Staphylococcal cassette chromosome mec containing a novel mec gene complex, B4

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Objectives: To describe a new subclass of mec class B complex identified in Staphylococcus epidermidis.

Methods: Four S. epidermidis isolates obtained from bloodstream infections in patients at University Medical Center Groningen (UMCG) were analysed by phenotypic antibiotic susceptibility testing and WGS.

Results: Sequence analysis revealed a new staphylococcal cassette chromosome mec (SCCmec) structure in isolate UMCG335. In this structure, plasmid pUB110 was found to be integrated into SCCmec IVc, creating a new SCCmec subtype, IVUMCG335. SCCmec IVc and a copy of plasmid pUB110 were found in other isolates, UMCG336 and UMCG341, respectively, indicating a probability that SCCmec IVUMCG335 could have evolved at the UMCG. SCCmec of UMCG337 contained a new genetic organization of the mec complex (IS431-ΔmecR1-mecA-IS431-pUB110-IS431-qIS1272) that we have named B4. This new subclass of mec class B complex originated by IS431-mediated inversion of the DNA segment encompassing the plasmid and most of the genes of the mec complex with the exception of IS1272. As the SCCmec organization in UMCG337 differed by the inversion of an ~10 kb sequence compared with SCCmec IVUMCG335, we have named it SCCmec subtype IVUMCG337. Isolates UMCG335 and UMCG337 carrying SCCmec IVUMCG335 and IVUMCG337, respectively, were associated with a restriction-modification system and a CRISPR-Cas system, creating a composite island of almost 70 kb.

Conclusions: Our findings highlight the importance of IS431 in the evolution of the SCCmec region. The increasing genetic diversity identified in the SCCmec elements imposes a great challenge for SCCmec typing methods and highlights possible difficulties with the SCCmec nomenclature.

Introduction

Methicillin-resistant staphylococci produce an altered PBP encoded by mecA (PBP2a) or mecC (PBP2c), with the genes located in a mobile genetic element called staphylococcal cassette chromosome mec (SCCmec). SCCmec elements integrate into the staphylococcal chromosome at a specific site at the end of the rlmH (orfX) gene and are flanked by direct and inverted repeat sequences (DRs and IRs, respectively). Fourteen types of SCCmec have been distinguished to date based on the combination of the mec gene complex class and the type of ccr gene complex, which is represented by the ccr gene allotype.1 The mec complexes differ by the presence or partial deletion of mecA regulatory genes (mecR1 and mecI) and the presence and location of insertion sequences IS431 and IS1172. SCCmec subtypes harbour variable J (‘junkyard’) regions, which contain other genetic elements, such as plasmids, transposons and antimicrobial and heavy metal resistance genes.

Specific staphylococcal species may be sources of particular SCCmec elements. In particular, Staphylococcus epidermidis appears to be a reservoir of SCCmec type IV that can spread to the most virulent staphylococcal species, Staphylococcus aureus.2 SCCmec type IV harbours class B mec and ccrAB allotype 2 complexes. At least five variants of the class B mec complex have been reported and they have been designated B, B1, B2, B3 and B(L).3,4 To date, 13 subtypes (IVa–IVo) have been described.5,6 In this study, we report a new subclass of class B mec originating from

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Materials and methods

Bacterial isolates

S. epidermidis isolates analysed in this study were obtained during an ongoing study on multiresistant strains recovered from bloodstream infections in patients at University Medical Center Groningen (UMCG), The Netherlands. Isolate UMCG364 was obtained from a 51-year-old patient in 2017, while isolates UMCG335, UMCG337 and UMCG341 were recovered from 40-year-old, 5-week-old and 56-year-old patients, respectively, in 2018.

Phenotypic antibiotic susceptibility testing

Resistance to β-lactam antibiotics (benzylpenicillin, oxacillin, cefoxitin and ceftaroline) was determined using Etest methodology (bioMérieux). MSSA ATCC 25923 was used as a control. Results were interpreted according to EUCAST clinical breakpoints.

Extraction of genomic DNA

The cells were lysed using lysozyme (100 mg/mL) and total DNA was purified using the MagAttract HMW DNA Kit (Qiagen). DNA was quantified using Qubit (ThermoFisher Scientific) and the quality assessed by TapeStation 2200 (Agilent Technologies). A NanoDrop 2000C spectrophotometer (ThermoFisher Scientific) was used to measure the purity of extracted DNA.

Illumina and Oxford Nanopore sequencing

Illumina genomic libraries were sequenced on a HiSeq system with a 2 x 150 paired-end protocol. Oxford Nanopore sequencing libraries were prepared using the Ligation Sequencing Kit (SQK-LSK109) and sequenced on a MinION device using flow cell type R9.4.1 (FLO-MIN106D).

Sequence assembly

For each isolate, nanopore data were sorted by length and the longest reads up to an expected depth of coverage of 100 de novo assembled with the XNG assembler (DNASTAR). Chromosomes were assembled into single contigs with a typical depth of coverage of 70, as were large plasmids. Contig consensus sequences were refined and small plasmids identified with an automated polishing workflow (DNASTAR) using the Illumina data. Polished assemblies were manually corrected using SeqMan Ultra (DNASTAR) and final sequences annotated with NCBI’s Prokaryotic Genome Annotation Pipeline (version 2020–02-06.build4373).

Data analysis

The DNA sequences were aligned using BLASTn software (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The MLST STs were assigned using the MLST server (www.cbs.dtu.dk/services/MLST). Clustered regularly interspaced short palindromic repeats (CRISPR) were identified using the CRISPRFinder program (https://crispr.i2bc.paris-saclay.fr/Server/). Preliminary typing of SCCmec elements was conducted by SCCmecFinder 1.2 (https://cge.cbs.dtu.dk/services/SCCmecFinder/).

Nucleotide sequence accession numbers

The chromosome sequences for UMCG335, UMCG337, UMCG341 and UMCG364 have been deposited in GenBank under the accession numbers CP064453, CP064640, CP064631 and CP064549, respectively.

Results

The four study isolates belonged to two STs: ST2 (UMCG341) and ST23 (UMCG335, UMCG337 and UMCG364). All isolates carried the mecA gene and were phenotypically methicillin resistant. The isolates were resistant to oxacillin, cefoxitin and benzylpenicillin, and susceptible to ceftaroline.

SCCmec of UMCG335 shared high nucleotide identity (99.9%) with SCCmec IVc of UMCG364 with an identical mec class B complex and ccrAB allele type 2, but differed by the presence of an integrated plasmid pUB110 downstream of mecA (Figure 1a). pUB110 carries genes for replication (rep), recombination (rec) and antimicrobial resistance, bleO and aadD, conferring resistance to bleomycin and aminoglycosides, respectively. The ST2 isolate, UMCG341, harboured SCCmec III with an integrated pUB110 plasmid (Figure 1a). The pUB110 sequences of isolates UMCG335 and UMCG341 were almost identical, differing by only two nucleotides, while the J regions of SCCmec were unrelated. This suggests that the plasmid was inserted into SCCmec IVc at UMCG. IS431 elements flanked both ends of pUB110 (Figure 1a), suggesting a role in plasmid integration. This SCCmec of UMCG335 contains a genetic structure not previously described. Therefore, we propose that this new SCCmec subtype should be classified as SCCmec IVUMCG335. BLASTn searches of the GenBank database with SCCmec of UMCG335 revealed the presence of an almost identical sequence in five S. aureus chromosomes (accession numbers CP051982, CP051972, CP019563, CP045439 and CP045435), but none in S. epidermidis.

The mec complex in UMCG337 resembled a mec class B complex, but its genetic organization was divergent, due to an inversion of the DNA segment spanning pUB110 and almost the entire mec complex with the exception of IS1272 (Figure 1b). It is likely that the IS431 transposase, Tnp431, catalysed IS431 movement to a new site and inversion of adjacent DNA as an additional IS431 element was found downstream of pUB110. Although, a truncated mecR1 was located upstream of mecA, and IS431 downstream of mecA, their gene order was inverted compared with the prototypic mec class B. Thus, SCCmec of UMCG337 contained a new mec complex organization (IS431-ΔmecR1-meca-IS431-pUB110-IS431-ψIS1272) that we have named B4 because of its similarity to mec complex B (IS431-mecA-ΔmecR1-ψIS1272). BLASTn searches did not identify any comparable sequences. As the SCCmec organization in UMCG337 differed by the inversion of an ~10 kb sequence compared with SCCmec IVUMCG335, we have named it SCCmec subtype IVUMCG337.

Isolates UMCG335 and UMCG337 carried SCCmec IV associated with a restriction-modification system and a CRISPR-Cas system, creating a large composite island of 69 075 and 69 878 bp in size, respectively (Figure 2). The regions located between short direct repeats DR3 and DR4, and DR4 and DR2 had already been identified together with SCCmec type II in a composite island of S. epidermidis RP62a, but have never been shown to be associated with SCCmec IV, as was determined for the isolates analysed in the current study. SCCmec of UMCG364 was not associated with any mobile element and was flanked by a core genome.

Discussion

The insertion, duplication and movement of IS431 as well as homologous recombination between different copies of IS431 play a
significant role in the continuous evolution of SCCmec. Katayama et al. revealed that the IS431 translocation associated with the deletion of mecI and mecR1 in the mecA gene complex correlated with phenotypic expression of methicillin resistance in mecA-carrying Staphylococcus haemolyticus. In another study, a potential role of IS431 in the excision of the mecA gene was shown. Conversely, characterization of a complex containing mecA, but lacking the ccr genes, in S. haemolyticus suggested that the structure containing mecA bracketed by two copies of IS431 in the same orientation might have formed a composite transposon.
could explain the absence of ccr in some staphylococcal isolates carrying mecA. IS431 was also implicated in the integration of plasmids into the chromosome.13 In this study, we found two IS431 copies flanking pUB110 and likely mediating its chromosomal integration.

When two IS elements of the same type are located in the same orientation and flank one or more genes they create a composite transposon (IS-unique_segment-IS). These IS elements can recombine between each other forming translocatable units (TU) consisting of the internal DNA segment of a composite transposon and one copy of a flanking IS (IS-unique_segment or unique_segment-IS).14 Such TUs recognize another IS of the same type as a target in the chromosome or on a plasmid. Subsequent recombination between TU and IS leads to directly oriented copies of IS at each end and the unique segment between them. This model was described for some IS elements, including IS26, IS257, IS1216 and, recently, IS431.15 The IS431-pUB110-IS431 structure found in SCCmec IVUMCG335 could result from the recombination of an IS431-pUB110 TU of unknown origin with the IS431 copy of UMCG364 SCCmec IVc (Figure 1A). Interestingly, pUB110 is commonly found inserted in SCCmec III, which is typically found in the MDR, hospital-adapted S. epidermidis ST2 BPH0662 lineage.16 At UMCG, we found a member of this lineage (UMCG341), a possible source of IS431-pUB110 TU (Figure 1a).

SCC elements are genomic islands widely prevalent in staphylococci that carry antibiotic and heavy metal resistance genes and also virulence-associated genes.17 Moreover, the epidemiological success of MRSA USA300 has been related to the acquisition of two mobile elements, the arginine catabolic mobile element (ACME) and the copper and mercury resistance element.18 Genome-wide analyses revealed that different mobile elements were co-transferred as the full identity of the entire ACME-SCCmec composite island in two distinct MRSA lineages was determined as well as SCCmec and SCC-like elements could be transferred independently.19,20 In this study, we identified composite islands in UMCG335 and UMCG337 consisting of SCCmec type IV together with restriction-modification and CRISPR-Cas systems responsible for protecting bacteria against foreign DNA. The presence of the CRISPR system has been rarely reported in S. epidermidis. Therefore, the prevalence of mobile elements containing the CRISPR-Cas system in the S. epidermidis ST23 lineage and its influence on mosaicisms of genomic islands should be explored.

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