Novel staphylococcal cassette chromosome *mec* (SCC*mec*) type XV (7A) and two Pseudo-SCC*mec* variants in foodborne methicillin-resistant *Staphylococcus aureus* in China

Running title: Novel SCC*mec* type XV(7A) and variants in China

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Background: Staphylococcal cassette chromosome *mec* (SCC*mec*) elements are highly diverse and have been classified into 14 types. Novel SCC*mec* variants have been frequently detected from humans and animals but rarely from food.

Objectves: To characterize a novel SCC*mec* type and two SCC*mec* variants identified from food-associated MRSA in China.

Methods: Three MRSA (NV_1, NT_611 and NT_8) collected from retailed foods in China were subjected to WGS and the SCC*mec* elements were determined.

Results: The novel SCC*mec*XV identified in NV_1 carried the *mec* gene complex class A (*mecI-mecR1-mecA-IS431*) and the *ccr* gene complex 7 (*ccrA1B6*), while a *Tn558*-mediated phenicol exporter gene *fexA* was detected in this SCC*mec*XV cassette. The pseudo-SCC*mec* elements ΨSCC*mec*$_{NT\_611}$ and ΨSCC*mec*$_{NT\_8}$ showed a truncated SCC*mec* pattern carrying the class C2 *mec* gene complex but missing the *ccr* genes. The ΨSCC*mec*$_{NT\_611}$ element shared more similarities with those of *S. haemolyticus* (AB478934.1) and carried a heavy metal resistance gene cluster *cadD-cadX-arsC-arsB-arsR-copA*. The ΨSCC*mec*$_{NT\_8}$ MRSA exhibited extensively MDR, showing an absence of a 19.3 kb segment comparing with the reference SCC*mec*XII element (CP019945.1). Notably, a 46 kb region containing multiple transposons encoding antimicrobial or metal resistance genes flanked by IS*431* or IS*256* was identified ~30 kb downstream from the *mec* gene complex in ΨSCC*mec*$_{NT\_8}$, which might explain such high MDR phenotype in MRSA NT_8.

Conclusions: Our finding of novel and pseudo-SCC*mec* elements reflected the ongoing intra/interspecies genetic rearrangements in staphylococci. Further study will be needed to investigate the biological significance and prevalence of those SCC*mec* variants along the food chain.

Introduction

MRSA has been considered to be a major healthcare-associated pathogen (HA-MRSA) since it was first reported as a typical 'superbug' in 1961.[1,2] Meanwhile, MRSA has been recently thought to be an important foodborne pathogen that leads to potential community infections (CA-MRSA).[3,4] Typically, MRSA is generated when a MSSA strain exogenously acquires a staphylococcal cassette chromosome *mec* (SCC*mec*) element that leads to the acquisition of β-lactam antibiotic resistance.[5,6] Typing of SCC*mec* element (SCC*mec* type) could help better understand the phylogenetic and epidemiological origin of MRSA lineages.[7,8]

SCC*mec* elements are composed of a methicillin resistance determinant (*mecA*, *mecB*, or *mecC*) contained within a *mec* gene complex and include site-specific recombinase genes (*ccrAB* or/and *ccrC*), which are responsible for the insertion of the SCC*mec* cassette into the core genome.[9] The *ccrA*, *ccrB*, and *ccrC* genes showed nucleotide similarities lower than 50%, while in general, *ccr* genes with nucleotide identities of more than 85% would be assigned to the same allotype. Based on different *ccr* allotypes and their combinations, nine *ccr* gene complex types have been identified in MRSA.[9,10]

SCC*mec* elements are highly diverse based on their combination of the *mec* gene class complex and the *ccr* gene complex.[7] Since its first identification and characterization in 1999,[11] novel MRSA genotypes have emerged due to the acquisition of variants of the SCC*mec* elements. According to the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC), a total of 14 SCC*mec* types (I–XIV) are recognized, based on the combinations of five *mec* complexes (class A, B, C1, C2 and E) and nine *ccr* gene complexes (types 1 - 9) so far.[7-10,12,13] Moreover, a rising number of variants of SCC*mec* elements that harbor composite cassettes as well as pseudo-SCC*mec* elements but do not harbor *ccr* genes were also detected in recent years.[14-16]

Our laboratory has been in charge of an ongoing investigation of *S. aureus* among retailed foods since 2010 in China. Annually, about 1,000 retailed food-associated *S. aureus* isolates were collected from supermarkets and farms' markets in over 30 provinces by local food safety control agencies. As previously reported, in 2015, a total of 1,150 *S. aureus* were cultured from 27,000 retail food samples across over 200 cities in China and 7.9% (91/1,150) were found to be MRSA.[3] More than 300 MRSA genomes have been obtained with Illumina

sequencing until 2018, most of which could be assigned the known SCC*mec* type by SCC*mec*Finder, with SCC*mec*IV and SCC*mec*V being the predominant SCC*mec* types.[17,18] However, a few isolates had non-typable SCC*mec* elements that cannot be assigned to any known SCC*mec* type from the assembled genomes. To obtain the complete genome and study the element diversity of these isolates, we re-sequenced the representatives through long read sequencing technique with the aim of trying to characterize a novel SCC*mec* type and SCC*mec* variants.

Materials and methods

Bacterial strains

A total of 360 MRSA were identified from approximately 8,000 *S. aureus* isolates from various food products between 2010 and 2018 in China.[3,17,18] Most MRSA strains could be typed by SCC*mec*_CLA (https://github.com/fesepc/SCCmec_CLA) and SCC*mec*Finder, while several strains lacked the *ccr* genes or seemed to carry the novel SCC*mec* elements. In total, nine strains were found to have non-typeable SCC*mec* elements, exhibiting three patterns grouped by ST types (ST5, ST88, and ST3686), and one strain from each of these three ST types coded NT_611, NT_8, and NV_1, respectively were selected for the following analysis **(Table S1)**.

Genome sequencing and Assenmbly

To obtain complete and accurate genome sequence of the SCC*mec* elements, both Illumina sequencing and Pacbio sequencing were performed for all strains used in this study. Genomic DNA (gDNA) was purified from the above three isolates using the Omega EZNA Bacterial DNA kit (Omega Bio-tek, Norcross, GA, USA). The gDNA was then dispatched for *de novo* sequencing using the Illumina NovaSeq PE150 platform at the Beijing Novogene Bioinformatics Technology Co., Ltd, and for whole genome sequencing using the SMRT® Pacific Biosciences RS II platform at Tianjin Biochip Corporation. The PacBio and Illumina sequencing reads were assembled *de novo* using a hybrid assembly algorithm implemented in Allpaths-LG software (v44620; http://www.broadinstitute.org/software/allpaths-lg/blog/).[19]

Identification of SCC*mec* elements and bioinformatics analysis

The boundaries of SCC*mec* were identified by the direct repeat (DR) sequences.[7] The ORF finder program (http://www.ncbi.nlm.nih.gov/orffinder) was further used to identify ORFs features of more than 75 bp in the SCC*mec*, and their functions were predicted by searching with the 14 known types of SCC*mec* using BLASTN.[20] The core SCC*mec* elements including the *mec* gene complex and *ccr* gene complex were

also identified by SCCmec_CLA (https://github.com/fesepc/SCCmec_CLA) using the default setting and SCC*mec*Finder with parameters set at -identity 90 -coverage 60.[18] A phylogenetic tree was constructed to determine the phylogenetic relations of *ccr* genes between our novel SCC*mec* and other reported SCC*mec*. The nucleotide sequences of 16 *ccrA* genes, 16 *ccrB* genes, and 9 *ccrC* genes were aligned using the ClustalX 2.1 program.[21] The phylogenetic tree was generated with the neighbor-joining method by creating 2,000 bootstrap replicates.[22] The tree was visualized with iToLv4.[23]

AST

All MRSA strains were subjected to AST using the Biofosun® Gram-negative panels (Fosun Diagnostics, Shanghai, China) by broth dilution method.[24] The panel of antimicrobial compounds tested (0.5 μg/mL-1024 μg/mL) included penicillin, oxacillin, erythromycin, daptomycin, vancomycin, linezolid, ciprofloxacin, chloramphenicol, clindamycin, tetracycline, gentamycin and kanamycin. *S. aureus* ATCC®29213 was used as the control for AST.

Sequence data accession numbers

The nucleotide sequences obtained by the SMRT® Pacific Biosciences RS II platform of three MRSA NV_1, NT_8 and NT_611 have been deposited in GenBank under the accession numbers CP080249, CP080222, and CP080251, respectively. While the sequence-read archive data for all three strains sequenced by the Illumina NovaSeq PE150 platform have also been deposited in NCBI SRA under the accession numbers SRR15222366, SRR15222365, and SRR15222364, respectively.

Results and discussion

A Novel *SCCmec* type XV(7A) with uncommon resistance gene in NV_1

A MRSA strain NV_1 (typed as ST5) recovered from a Chinese salad sample carried one untypable SCC*mec* element that did not match with any of the 14 known SCC*mec* types. Among the complete genome, a total length of 33.2 kb region was identified as a SCC*mec* cassette by two integration site sequences (ISS) comprising DR sequences **(Figure 1)**. The element was acknowledged as novel by the IWG-SCC and designated SCC*mec* type XV (7A). In detail, this SCC*me*XV cassette carried the *mec* gene complex class A (*mecI-mecR1-mecA-IS431*) and the *ccr* gene complex 7 (*ccrA1* and *ccrB6*). The *ccrA1* and *ccrB6* genes in NV_1 showed 93% nucleotide identity with *ccrA1* and 90% nucleotide identity with *ccrB6* in SCC*mec* type X (AB505630.1), respectively **(Figure 1)**.[22] According to the IWG-SCC, the *mec* gene complex class A

is present in type II (D86934.2), III (AB037671.1), VIII (FJ390057.1), and XIII (MG674089.1) SCC*mec* cassettes along with different *ccr* gene complexes including *ccrA2B2*, *ccrA3B3*, *ccrA4B4*, and *ccrC2*, respectively **(Figure 1)**.[12,25,26] Moreover, the phylogenetic tree developed by known *ccr* genes depicts a clustering of the *ccrA1* and *ccrB6* genes, supporting the proposed nomenclature **(Figure S1)**. Therefore, this type of SCC*mec* was designated as a novel type XV SCC*mec* (7A). In addition, *Tn558* which was previously detected on a stahoylococcal plasmid but rarely reported from the SCC*mec* elements, was detected along with the phenicol exporter gene *fexA* in this SCC*mec* cassette. This suggests that this genetic vehicle might act as a tool to capture resistance genes in the future.[27]

A pseudo-SCC*mec* lacking the *ccr* gene complex in NT_611

It has been well documented that the *mecA* gene becomes immobilized on the chromosome after losing the *ccr* gene complex and leads to a lower cost for SCC*mec* maintenance, which plays a role in the adaption of the host bacteria under antibiotic pressure.[28] In this study, the MRSA strain NT_611 (typed as ST88) recovered from a pork sample showed a truncated SCC*mec* pattern carrying the *mecA* gene but without *ccr* gene complex. This type of pseudo staphylococcal cassette chromosome (ΨSCC*mec*), designated as "ΨSCC*mec*$_{NT\_611}$", contained a class C2 *mec* gene complex that could entirely align with both the class C2 *mec* gene complex in SCC*mec* type V (KF593809.1) with 99.89% identity and in SCC*mec* type IX (AB505628.1) with 99.74% identity.[22,29] **(Figure 2)**. The ΨSCC*mec*$_{10A611}$ element (~17 kb) also harbored the heavy metal resistance genes including the cadmium operons (*cadD* and *cadX*), arsenical resistant complex genes (*arsC*, *arsB*, and *arsR*), and copper resistance gene *copA*. These are homologous to that of the SCC*mec* type IX in *S. aureus* JCSC6943 (AB505628.1) with 99.19% identity, and the staphylococcal cassette in *Staphylococcus haemolyticus* SH621 (AB478934.1) with 99.34% identity **(Figure 2)**. We further compared each coding sequence in ΨSCC*mec*$_{NT\_611}$ with the above two strains (AB505628.1 and AB478934.1) **(Table 1)** and found that more similarities were shared with *S. haemolyticus* coding sequences. Moreover, similar complex contexts containing *mecA* but lacking *ccr* genes have been frequently reported from *S. haemolyticus* but occasionally identified in MRSA.[30-33] Previous studies also revealed that *S. haemolyticus* and other CoNS harbor more diverse SCC*mec* elements than *S. aureus* and are believed to serve as the potential reservoirs for SCC*mec* elements transfering to *S. aureus*.[30,34]

A pseudo-SCC*mec* lacking the *ccr* gene complex in the high resistant MRSA strain NT_8

A novel pseudo-SCC*mec* (ΨSCC*mec*$_{NT\_8}$) was also identified in the MRSA strain NT_8 recovered from a chicken sample, typed as ST3686 (a single-locus variant of ST9). A recent study characterized two SCC*mec* variants, including a pseudo-SCC*mec* (*S. aureus* NX-T55, CP031839) and a rearrangement of SCC*mec* XII (*S. aureus* QD-CD9, CP031838) **(Figure 3)**.[35] The ΨSCC*mec*$_{NT\_8}$ element in this study contained a class C2 *mec* gene complex but negative for *ccr* genes. The class C2 *mec* gene complex regions were in the same orientation among NT_8, NX-T55 (CP031839), and QD-CD9 (CP031838), where the SCC*mec* XII (*S. aureus* BA01611, CP019945.1) was reversed.[9,35] In addition, the rearrangement pattern of an absence of a 19.3 kb segment was found in NT_8 and NX-T55 in comparion with those in QD-CD9 and the type strain SCC*mec*XII.

The results of AST showed that NT_8 exhibited an extensively multi-drug resistant (MDR) phenotype to a panel of antimicrobials employed with a minimum inhibitory concentration (MIC) ranging from 16 μg/mL to 256 μg/mL but susceptible to daptomycin, vancomycin, linezolid and chloramphenicol **(Table S2)**. To reveal the MDR mechanism of NT_8, the antimicrobial-resistant genes were screened . A 46 kb region that contains multiple transposons encoding antimicrobial or metal resistances flanked by insertion sequences of IS*431* or IS*256* located at ~30 kb downstream from the *mec* gene complex was found **(Figure 3)**. In this 46 kb multi-resistant region, downstream from two arsenic resistant genes *arsM-arsR*, the transposon Tn*552* that encodes the β-lactamase operon *blaI-blaR1-blaZ* and the serine resolvase gene *bin3* were identified. Of note, Tn*552* and sequences derived from it are almost ubiquitous in *S. aureus* isolates.[36] Moreover, it has already been revealed that Tn*552* insertion adjacent to transposons, within other transposons, or near the *res* sites can create DNA segments and then can be inverted or deleted by Bin.[37,38]

Two resistant genes *aacA-aphD* and *erm(C)* downstream Tn*552* were detected. The bifunctional AAC/APH gene of *aacA-aphD* located on the non-conjugative composite transposon Tn*4001* that confers resistance to aminoglycosides including gentamicin, tobramycin, and kanamycin was found. This resistant gene was flanked by two copies of the IS*256* elements in opposite orientations. Interestingly, the *aacA-aphD* gene within the Tn*4001* has been frequently detected from staphylococci of poultry origin,[39,40] suggesting persistent contamination of this resistant strains

within poultry industry. Additionally, the *erm(C)* gene was flanked by two copies of the IS*431* elements in the same orientation, recently found to be a translocatable unit (TU).[41] Moreover, the *erm(C)* gene is commonly located on various small plasmids (2.3 kb-4.0kb) in staphylococci, which can form cointegrates with other small resistance plasmids by using the staphylococcal recombination site A(RS_A).[42] Similar structure of this erm(C)-carrying TU region is also found in the corresponding chromosomal regions of *S. aureus* NX-T55 (CP031839) from swine and pSA01-tet (CP053076) in *S. aureus* SA01 from chicken, indicating that these MRSA have already been prevalent among food-producing animals. [35,40]

A *tet(L)* gene that encode efflux proteins conferring resistance to tetracycline, downstream from *erm(C)*-carrying TU and flanked by one IS*431* on the right was identified.[40] , A plasmid recombination enzyme type 2-encoding gene *pre* that could serve a function in plasmid maintenance and contribute to the distribution of small antibiotic resistance plasmids among Gram-positive bacteria upstream *tet(L)* element was identified followed by an aminoglycoside resistant gene cluster *aadK-apt-ant1* and the *lsa(E)* gene. The *lsa(E)* gene can encode an ABC transporter which confers resistance to pleuromutilins, lincosamides and streptogramin A antibiotics.[43] Nine genes downstream of *lsa(E)* gene, the kanamycin nucleotidyltransferase encoding gene *knt* flanked by two IS*431* elements in the same orientations was identified. Within this transposon, a *rep* gene that codes the initiator of plasmid replication was also identified.[44] At the end of this region is another arsenic resistant gene cluster *arsR-arsB-arsC* flanked by *bin3* upstream.

IS*431* can mediate the integration of small plasmids into either larger plasmids or the chromosomal DNA.[45,46] While, IS*256* that was originally identified as a bordering component of the composite aminoglycoside resistance-mediating transposon Tn*4001*, is a highly active in multi-resistant staphylococci and enterococci.[47] Therefore, the high drug resistance phenotype of NT_8 may be due to the transposons with high antimicrobial resistance features it contains. The resistant plasmids as well as the mobile elements inserted into the chromosome could give rise to high level of drug resistance of MRSA. Moreover, the right end (*attR*) of ΨSCCmec$_{NT_8}$ could not be identified. The most plausible explanation for this phenomenon might be owing to that the insertion of the 46 kb region enriched with antimicrobial and metal resistance genes lead to a deletion on the right end of ΨSCCmec$_{NT_8}$.

Conclusions

One novel SCC*mec* type XV (7A) and two Pseudo-SCC*mec* variants were identified from food-associated MRSA in this study. These uncommon and novel SCC*mec* elements reflect the ongoing intra/interspecies genetic rearrangements in staphylococci. To our best knowledge, this is the first report on Tn*558* containing *fexA* in a SCC*mec* element. The acquirement of antimicrobial resistant transposons in SCC*mec* might enhance the MDR phenotype of the novel pseudo-SCC*mec* ΨSCC*mec*$_{NT\_8}$. Further surveillance for these foodborne SCC*mec* variants along food chains is necessary in order to clarify the biological significance of these elements.

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Transparency declarations

None to declare.

Supplementary data

Figures S1, Tables S1 and Table S2 are available as Supplementary data at JAC Online.

References

1. Nada T, Ichiyama S, Osada Y, *et al*. Comparison of DNA fingerprinting by PFGE and PCR-RFLP of the coagulase gene to distinguish MRSA isolates. *J Hosp Infect* 1996; **32**:305-317.
2. Chen CJ, Huang YC. New epidemiology of *Staphylococcus aureus* infection in Asia. *Clin Microbio Infect* 2014; **20**:605-623.
3. Wang W, Baloch Z, Jiang T, *et al*. Enterotoxigenicity and antimicrobial resistance of *Staphylococcus aureus* isolated from retail food in China. *Front Microbiol* 2017; **8**:2256.
4. Umeda K, Nakamura H, Yamamoto K, *et al*. Molecular and epidemiological characterization of staphylococcal foodborne outbreak of *Staphylococcus aureus* harboring *seg*, *sei*, *sem*, *sen*, *seo*, and *selu* genes without production of classical enterotoxins. *Int J Food Microbiol* 2017; **256**:30-35.
5. Lakhundi S, Zhang K. Methicillin-resistant *Staphylococcus aureus*: molecular characterization, evolution, and epidemiology. *Clin Microbiol Rev* 2018; **31**:e00020-18.
6. Peacock SJ, Paterson GK. Mechanisms of Methicillin Resistance in *Staphylococcus aureus*. *Annu Rev Biochem* 2015; **84**:577-601.
7. International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC). Classification of staphylococcal cassette chromosome *mec* (SCC*mec*): guidelines for reporting novel SCC*mec* elements. *Antimicrob Agents Chemother* 2009; **53**:4961-4967.
8. Liu J, Chen D, Peters BM, *et al*. Staphylococcal chromosomal cassettes *mec* (SCC*mec*): A mobile genetic element in methicillin-resistant *Staphylococcus aureus*. *Microb Pathog* 2016; **101**:56-67.
9. Katayama Y, Ito T, Hiramatsu K. A new class of genetic element, *Staphylococcus* cassette chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2000; **44**:1549-1555.
10. Wu Z, Li F, Liu D, *et al*. Novel type XII staphylococcal cassette chromosome *mec* harboring a new cassette chromosome recombinase, CcrC2. *Antimicrob Agents Chemother* 2015; **59**: 7597-7601.
11. Ito T, Katayama Y, Hiramatsu K. Cloning and nucleotide sequence determination of the entire mec DNA of pre-methicillin-resistant Staphylococcus aureus N315. *Antimicrob Agents Chemother* 1999; **43**:1449 –1458.
12. Baig S, Johannesen TB, Overballe-Petersen S, *et al*. Novel SCC*mec* type XIII (9A) identified in an ST152 methicillin-resistant *Staphylococcus aureus*. *Infect Genet Evol* 2018; **61**:74-76.
13. Urushibara N, Aung MS, Kawaguchiya M, *et al*. Novel staphylococcal cassette chromosome *mec* (SCC*mec*) type XIV (5A) and a truncated SCC*mec* element in SCC composite islands carrying speG in ST5 MRSA in Japan. *J Antimicrob Chemother* 2020; **75**:46-50.

14. Wilson LK, Coombs GW, Christiansen K, *et al*. Characterization of a novel staphylococcal cassette chromosome composite island from community-associated MRSA isolated in aged care facilities in Western Australia. *J Antimicrob Chemother* 2016; **71**:3372-3375.
15. Perreten V, Chanchaithong P, Prapasarakul N, *et al*. Novel pseudo-staphylococcal cassette chromosome *mec* element (ψSCCmec57395) in methicillin-resistant *Staphylococcus pseudintermedius* CC45. *Antimicrob Agents Chemother* 2013; **57**:5509-5515.
16. Heusser R, Ender M, Berger-Bächi B, *et al*. Mosaic staphylococcal cassette chromosome *mec* containing two recombinase loci and a new *mec* complex, B2. *Antimicrob Agents Chemother* 2007; **51**:390-393.
17. Wang W, Baker M, Hu Y, *et al*. Whole-genome sequencing and machine learning analysis of *Staphylococcus aureus* from multiple heterogeneous sources in China reveals common genetic traits of antimicrobial resistance. *mSystems* 2021; **6**:e01185-20.
18. Kaya H, Hasman H, Larsen J, *et al*. SCC*mec*Finder, a web-based tool for typing of staphylococcal cassette chromosome *mec* in *Staphylococcus aureus* using whole-genome sequence data. *mSphere* 2018; **3**:e00612-17.
19. Gnerre S, Maccallum I, Przybylski D, *et al*. High-quality draft assemblies of mammalian genomes from massively parallel sequence data. *Proc Natl Acad Sci U S A* 2011; **108**:1513-1518.
20. Zhang Z, Schwartz S, Wagner L, *et al*. A greedy algorithm for aligning DNA sequences. *J Comput Biol* 2000; **7**:203-214.
21. Larkin MA, Blackshields G, Brown NP, *et al*. Clustal W and Clustal X version 2.0. *Bioinformatics* 2007; **23**:2947-2948.
22. Li SS, Skov RL, Han X, *et al*. Novel types of staphylococcal cassette chromosome *mec* elements identified in clonal complex 398 methicillin-resistant *Staphylococcus aureus* strains. *Antimicrob Agents Chemother* 2011; **55**:3046-3050.
23. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res* 2019; **47**:W256-W259.
24. CLSI. Performance *Standards for Antimicrobial Susceptibility Testing: Twenty-Fifth Edition: M100.* 2015.
25. Ito T, Okuma K, Ma XX, *et al*. Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: genomic island SCC. *Drug Resist Updat* 2003; **6**:41-52.
26. Zhang KY, McClure JA, Elsayed S, *et al*. Novel staphylococcal cassette chromosome *mec* type, tentatively designated type VIII, harboring class A mec and type 4 *ccr* gene complexes in a Canadian epidemic strain of methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2009; **53**:531-40.
27. Kadlec K, Feßler AT, Hauschild T, *et al*. Novel and uncommon antimicrobial resistance genes in livestock-associated methicillin-resistant *Staphylococcus aureus*. *Clin Microbiol Infect* 2012; **18**:745-755.

28. Zong ZY. Characterization of a complex context containing *mecA* but lacking genes encoding cassette chromosome recombinases in *Staphylococcus haemolyticus*. *BMC Microbiol* 2013; **13**:64.
29. Vandendriessche S, Vanderhaeghen W, Larsen J, *et al*. High genetic diversity of methicillin-susceptible *Staphylococcus aureus* (MSSA) from humans and animals on livestock farms and presence of SCC*mec* remnant DNA in MSSA CC398. *J Antimicrob Chemother* 2014; **69**:355-362.
30. Berglund C, Soderquist B. The origin of a methicillin-resistant *Staphylococcus aureus* isolate at a neonatal ward in Sweden-possible horizontal transfer of a staphylococcal cassette chromosome *mec* between methicillin-resistant *Staphylococcus haemolyticus* and *Staphylococcus aureus*. *Clin Microbiol Infect* 2008; **14**:1048-1056.
31. Hanssen AM, Sollid JUE. Multiple staphylococcal cassette chromosomes and allelic variants of cassette chromosome recombinases in *Staphylococcus aureus* and coagulase-negative staphylococci from Norway. *Antimicrob Agents Chemother* 2007; **51**:1671-1677.
32. Ruppé E, Barbier F, Mesli Y, *et al*. Diversity of staphylococcal cassette chromosome *mec* structures in methicillin-resistant *Staphylococcus epidermidi*s and *Staphylococcus haemolyticus* strains among outpatients from four countries. *Antimicrob Agents Chemother* 2009; **53**:442-449.
33. Yoon EJ, Lee H, Kim D, *et al*. Methicillin-resistant *Staphylococcus aureus* blood isolates harboring a novel pseudo-staphylococcal cassette chromosome *mec* element. *Front Microbiol* 2019; **10**:540.
34. Otto M. Coagulase-negative staphylococci as reservoirs of genes facilitating MRSA infection: Staphylococcal commensal species such as *Staphylococcus epidermidis* are being recognized as important sources of genes promoting MRSA colonization and virulence. *Bioessays* 2013; **35**: 4-11.
35. Jiang N, Li J, Feßler AT, *et al*. Novel pseudo-staphylococcal cassette chromosome *mec* element (φSCC*mec*T55) in MRSA ST9. *J Antimicrob Chemother* 2019; **74**:819-820.
36. Eto KY, Firth N, M. Davis A, *et al*. Evolution of a 72-kilobase cointegrant, conjugative multiresistance plasmid in community-associated methicillin-resistant *Staphylococcus aureus* isolates from the early 1990s. *Antimicrob Agents Chemother* 2019; **63**:e01560-19.
37. Rowland SJ, Dyke KG. Characterization of the staphylococcal beta-lactamase transposon Tn*552*. *EMBO J* 1989; **8**:2761-2773.
38. Rowland SJ, Dyke KG. Tn*552*, a novel transposable element from *Staphylococcus aureus*. *Mol Microbiol* 1990; **4**:961-975.
39. Lange CC, Werckenthin C, Schwarz S. Molecular analysis of the plasmid-borne *aacA*/*aphD* resistance gene region of coagulase-negative staphylococci from chickens. *J Antimicrob Chemother* 2003; **51**:1397-1401.

40. Zhu Y, Wang C, Schwarz S, *et al*. Identification of a novel tetracycline resistance gene, *tet(63)*, located on a multiresistance plasmid from *Staphylococcus aureus*. *J Antimicrob Chemother* 2021; **76**:576-581.
41. Zhu Y, Zhang W, Liu S, *et al*. Identification of an IS*431*-derived translocatable unit containing the *erm(C)* gene in *Staphylococcus aureus*. *J Antimicrob Chemother* 2021; **76**:1102-1104.
42. Hauschild T, Lüthje P, Schwarz S. Staphylococcal tetracycline-MLSB resistance plasmid pSTE2 is the product of an RSA-mediated in vivo recombination. *J Antimicrob Chemother* 2005; **56**:399-402.
43. Wendlandt S, Lozano C, Kadlec K, *et al*. The enterococcal ABC transporter gene *lsa(E)* confers combined resistance to lincosamides, pleuromutilins and streptogramin A antibiotics in methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother* 2013; **68**:473-475.
44. Venkova-Canova T, Pátek M, Nešvera J. Control of *rep* gene expression in plasmid pGA1 from *Corynebacterium glutamicum*. *J Bacteriol* 2003; **185**:2402-2409.
45. Firth N, Skurray RA. Mobile elements in the evolution and spread of multiple-drug resistance in staphylococci. *Drug Resist Updat* 1998; **1**:49-58.
46. Schwarz S, Fessler AT, Hauschild T, *et al*. Plasmid-mediated resistance to protein biosynthesis inhibitors in staphylococci. *Ann N Y Acad Sci* 2011; **1241**:82-103.
47. Loessner I, Dietrich K, Dittrich D, *et al*. Transposase-dependent formation of circular IS*256* derivatives in *Staphylococcus epidermidis* and *Staphylococcus aureus*. *J Bacteriol* 2002; **184**:4709-4714.

Figure legends-

Figure 1 Comparison of novel SCC*mec* element with *mec* gene complex A including SCC*mec* type II, III, VIII, XIII and SCC*mec* type X with *ccr* gene complex 7 (A1B6). Genes are shown with the direction of transcription and color coded according to their gene name. The *orfX* gene was colored in black, *mec* gene complex was colored in red, *ccr* gene complex was colored in blue, IS genes were colored in aqua, plasmid related genes were colored in light orange, antimicrobial resistance genes were colored in pink, metal resistance genes were colored in orange, and other functional ORFs were colored in grey. The flags represent the DR sequence of the SCC*mec* elements. The *mec* gene complex was shaded and colored by sky blue.

Figure 2 Comparison of pseudo-SCC element with SCC*mec* type V(5C2), IX and *S. haemolyticus*. The *orfX* gene was colored in black, *mec* gene complex was colored in red, *ccr* gene complex was colored in blue, IS genes were colored in aqua, plasmid related genes were colored in light orange, antimicrobial resistance genes were colored in pink, metal resistance genes were colored in orange, and other functional ORFs were colored in grey. The flags represent the DR sequence of the SCC*mec* elements. The *mec* gene complex was shaded and colored in sky blue and the metal resistance gene cluster was shaded and colored in pale red.

Figure 3 Comparison of pseudo-SCC element with SCC*mec* elements from MRSA isolates NX-T55, QD-CD9 and BA01611 (*SCCmec* type XII). The *orfX* gene was colored in black, *mec* gene complex was colored in red, *ccr* gene complex was colored in blue, IS genes were colored in aqua, plasmid related genes were colored in light orange, antimicrobial resistance genes were colored in pink, metal resistance genes were colored in orange, and other functional ORFs were colored in grey. The flags represent the DR sequence of the SCC*mec* elements. The *mec* gene complex was shaded and colored in sky blue and regions of >99% nucleotide sequence identity are indicated by shading in grey.

Figure S1 Phylogenetic relations of ccr genes. The *ccrA*, *ccrB*, and *ccrC* genes from the following strains were used. DDBJ/EMBL/GenBank database accession numbers are indicated in parentheses, and species are not indicated in the cases of *S. aureus* strains. ***ccrA1* and *ccrB1***, NCTC10442 (AB033763), MSSA476 (BX571857), and GIFU12263 (AB063171); ***ccrA2* and *ccrB2***, N315 (D86934), CA05 (AB063172), and MW2 (NC003923); ***ccrA3* and *ccrB3***, 85/2082 (AB037671); ***ccrA4* and *ccrB4***, HDE288 (AF411935), ATCC 12228 (AE015929), CHE482-1 (EF126185), and

CHE482-2 (EF126186); ***ccrA1*** **and** ***ccrB6***, ATCC 15305 (NC007350); JCSC6945 (AB505630); ***ccrA1*** **and** ***ccrB7***, TSU33 (AB353724); ***ccrA5*** **and** ***ccrB3***, KM241 (AM904731); ***ccrC1-ccrC9***, WIS (AB121219), TSGH17 (AY894416), 85/2082 (AB037671), M (U10927), *S. haemolyticus* JCSC1435 (AP006716), *S. haemolyticus* 25-60 (EF190467), *S. epidermidis* 13-48 (EF190468), PM1 (AB462393.1), and *S. saprophyticus* ATCC 15305 (AP008934.1).

448 **Table 1 Comparison of ORFs from pseudo-SCC element with *S. aureus* and *S.***
449 ***haemolyticus*.**

ID	Size (nt)	Direction	Predicted function	*S. aureus* IX (AB505628.1)		*S. haemolyticus* (AB478934.1)	
				coverage	identity	coverage	identity
ORF1	479	+	Ribosomal RNA large subunit methyltransferase H	100	**97.9**	100	82.6
ORF2	674	-	Transposase for insertion sequence-like element IS*431*mec	100	98.07	100	**99.4**
ORF3	167	+	Hypothetical protein	100	**100**	100	**100**
ORF4	743	+	Glycerophosphoryl diester phosphodiesterase	100	**100**	100	**100**
ORF5	428	+	Uncharacterized protein YdeM	100	**100**	100	**100**
ORF6	2006	-	Beta-lactam-inducible penicillin-binding protein	100	**100**	100	99.95
ORF7	674	+	Transposase for insertion sequence-like element IS*431*mec	100	98.36	100	**99.11**
ORF8	347	-	Uncharacterized HTH-type transcriptional regulator HI_0186	100	**100**	100	99.42
ORF9	683	+	Probable chaperone protein HSP31	100	**100**	100	**100**
ORF10	665	+	Uncharacterized sugar epimerase YhfK	100	**100**	100	**100**
ORF11	1004	+	Putative NADP-dependent oxidoreductase YfmJ	100	**100**	100	**100**
ORF12	365	+	Hypothetical protein	100	**100**	100	**100**
ORF13	617	+	Hypothetical protein	100	**99.84**	100	**99.84**
ORF14	341	+	Cadmium resistance transcriptional regulatory protein CadC	100	**99.41**	100	**99.41**
ORF15	401	-	Protein ArsC 1	100	**98.75**	100	**98.75**
ORF16	1289	-	Arsenical pump membrane protein	100	**100**	100	99.92
ORF17	317	-	Arsenical resistance operon repressor	100	**99.68**	100	**99.68**
ORF18	2060	+	Probable copper-transporting P-type ATPase B	100	97.87	100	**99.27**
ORF19	545	+	Uncharacterized protein YdhK	88	98.33	**100**	**98.53**

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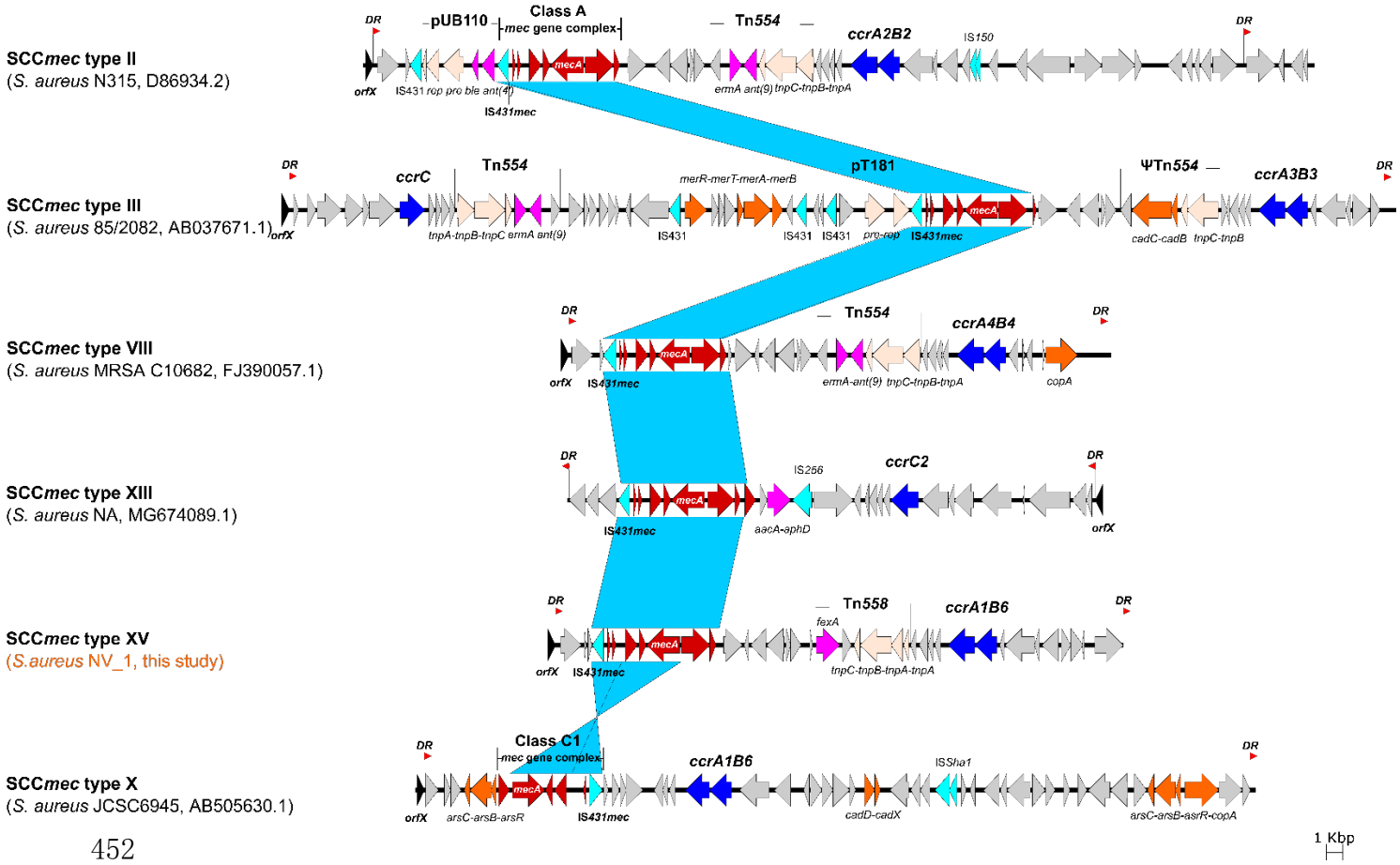
451 Figure 1



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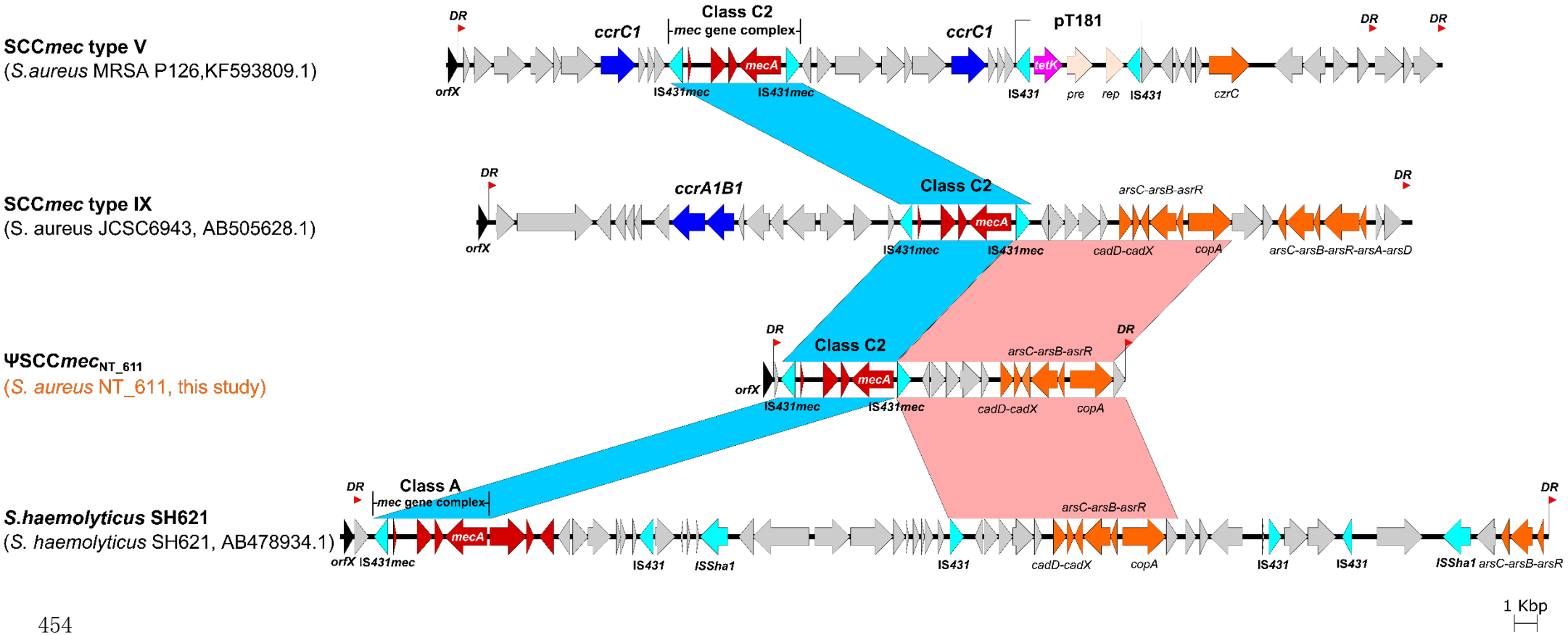
453 Figure 2



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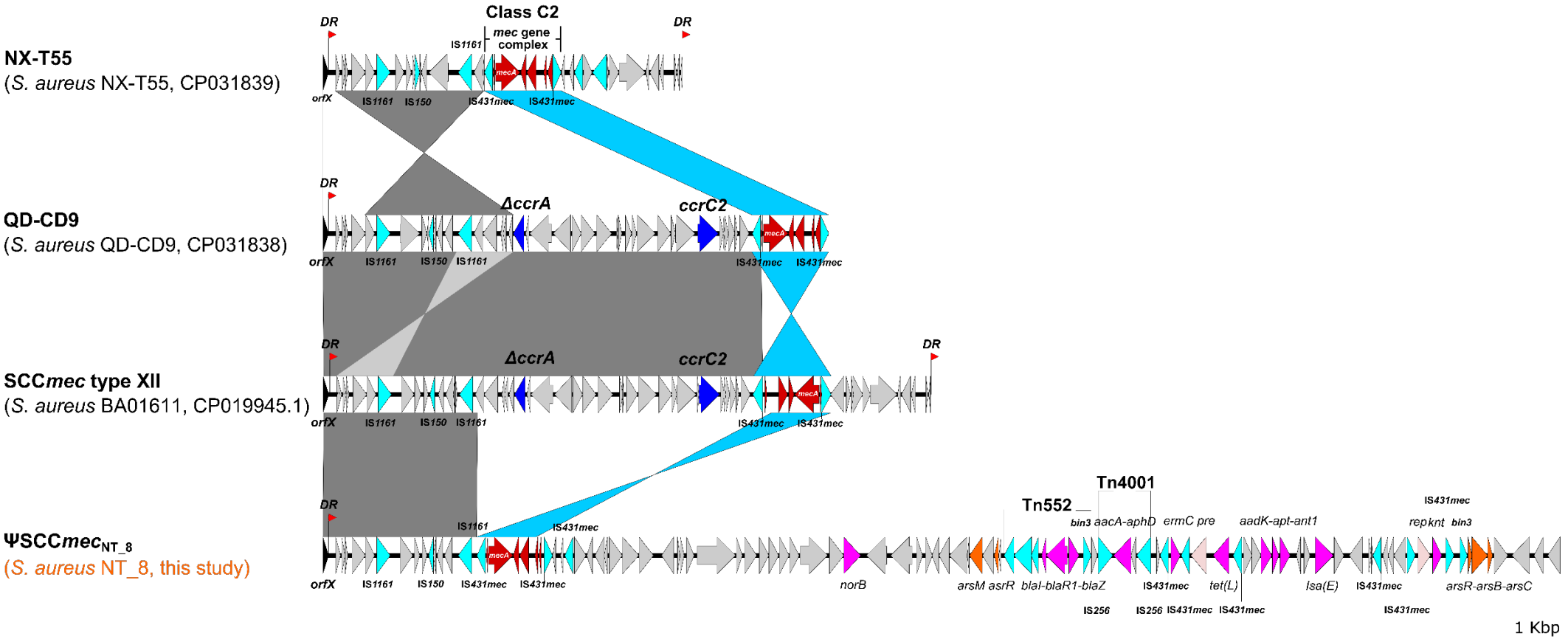
456 Figure 3



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