending into IS431.
* The *tnpA* of IS431 was terminated prematurely due to internal point mutation.
X SCCmec. R1 and R2 had different origins and were separated by a single copy of IS431, suggesting that IS431 served as a joining point that brought the two regions together.

The large region between IR2 and IR3 was designated R3. The two genes, acf and orf27 (putatively encoding a type I restriction endonuclease), adjacent to IR2 had 96.8% identities to the counterparts of a SCC element on the chromosome of S. haemolyticus JCSC1435. At the other end of R3, there was a second copy of the ars operon, which was closest to those on a few S. aureus plasmids, e.g. pl258 (GenBank accession no. GQ900378) and pK59 (GenBank accession no. GQ900488) with 92.0% identity and had only 86.4% identity with the first ars operon in R2 of WCH1. The intervening genetic components in R3 had lower than 80% identity with the closest matches identified by BLAST and were absent from the chromosome of S. haemolyticus JCSC1435. All above findings suggest that all genetic components in R3 had origins other than S. haemolyticus.

Of note, there were two copies of IS431 and an ISShaI in R3 but no DR could be identified, suggesting that these MGEs were likely to have resulted from homologous recombination rather than direct insertion.

Of note, R3 contained several possible virulence factors. A putative proline permease-encoding putP gene was present on R3 and had 78% identity with that of Staphylococcus saprophyticus strain ATCC 15305 [23]. putP has been identified as a virulence factor in S. aureus, contributing to invasive infection [24]. R3 also contained a feoB-like gene that was 68% identical to the counterpart of Staphylococcus carnosus strain TM300 (GenBank accession no. AM295250). feoB has been known as a virulence factor in Gram-negative bacteria, while its virulence status in Gram-positive remains controversial since it has been found conferring virulence in Streptococcus suis [25] but not in Listeria monocytogenesis [26] and there is no study of feoB for staphylococci. In addition, orf32 encodes a putative ABC-type bacteriocin transporter, which might involve in the regulation of virulence factor expression.

In addition, a number of genes encoding products for metabolism, transporting nutrients or detoxifying harmful substances were present in this large region carrying mecA (Table 1). The presence of these features could enhance the adaptation of the host strain to variable environment and therefore provided advantages in fitness. Of note, it has been reported that staphylococci are resistant to chromates. To our knowledge, it is the first time to identify a chromate transporter gene in staphylococci. It also suggests that additional mechanisms are responsible for the chromate resistance in staphylococci.

Although the genetic context of mecA was characterized in detail, the exact reason for the absence of ccr genes in WCH1 remains undetermined. It is possible that mecA was originally carried by a SCCmec element with ccr genes and the subsequent insertion of an additional IS431 upstream of mecA could give rise to the
Table 2: Primers used for PCR

| Primer     | Sequence (5'-3')a | Target/locationb | Reference |
|------------|-------------------|------------------|-----------|
| acf-R1     | TCTCGAGCATCCTTTTCTCA | acf              | This study |
| ars-up1    | TGTTAGCTCATGGAAATTGAGGA | upstream of arsR | This study |
| fecB-F1    | ATGGTCTCCAAAGCGATGA | fecB             | This study |
| fecB-F2    | AAGACAGGAACACGGAACGA | fecB             | This study |
| fecB-R1    | TGGGGCTATGATTACCTGA | fecB             | This study |
| IRL-scc    | TACTCGWTRATGATGAGGTTT | IRL of SCCmec   | [7]       |
| IS2        | TGAGGTATTAGATATTTGCATGT | IS431          | [31]      |
| MecA147-F  | GTGAAAGATACAAGGATTGATT | mecA          | [30]      |
| MecA147-R  | ATGCCCTATAGATGAAAGGA | mecA             | [30]      |
| orf_1-F1   | GAAATAGGCTGAAAGAC | ip                | This study |
| Orf2-1-R1  | TTGGAGGTTCCTCCCCCATC | orf24, a Putative multicopper oxidases gene | This study |
| Orf24-1    | CCAAATAGGTTAGCTTGTTGTTG | spacer between putP and IS431Δ | This study |
| orfX-F1    | GAAAAACGACWGAAMATGAG | orfX             | [7]       |
| SH0128-R1  | TTTTGTGGTTGGAGGTGTTGATG | orf45, locus SH0128 in AP006716 | This study |
| ZZ-3       | GTGAGGCTTCTGGTATGATGTTG | spacer between putP and IS431Δ | This study |
| ZZ-4       | GCCGGTCTCCTGGTATGATGTTG | FAD             | This study |
| ZZ-11      | TATCTCGGGAACATCGATAAAA | spacer between IS431 and orf32 | This study |
| ZZ-12      | GTTGAAGGAACACAAAAACTACG | spacer between IS431 and orf32 | This study |
| ZZ-16      | CGGATACGTATCCTATCCTATC | orf32, ABC-type bacteriocin transporter gene | This study |
| ZZ-24      | AGCAGCAGAACAAAGACATC | orf33           | This study |
| ZZ-28      | TGGAGGAGGATTGGTTCCTA | orf35, chromate transporter | This study |
| ZZ-29      | TACGAGACAGACTCCCTA | orf35, chromate transporter | This study |
| ZZ-30      | TGGATCTGCTGCTGCATTGTC | orf35, chromate transporter | This study |
| ZZ-31      | GCTGACAGCTCAGTCTGTTA | orf35, chromate transporter | This study |
| ZZ-32      | TGGAGGTATCATCTGTCTATG | orf33           | This study |

a D: A, G or T; H: A, C or T; M: A or C; R: A or G; W: A or T; Y: C or T; V: A, C or G.
b More information about orfs listed here is available in Table 1.

potential composite transposon, Tn6191, together with the already-existed IS431 downstream of mecA. Tn6191 might have mobilized mecA into a new genomic location or alternatively, ccr genes could have been deleted due to homologous recombination between multiple copies of IS431 that were present in WCH1.

Conclusions

mecA was identified in a 40-kb region that contained IR of SCC elements but no ccr genes. This large region was very complex in structure and contained multiple genetic components with different origins. Genetic components with various origins were likely introduced in tandem by SCC elements and insertion sequences through insertion and homologous recombination. Two copies of IS431 bracketed mecA and were flanked the characteristic 8-bp direct repeat sequence, suggesting that the two IS431 might have form a composite transposon with the potential to be active. The IS431-formed composite transposon might represent a new mechanism for the mobilization of mecA independent of the action of SCCmec. The present study aimed to illustrating the complex context of mecA but further experiments to demonstrate the activity of Tn6191 in transposing mecA are warranted to confirm the proposed new mechanisms for the mobilization of mecA.

Methods

Strain and SCCmec typing
Clinical isolate WCH1 was recovered from blood collected in West China Hospital, Chengdu, western China, and was obtained as part of standard care. WCH1 was identified as S. haemolyticus by partially sequencing the 16S rRNA gene amplified with the universal primers 27 F and 1492R [28]. WCH1 could grow on plates containing 4 μg/ml cefoxitin (Sigma, St Louis, MO). The MIC of cefoxitin against WCH1 was determined using the broth dilution method following the Clinical and Laboratory Standards Institute guidelines [29]. The mecA gene and its regulatory genes mecI and mecR1 were detected by PCR [30]. The SCCmec typing was carried out using
multiplex PCR [30]. The presence of ccr genes were also examined by PCR using multiple universal primers as described previously [9].

**PCR mapping and inverse PCR**

Two overlapping long-range PCR (Fermentas, Burlington, ON, Canada) were used to obtain two regions, one between mecA and orfX and the other between mecA and an inverted repeat (IR) sequence of SCC (Figure 1). A few inverse PCR reactions were employed to identify the genetic context of mecA with pairs of outwards-facing primers (Table 2 and Figure 1). Genomic DNA of WCH1 prepared using a commercial kit (Tiangen, Beijing, China) was restricted with a certain restriction enzyme (Figure 1), self-ligated with T4 DNA ligase (New England Biolabs, Ipswich, NY) and then used as a template for inverse PCR. The links between genetic elements were confirmed by overlapping long-range PCR (Figure 1, primers listed Table 2).

**Sequencing**

Amplicons were sequenced by primer walking using an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA) at the Beijing Genomics Institute (Beijing, China). Sequences were assembled using the SeqMan II program in the Lasergene package (DNASTAR Inc, Madison, WI) and similarity searches were carried out using BLAST programs (http://www.ncbi.nlm.nih.gov/BLAST/). The putative function of proteins was analyzed using the InterProScan tool (http://www.ebi.ac.uk/Tools/pi/ipsrscan/).

**Nucleotide sequences accession number.** The complete sequence of the genetic context of mecA in WCH1 has been deposited in GenBank as JQ764731.

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