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Identification and detection of USA300 methicillin-resistant *Staphylococcus aureus* clones with a partial deletion in the *ccrB2* gene on the type IV SCC*mec* element

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ABSTRACT

Panton-Valentine leukocidin-positive highly pathogenic USA300 methicillin-resistant *Staphylococcus aureus* carries type IV staphylococcal cassette chromosome (SCC) *mec*. Here, we found USA300-like strains (named as ΨUSA300), which could not be identified as SCC*mec* type IV by the conventional PCR method due to a 12 bp deletion on *ccrB2*.

USA300 clones, which are highly pathogenetic and global epidemic community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) clone, have spread rapidly worldwide (Nimmo, 2012; Tenover and Goering, 2009). USA300 clones carry the Panton-Valentine leukocidin (PVL) genes and arginine catabolic mobile element (ACME) and are sequence type (ST) 8-staphylococcal cassette chromosome (SCC) *mec* type IVa. Recently, a highly virulent clone, USA300-LV, which is closely related to USA300 and has spread rapidly through Latin American countries, has been reported (Arias et al., 2008; Planet et al., 2015; Reyes et al., 2009). USA300-LV has the following different genetic features, which are in contrast to those of USA300: it is ACME-negative and it has the SCC*mec* type IVc element; this strain shows <80% pulsed-field gel electrophoresis (PFGE) similarity with USA300. Performing multilocus sequence typing (MLST) and PFGE is essential to identify the USA300 clones. Additionally, PCR screening for ACME and SCC*mec* typing are important methods used to rapidly screen USA300 clones.

During our previous study, some USA300-like strains (named as ΨUSA300), which showed PVL- and ACME-positive, ≥80% similarity with the USA300 clone by PFGE, and ST8 but could not be identified as SCC*mec* type IV by conventional multiplex PCR, were found (Takadama et al., 2017; Takadama et al., 2018). Unlike USA300-LV, the ΨUSA300 strains exhibited the same genetic features as USA300 clones, except for the SCC*mec* type. Here, we investigate the reason for the failure of the SCC*mec* typing of the ΨUSA300 strains.

A total of 82 PVL and ACME-positive ST8 MRSA strains which were isolated from multiple hospitals in Tokyo, Japan, between 2012 and 2016 were used. Among them, strains isolated in 2012–2015 have already been reported in our previous study (Takadama et al., 2018). To compare PFGE patterns, CA-MRSA JCSC6774 (USA300 clone) was used (Watanabe et al., 2009). SCC*mec* typing by multiplex PCR was performed according to the method described by Boye et al. (2007). The ΨUSA300 genome was analyzed by the Illumina Miseq System (Illumina, CA, USA) (Nakaminami et al., 2017). The obtained contigs were annotated by a CLC Genomics Workbench II (QIAGEN bioinformatics, Aarhus, Denmark) using the USA300 FPR3757 genome (Accession no. CP 000255) as the reference sequence. The genome sequence of ΨUSA300 was deposited into the GenBank accession no. DRA007443. PCR amplification was performed using the Q5® High-Fidelity DNA Polymerase (New England BioLabs, MA, USA) and specific primers [ccrB2-seqF; 5′-TGACATATCCTTTGTGATTCAATG-3′ + α2 (Boye et al., 2007)] with the following cycles: 98 °C for 30 s; 30 cycles of 98 °C for 10 s, 55 °C for 30 s, and 72 °C for 30 s; and 72 °C for 2 min. Analyses of the nucleotide and amino acid sequences were performed using ATGC ver. 6 software (Genetyx, Tokyo, Japan) and Genetyx ver. 10 (Genetyx), respectively.

The conventional SCC*mec* typing showed that 63 isolates (76.8%) were identified as SCC*mec* type IV (USA300), whereas 19 isolates (23.2%) could not be identified as SCC*mec* type IV (ΨUSA300). Genome analysis of TSI637, which was one of the ΨUSA300 strains, showed a 12 bp deletion. The deletions existed in the *ccrB2* gene sequence of the SCC*mec* type IVa element (Fig. 1). Currently, the SCC*mec* typing by multiplex PCR is widely used for the molecular epidemiological classifications of MRSA strains, and the method described by Boye et al., is one

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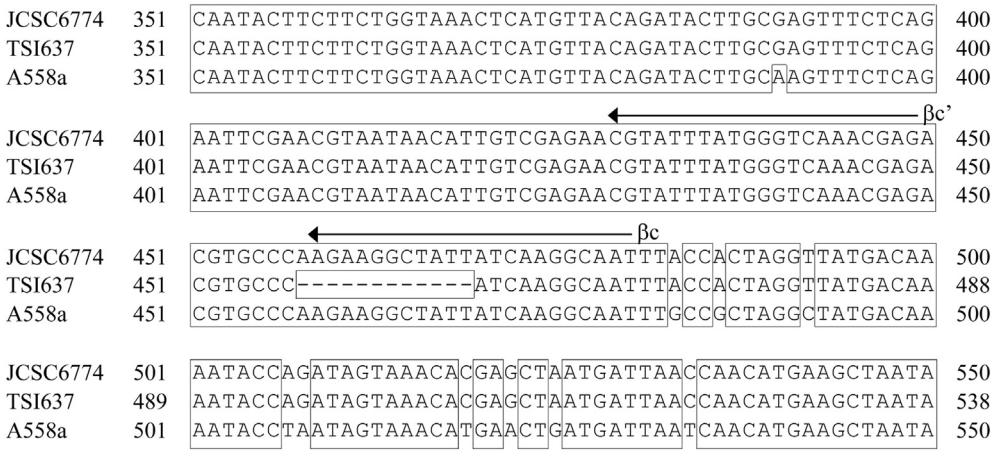

Fig. 1. Comparison of the DNA sequence alignment of *ccrB2*.
JCSC6774, USA300; TSI637, ΨUSA300; A558a, SCC*mec* type IV strain.
The arrows indicate the primer binding sites.

of the often used (Boye et al., 2007). In this method, primers βc and α2 are used for amplification of the *ccr* region. However, the 12 bp deletion in ΨUSA300 was located at the βc primer binding site (Fig. 1). Therefore, the reason for the failure of the SCC*mec* typing of ΨUSA300 strain was this deletion. Furthermore, the nucleotide sequencing of *ccrB2* from the other 18 ΨUSA300 strains indicated that all of them had the same deletions as TSI637.

The 12 bp deletion on *ccrB2* in ΨUSA300 causes a Q153H substitution and a deletion of three amino acids (154 to 156), but these were in-frame deletions. CcrB2 is one of the cassette chromosome recombinases which plays a key role in the site-specific excision of SCC*mec* (Zhang et al., 2015). Amino acids 123 to 152 in CcrB2 form a flexible extended helix loop arm, which is considered to be involved in recombination (Wang et al., 2015). Among these amino acids, arginine at position 151, in particular, is said to be involved in the functioning of the DNA-binding roop. The 12 bp deletion in *ccrB2* in ΨUSA300 was adjacent to the region that is important for recombination, but the downstream amino acid sequence was maintained due to this deletion being an in-frame deletion. Further studies are necessary to clarify the influence of the substitution and deletion of amino acids 153 to 156 in CcrB2.

To identify the SCC*mec* type of the ΨUSA300 accurately, a new primer; βc′, 5′-CTCGTTTGACCCATAAATACG-3′, was designed according to the *ccrB2* nucleotide sequence of ΨUSA300 (Fig. 1). The new primer could accurately detect the SCC*mec* type of both USA300 and ΨUSA300 strains. Furthermore, the new primer could also amplify SCC*mec* type IV strains other than USA300, because it was designed using highly conserved sequences found in various SCC*mec* type IV strains (data not shown). Other conventional SCC*mec* typing by multiplex PCR described by Kondo et al., and Zhang et al., are also widely used (Kondo et al., 2007; Zhang et al., 2005). The conventional primer, βc, which was designed by Kondo et al., is used in all the methods for amplifying the *ccr* region (Boye et al., 2007; Kondo et al., 2007; Zhang et al., 2005). The new primer designed in this study can be used for the identification of not only ΨUSA300 strains, but also for the identification of other SCC*mec* type IV strains. The clinical significance caused by a 12 bp deletion in *ccrB2* is still unclear. Further studies are necessary to clarify it.

In conclusion, our findings showed that at least 20% of the USA300 clones have a partially defective *ccrB2* gene. The new primer designed in this study is useful for screening USA300 clones.

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Transparency declaration

None to declare.

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