Structures of SCC*mec* elements in methicillin-resistant *Staphylococcus lugdunensis* are closely related to those harbored by community-associated methicillin-resistant *Staphylococcus aureus*

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Abstract

Methicillin-resistant *Staphylococcus lugdunensis* (MRSL) has been increasing recognized in healthcare and community settings. This study characterized the structure of SCC*mec* elements harboured by 36 MRSL isolates from diverse sources in Hong Kong during 2008 to 2017 by whole genome sequencing. The ST-SCC*mec* combinations in the 36 MRSL isolates were as follows: ST3-SCC*mec* IV (*n*=2), ST3-SCC*mec* V (*n*=28), ST27-SCC*mec* V (*n*=5) and ST42-SCC*mec* V (*n*=1). The two SCC*mec* IV elements were highly similar to the SCC*mec* IV element harbored by the community-associated methicillin-resistant *S. aureus* (CA-MRSA) JCSC6668. The J3-*mec* complex-J2 regions in the SCC*mec* V elements were highly similar to the corresponding regions in CA-MRSA strain PM1 (n=13) or WIS (n=21). Based on the J1 to J3 sequences, the SCC*mec* V elements can be categorized into nine different subtypes. Our findings highlight the diversified structures of SCC*mec* elements among MRSL strains and their close relationship with SCC*mec* elements harboured by CA-MRSA.

INTRODUCTION

Staphylococcus lugdunensis is unique among the coagulase-negative staphylococci in that infections caused by this organism are similar in type and severity to those caused by *S. aureus* [1]. This organism remains sensitive to most antibiotics, contrary to other staphylococci [1,2]. However, multidrug resistant strains have been increasingly identified and may be partly related to better recovery and identification methods [3-7]. Our previous work showed that emerging resistance in the organism is associated with the expansion of the sequence type (ST) 3 clone and mosaic multidrug-resistant plasmids [5,6]. ST3 is one of the major lineages identified in *S. lugdunensis* populations [8]. In Hong Kong, ST3 isolates were highly prevalent in healthcare settings and have also been detected in clinical specimens from patients with community-associated infections [4,5]. In Taiwan and Europe, ST3 *S. lugdunensis* has been found to cause skin and soft tissue, osteoarticular and bacteremic infections of community- and healthcare-associated origins [8,9].

In *S. lugdunensis*, the emergence of methicillin resistance has found to be associated with acquisition of SCC*mec* IV or V elements [3,10]. In Taiwan, ST6 carrying SCC*mec* V has been reported to be endemic in some hospitals [3]. However, only limited information is available on the sequences of the SCC*mec* elements carried by *S. lugdunensis* [11]. To obtain a better understanding of the emergence of methicillin-resistant *S. lugdunensis* (MRSL) in Hong Kong, this study was performed to investigate the structure and content of SCC*mec* elements in a collection of MRSL isolates of diverse healthcare-associated and community-associated origins by whole genome sequencing.

METHODS

A total of 36 MRSL isolates from several published collections and blood culture archives of three acute, regional hospitals in Hong Kong were included [4-6,10]. Twenty-one isolates were obtained from patients hospitalized for clinical infections (12 skin and soft tissue infections, 7 bacteremia, 1 continuous ambulatory peritoneal dialysis peritonitis and 1 pneumonia) during 2008-2014 [4]. Fifteen isolates were collected from patients on long-term renal dialysis (n=13, 2013-2014) and medical students (n=2, one each from 2015 and 2017) with asymptomatic carriage [5,6,10]. Medical students expose to the healthcare environment and they represent a special category that might be at risk of carriage of both community-associated and healthcare-associated MRSL. Thus, they were involved in the present study and expected to extend the diversity of SCC*mec* elements in MRSL. Each isolate originated from a different individual and all viable isolates were included. The number of isolates with healthcare-associated (from an inpatient after >2 days of hospitalization, hospitalization in past 6 month, old age home residents and/or on long-term renal dialysis) and community-associated origin (from an outpatient or an inpatient within 2 days of hospitalization and in which healthcare-associated risk factors were absent) was 22 and 14, respectively. The isolates were identified by MALDI-TOF and antimicrobial susceptibility to methicillin, chloramphenicol, erythromycin, fusidic acid, gentamicin and tetracycline and mupirocin determined using the CLSI's disc diffusion and/or Etest, as previously reported [4-6,10].

At least 10 colonies for each isolate were inoculated into culture broth and genomic DNA was extracted using EZ1 DNA Investigator kit (Qiagen, Hilden, Germany). The isolates were sequenced using an Illumina HiSeq 1500 Platform (Illumina, California, USA) at the Genome Research Center of the University of Hong Kong at >50-fold coverage. A commercial software package (CLC Genomics Workbench 9.01) was used for *de novo* assembly. A methicillin-susceptible *S. lugdunensis* strain K93G (GenBank CP017069) was used as a

reference [6]. Contigs that could not be mapped onto the reference were further analyzed using BLAST search. Overlapping contigs were concatenated into supercontigs and the SCC*mec* assembly was further improved using a Sanger pipeline [6,12]. Any remaining gaps between SCC*mec* contigs were closed by PCR and Sanger sequencing (Table S1).

Genes were annotated by RAST [13]. SCC*mec* types and subtypes were assigned according to published guidelines [14]. Subtypes were defined by the presence of specific DNA sequences located in the J regions and designated using Arabic numbers in order of J1, J2 and J3 with periods in between [14]. Where a J region was absent, 0 was assigned to indicate the deletion. In *S. aureus*, three SCC*mec* V subtypes have been described including subtype V.1.1.1 (strain WIS), subtype V.2.2.2 (strain PM1) and subtype V.3.1.2 (strain JCSC6944) [14,15]. The *ccr* gene allotypes and alleles were assigned as previously described [14,16]. Core genome alignment, variant calls and phylogenetic tree construction were performed as previously described [17]. In brief, after mapping quality filtered and trimmed reads to *S. lugdunensis* strain K93G using SMALT v0.7.6, SNPs were called and filtered with QUAL>50, depth of coverage ≥50 and a minimum alternate allele frequency ≥0.9 using SAMtools v1.3.1 [18] and VarScan [19]. A maximum likelihood (ML) phylogeny was constructed with an General Time Reversal (GTR) nucleotide substitution model and 1000 times of bootstrap test using PhyML v3.0 [20].

RESULTS

The ST-SCC*mec* combinations in the 36 MRSL isolates were as follows: ST3-SCC*mec* IV (*n*=2), ST3-SCC*mec* V (*n*=28), ST27-SCC*mec* V (*n*=5) and ST42-SCC*mec* V (single locus variant of ST27, *n*=1). Resistance to non-beta-lactam drugs (11-39%) was common among the isolates and correlated with the presence of plasmid contigs encoding *cat* (n=4), *ermC* (n=6), *aacA-aphD* (n=14), *ileS2* (n=5) and *tetK* (n=13) genes (Table S2).

The full lengths of SCC*mec* elements in the 36 MRSL isolates were assembled. According to their junctional sequences, the SCC*mec* elements were further categorized into 10 different subtypes. The two ST3-SCC*mec* IV elements belonged to subtype IV.7.1.1 (size 23.6 kb). The 28 ST3-SCC*mec*V elements belonged eight different subtypes including V.4.1.1 (size~70.5 kb, *n*=13), V.4.2.2 (size 80.4 kb, *n*=4), V.6.1.1 (size 53.3 kb, *n*=3), V.7.1.1 (size 23.6 kb, *n*=3), V.5.2.2 (size 63.3 kb, *n*=2) and one each of V.4.1.3 (size 70 kb), V5.1.1 (size 51.4 kb) and V.9.0.2 (size 14.1 kb). Subtype V10.0.2 (size 28.9 kb) was identified in the ST27-SCC*mec* V (*n*=5) and ST42-SCC*mec* V (*n*=1) isolates.

Subtype IV.7.1.1 element harbored by the two ST3/SCC*mec* IV isolates was highly identical to the SCC*mec* IV.7.1.1 element harbored by the community-associated methicillin-resistant *S. aureus* (CA-MRSA) JCSC6668 (GenBank accession AB425823, 91.5% coverage and 99.4% identity; from Sweden in 1999) [21]. Sequence alignment and blast search showed that four SCC*mec* V subtypes harbored by our isolates (including 7 ST3, 5 ST27 and 1 ST42) shared high identity with the SCC*mec* V.2.2.2 harbored by the CA-MRSA strain PM1 (GenBank accession AB462393, from Taiwan in the 2000s) [22] over the J3-*mec* complex-J2 region (97.5%-99.8% identities, Figure 1). Subtype V10.0.2 harbored by our isolates (5 ST27 and 1 ST42) was highly similar to SCC*mec*$_{\text{CMUH-22}}$ (KP307925, 96.3% coverage and 99.9% identity) harboured by a Taiwanese MRSL. Variations in the J1 regions of the subtypes seem to have occurred from genetic events involving insertion and deletion and modules with high sequence identities to the ACME element in *S. epidermidis* I23OR2 (GenBank MH188478) [23] and SCC*mec* in *S. aureus* COL (GenBank CP000046) [24] were found (Figure 1). The SCC*mec* elements were demarcated by two 18-bp direct repeats, DR1 on the right chromosomal-SCC*mec* junction (DR$_{\text{SCC}}$-R) and DR3, DR4 or DR5 at the left SCC*mec*-

chromosomal junction (DR_{SCC}-L). Subtypes with the same pair of DR_{SCC}-R and DR_{SCC}-L also shared an identical *ccrC*1 allele (either *ccrC*1-3 or *ccrC*1-8) in the J3 region. In some of the subtypes, additional 18-bp DRs (DR2, DR3, DR6) could be identified in the J1 region.

The remaining five SCC*mec* V subtypes were harboured by 21 ST3 isolates (Figure 2). Sequence comparison revealed that they shared high identity with SCC*mec* V.1.1.1 harbored by the CA-MRSA WIS (GenBank accession AB121219, from West Australia in the 1990s) [25] over the J3-*mec* complex-J2 region (93.9%-100% identities). However, the *ccrC*1 allele in our *S. lugdunensis* isolates (*ccrC*1-11, Figure S1) was different from that in strain WIS (*ccrC*1-1). Despite the dissimilarities in the gene content of the subtypes with WIS-like and PM1-like J3-*mec* complex-J2 regions, they shared similar modules of genes in their J1 regions, including the direct repeats (DR2, DR3) within J1 region. In 20 isolates, the harbored SCC*mec* elements have an identical pair of DR_{SCC}-R (DR7) and DR_{SCC}-L (DR4). The DR_{SCC}-R and DR_{SCC}-L in the remaining isolate were DR6 and DR4, respectively. The direct repeats at the two ends of $SCCmec_{WIS}$ were different (DR8 and DR9). Overall, 23 and 30 SCC*mec* V elements had the restriction-modification *hsd* system and *pls* surface protein gene, respectively within the J1 regions. However, the *hsd* genes in the *S. lugdunensis* SCC*mec* elements were different from that those in $SCCmec_{PM1}$ (42.3% identity) or $SCCmec_{WIS}$ (41.3% identity). According to the specific sequences in the J regions, eight SCC*mec* V subtypes (including V.4.2.2, V.5.5.5, V.9.0.2, V.4.1.3, V.4.1.1, V.5.1.1, V.6.1.1 and V.7.1.1) in our isolates were novel.

To investigate the phylogenetic relationship of the 36 isolates carrying different SCC*mec* subtypes, we constructed a Maximum Likelihood tree based on 10,496 total SNPs in their genomes (Figure S2). The tree topology and the pairs of DR_{SCC}-R/DR_{SCC}-L were compatible

with five separate SCC*mec* insertional events in the isolates: group 1 (*n*=2, subtype IV.7.1.1 and DR2/DR4), group 2 (*n*=1, subtypes V.4.1.3 and DR6/DR4), group 3 (*n*=20, subtypes V4.1.1, V5.1.1, V6.1.1, V7.1.1 and DR7/DR4), group 4 (*n*=7, subtypes V4.2.2, V5.2.2, V.9.0.2 and DR1/DR4) and group 5 (*n*=6, subtype V.10.0.2 and DR1/DR3).

DISCUSSION

This study showed that emerging SCC*mec* IV and V elements in *S. lugdunensis* were related to those harbored by CA-MRSA. Based on the sequence over the J3-*mec* complex-J2 region, the SCC*mec* V subtypes may be considered to be variants of two prototype elements, SCC*mec*$_{PM1}$ and SCC*mec*$_{WIS}$ in *S. aureus*. The analysis further suggests that similar SCC*mec* elements have inserted multiple times into the ST3 background. The most common SCC*mec* subtype was V.4.1.1. Correlation of the phylogenetic tree with the SCC*mec* subtypes suggested that SCC*mec* sequence variations have occurred after the chromosomal insertion of SCC*mec*$_{PM1}$ and SCC*mec*$_{WIS}$ -like elements (Figure S2) and multiple subtypes were identified in isolates of both healthcare-associated and community-associated origins. Interestingly, isolates carrying the same subtypes were detected in both healthcare-associated and community-associated infections, raising the possibility that MRSL is emerging in multiple settings.

The *hsd* system and *pls* gene [16,25], which may contribute to SCC*mec* stability and host colonization, respectively, were detected within the J1 region in 64% (23/36) and 83% (30/36), respectively of the isolates in the collection (Figure 1 and 2). In the SCC*mec* harboured by the two CA-MRSA strains PM1 and WIS, the *hsd* system but not the *pls* gene was found in the J1 region.

In both SCC*mec*$_{PM1}$ -like and SCC*mec*$_{WIS}$ -like groups of subtypes, the variable J1 region is mosaic and common DRs (DR2, DR3, DR6) were observed. Since integration site sequence for the *ccr* gene complex were found within these DRs, it is likely that the J1 sequence diversity is partly a result of *ccr*-mediated recombination. Previous studies have described multiple DRs and mosaic modular structures within diversified ACME elements in *S. epidermidis* [23]. In some of the isolates, ACME elements were found with adjoining SCC*mec* or other operons as composite islands [23]. Based on the DR$_{SCC}$-R/DR$_{SCC}$-L analysis, we assigned the mosaic sequences to the J1 region and not as modules outside the SCC*mec* element. As in the prototype SCC*mec* V elements harboured by *S. aureus*, the variant subtypes harbored by *S. lugdunensis* do not carry any antibiotic resistance genes besides *mecA*. The multidrug-resistance phenotype in the isolates was caused by additional resistance genes harboured on plasmids.

In conclusion, our findings highlight the diversified structures of SCC*mec* elements among MRSL strains of the emerging ST3 lineage.

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Figure 1. Linear maps of 4 SCC*mec* V subtypes harbored by 13 *S. lugdunensis* isolates in this study. Previously described SCC*mec* elements in CA-MRSA PM1 (AB462393, from Taiwan in the early 2000s), *S. lugdunensis* CMUH-22 (KP307925, from Taiwan in 2010) and *S. lugdunensis* CHUH-25 (KP307924, from Taiwan in 2010) were included for comparison. The *mec* class and the *ccr* gene complex were indicated by red and blue shading, respectively. The joining J1, J2 and J3 regions, and regions with identities to partial sequence of ACME element in *S. epidermidis* I23OR2 (MH188478) and SCC*mec* element in *S. aureus* COL (CP000046) were indicated on top. The host bacterial species, representative SCC*mec* element with the strain name in subscript, SCC*mec* V subtype and number of isolates with the subtypes are labeled for each. The DRs are indicated by thin arrows and correspond to DR sequences in Table S3 in the supplementary file.

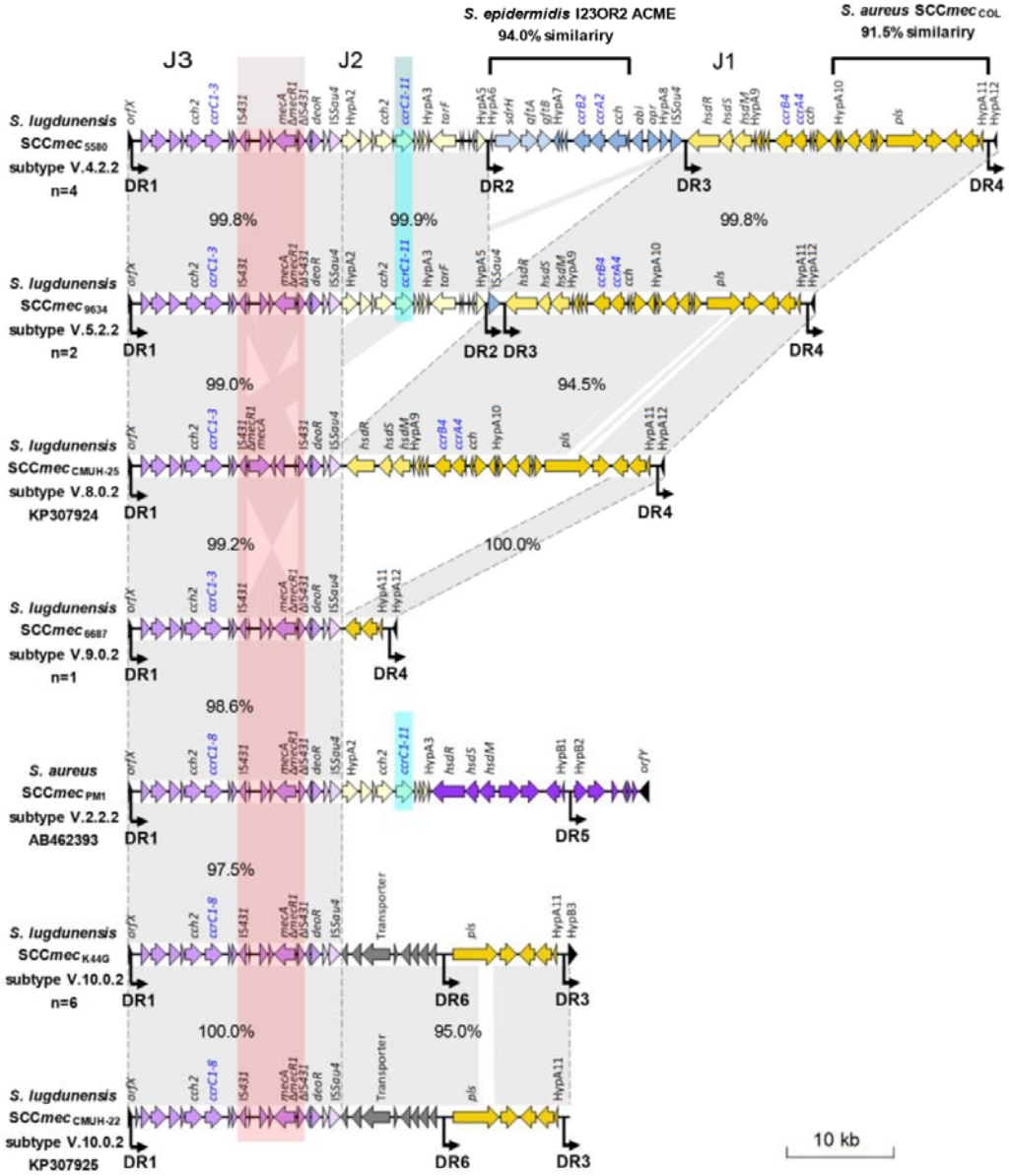

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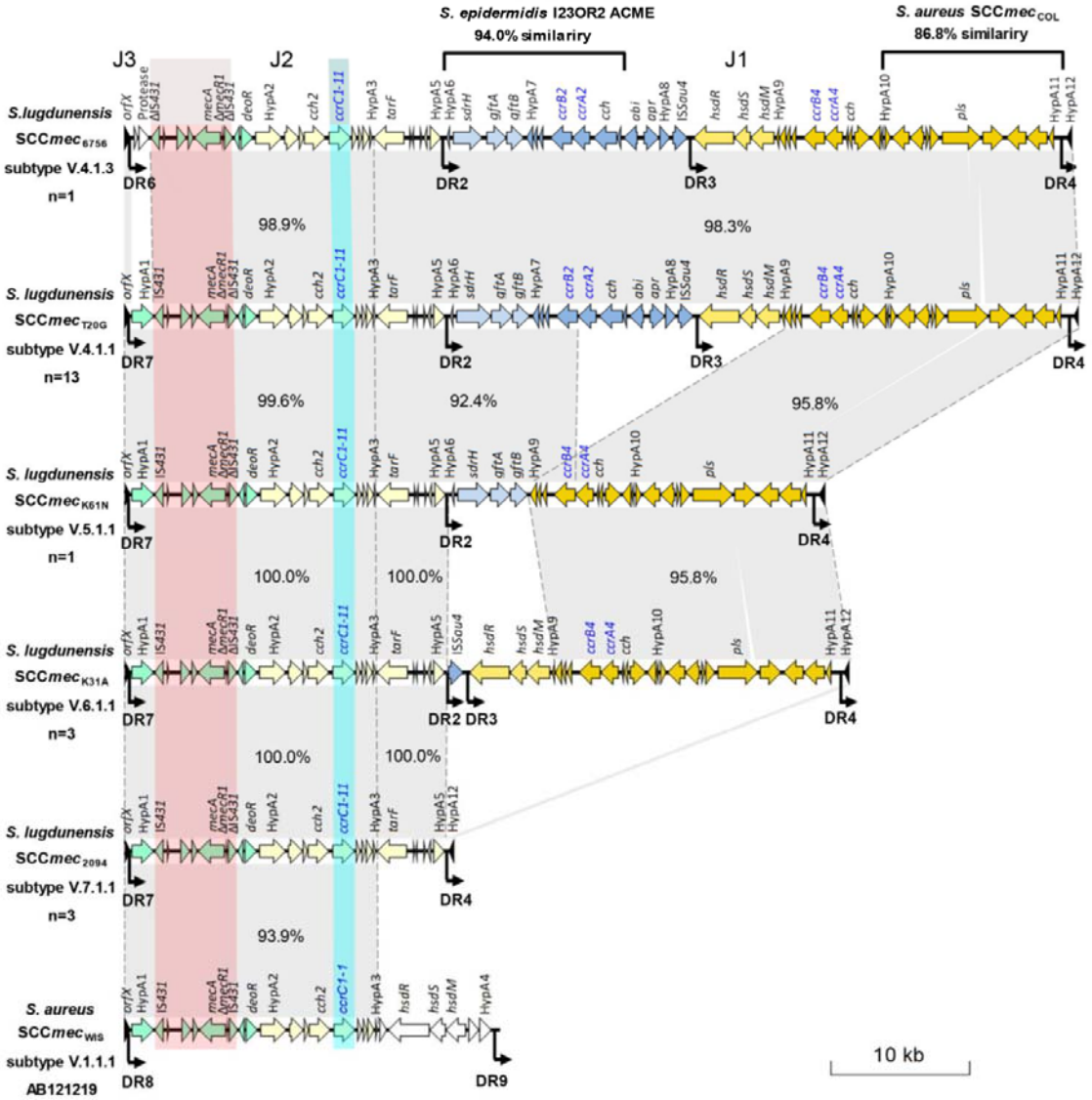
Figure 2. Schematic representation of five SCC*mec* V subtypes harbored by 21 *S. lugdunensis* isolates in this study. Previously described SCC*mec* element in CA-MRSA WIS (AB121219, from West Australia in 1990s) was included for comparison. The *mec* class and the *ccr* gene complex were indicated by red and blue shading, respectively. The joining J1, J2 and J3 regions, and regions with identities to partial sequence of ACME element in *S. epidermidis* I23OR2 (MH188478) and SCC*mec* element in *S. aureus* COL (CP000046) were indicated on top. The host bacterial species, representative SCC*mec* element with the strain name in subscript, SCC*mec* V subtype and number of isolates with the subtypes are labeled for each. The DRs are indicated by thin arrows and correspond to DR sequences in Table S3 in the supplementary file.



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