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**Title: Novel Staphylococcal Cassette Chromosome composite island (SCC-CI)
with a new subtype of *SCCmecVI* cassette found in ST5 MRSA in France**

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Abstract

An emergent kanamycin-susceptible ST5-MRSA lineage has been identified in France. Whole genome sequencing revealed a 40 kb SCC composite island with a mosaic structure including 3 SCC elements: a Ψ SCC_{cop/ars}, a SCC_{Lim88A} with a *ccrC* recombinase, and a novel subtype of SCC_{mec} type VI (VIb). This mosaic structure suggests a high recombination rate of SCC elements from distinct staphylococci species.

Keywords: *Staphylococcus aureus*, Antimicrobial resistance, MRSA, *mecA*, novel SCC_{mec}

1. Introduction

Methicillin resistance in *Staphylococcus aureus* (MRSA) is due to the acquisition of an alternative penicillin-binding protein PBP2a which has a low affinity for β -lactam antibiotics [1]. This enzyme, encoded by the *mecA* gene, is horizontally transferred within staphylococci by a large mobile genetic element named the “staphylococcal cassette chromosome *mec*” (SCC_{mec}) [2]. SCC_{mec} elements integrate into the genome of *S. aureus* within the *orfX/rlmH* gene, via recombination events mediated by the site-specific recombinases (*ccr* complex) encoded by the SCC_{mec} element itself [3]. Majority of MRSA clones detected worldwide belongs to a few common clonal complexes (CCs): 1, 5, 8, 22, 30 and 45 [4,5]. The CC5 is one of the CCs clustering the higher number of MRSA clones that have a worldwide distribution. Each of these MRSA clones harbors distinct SCC_{mec} elements such as the ones found in the pandemic ST5-MRSA type II (New York/Japan clone), ST5-MRSA type IV (Paediatrics

clone), ST5-MRSA type VI (the New Paediatrics clone), ST5-MRSA type I (EMRSA-3) and ST5-MRSA type I (Geraldine clone) [6–9].

2. Materials and Methods

In July 2014, a 90 year-old man was admitted at the Limoges University Hospital Center, France, for a sepsis whose origin was a heel bed sore. Blood cultures were positive with Gram-positive cocci in clusters. They were directly tested for the presence of *S. aureus*/MRSA with the Xpert® MRSA/SA Blood Culture kit (Cepheid), as recommended by the manufacturer. The presence of *S. aureus* was confirmed by *spa* gene detection, but the presence of a MRSA was not retained as positive, despite a positive *mecA* PCR result, due to the absence of amplification for the *orfX-SCCmec* junction. The day after, antimicrobial susceptibility testing revealed a MRSA profile (strain LIM88). Whole-genome sequencing (WGS) of LIM88 was performed using the Ion Proton™ system (ThermoFisher Scientific), according to the manufacturer's instructions. The reads were assembled using MIRA (Mimicking Intelligent Read Assembly). WGS analysis, using both the SRST2 v0.2.0 [10] and RidomsStaphType v2.0 (Ridom GmbH), revealed that LIM88 belonged to the ST5 genetic background and that it had a *spa*-type t777.

Characterization of the SCC elements was performed by searching for site-specific insertion sequences (ISS) characteristic of SCC elements [11] and associated direct and inverted repeats. Open reading frame (ORF) detection and annotation was performed using the RAST service [12] and all proteins were blasted [13] against the NCBI *nr* nucleotide database to search for the closest protein hits. Genomic comparison between SCC elements was conducted using a combination of Mauve Progressive [14] and *blastn* results [13].

73 Screening for the new SCC*mec* element in 40 MRSA isolated from blood cultures,
74 during 2015 in the teaching hospital of Limoges (CHU Limoges), was performed using
75 Kondo's PCR [15] primers targeting the concomitant presence of the recombinases
76 complexes *ccrC* and *ccrAB* type 4.

77 3. Results and Discussion

78 We identified the presence of 4 ISS comprising direct repeat (DR) sequences typical
79 of SCC-like cassettes. Thus, LIM88 carries a SCC composite island of 39.6 kb with 3
80 SCC elements in tandem: a Ψ SCC_{*cop/ars*} element (without recombinase genes), a SCC
81 element with a *ccrC* recombinase (called SCC_{LIM88A}) and a SCC*mec* element with a
82 type 4 *ccrAB* complex (Figure 1). The nucleotide sequence of this novel SCC
83 composite island was deposited in the NCBI database under the accession number
84 GenBank KX646745.

85 Comparison of the 16.6 kb SCC*mec* (ORF25-40, Table S1) element including 16 ORFs
86 found in strain LIM88 with the 23kb type VI SCC*mec* element of the New Paediatric
87 ST5, t777 MRSA clone (strain HDE288) [9] reveals a high nucleotide similarity (>89%)
88 of the "non-essential" region J3, the type B1 *mec* complex (IS431-*mecA*- Δ *mecR*-
89 IS1272), the "non-essential" region J2 and the *ccrAB* (type 4) complex found in both
90 SCC*mec* elements (Figure 2A). Only the J1 regions are distinct (Figure 2A).
91 Interestingly, the J1 region of LIM88 SCC*mec* type VI element shows an overwhelming
92 similarity (more than 90% nucleotide Identity) with a region of a SCC element found in
93 *S. epidermidis* strain ATCC12228 (Figure 2B), which encompasses the *ccrA4B4*
94 complex and a *copA/cadA* resistance gene. Based on these data, the SCC*mec*
95 element found in strain LIM88 can be classified as a novel subtype of SCC*mec* type
96 VI element, named SCC*mec*VIb after consulting and with the agreement of the

97 International Working Group on the Classification of Staphylococcal Cassette
98 Chromosome Elements (IWG-SCC).

99 Immediately downstream from the SCC_{mec} element, a 11.6 kb SCC element (ORF13-
100 24, Table S1) carrying 12 ORFs, among which a *ccrC* recombinase (allele 7) and no
101 known specific virulence or resistance markers, was identified. This element was
102 named SCC_{Lim88A}. Genomic comparison reveals that this region is highly similar to the
103 *rlmH* proximal J3 region of SCC_{mec}_{ZH47}, a SCC_{mec} element previously described in a
104 CC5 strain isolated from drug-users in Switzerland [16] but, also, to the J3 region found
105 in a composite island of SCC elements (SCC-CI) of the *S. haemolyticus* NW19A
106 (GenBank KM369884) [177], a strain isolated from bovine milk (Figure 2C). This
107 mosaic structure is even more surprising when we look at the SCC_{mec} element
108 inserted immediately downstream SCC_{Lim88A}. The J3 and *mecA* complex itself of the
109 SCC_{mec} element are highly similar (90 % nucleotide identity) to the *mecA* complex
110 and its proximal J3 region as found in 3 different SCC_{mec} elements: i) the SCC_{mec}_{ZH47}
111 (SCC_{mec} new subtype IV) but without the aminoglycoside resistance transposon
112 Tn4001 inserted in the *mecR* gene (Figure 2C); ii) the SCC-CI of *S. haemolyticus*
113 NW19A (Figure 2C) and the SCC_{mec} type VIa element of strain HDE288 of the ST5-
114 MRSA New Paediatrics clone, as presented in Figure 2A.

115 Finally, the third SCC element is, in fact, a pseudo-SCC, called Ψ SCC_{cop/ars}, as it does
116 not carry any recombinase. This 11.1 kb region, located immediately downstream from
117 the *rlmH* gene, harbors 12 ORFS (ORF 1-12, Table S1). Almost all of them encode for
118 heavy metal detoxification functions: two copper related resistance genes (ORF1 and
119 ORF2) and a complete arsenic resistance operon (*ars* operon, ORF4-8, ORF10). As
120 presented in Figures 2B and 2C, this detoxification cluster is present in SCC elements

found in other staphylococci species, such as the SCC composite island (SCC-CI) found in the *SCCmec* element of a *S. epidermidis* strain M13/0453 (GenBank MF062491.1) [18] or the SCC-CI of *S. haemolyticus* strain NW19A (GenBank KM369884). The comparison with other simple or composite SCC elements does not help to clarify the origin of this heavy metal resistance cluster as this element is part of canonical SCC elements (carrying within them a cassette chromosomal recombinase) (Figure 2B) but also is part of longer pseudo-SCC elements (SCC elements without the site specific recombinases) (Figure 2C). Nonetheless, the closeness of both functional and truncated IS nearby this heavy metal cluster in almost all SCC elements suggest that they may play a role in the transfer and insertion of this resistance cluster. No intermediate SCC elements were found in public databases including NCBI or EMBL. The shared similarity with different SCC elements from different staphylococcal species suggests an array of recombination events before the final structure of the SCC-CI, as found in strain LIM88, with no certainty as to the ancestral donor-SCC elements. The presence of this atypical composite island $\Psi\text{SCC}_{\text{cop/ars}}\text{-SCC}_{\text{Lim88A}}\text{-SCC}_{\text{mecVIb}}$ composed by 3 distinct SCC elements, with a non-*SCCmec* cassette inserted at the end of the *rlmH* gene, is likely the reason for the absence of amplification of the *orfX*-*SCCmec* junction when using the Xpert® MRSA/SA Blood Culture kit. Phylogenetic analysis of LIM88 and other CC5 MRSA strains, based on 2288 core SNP, suggests the emergence of a novel clonal ST5 MRSA population harboring this novel composite SCC-CI (Figure S1). A preliminary screening of 40 MRSA isolated from blood culture during 2015, suggests that the new ST5-MRSA lineage, carrying the novel SCC-CI $\Psi\text{SCC}_{\text{cop/ars}}\text{-SCC}_{\text{Lim88A}}\text{-SCC}_{\text{mecVIb}}$ element, represents 15% (n=6 isolates) of blood culture

MRSAs isolated in the CHU Limoges. LIM88 and the other 6 MRSA strains were coresistant to fluoroquinolones but susceptible to all other antibiotic classes, notably to kanamycin. One out of the other 6 strains was also resistant to fosfomycin.

4. Conclusion:

We report an original mosaic structure of the staphylococcal chromosome cassette composite island Ψ SCC_{cop/ars}-SCC_{LIM88A}-SCC_{mecVIb} element carried by a ST5 MRSA (strain LIM88) isolated in Limoges, France. Investigations are now ongoing to accurately characterize this novel lineage and to determine the prevalence and the regional *versus* national distribution of this emerging ST5 lineage in France.

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Declarations

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Competing Interests : No conflict of Interest for all authors

Ethical Approval : Not required

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229 **Figure 1. Schematic representation of the SCC composite island Ψ SCC_{cop/ars}-**
 230 **SCC_{Lim88A}-SCC_{mec} Vlb element in ST5 MRSA LIM88.**

231 The sequence of the Ψ SCC_{cop/ars}-SCC_{Lim88A}-SCC_{mec} composite island of strain
 232 LIM88 is represented with open reading frames (ORFs) shown as gray arrows
 233 indicating the transcription direction. Colored arrows highlight specific genes according
 234 to the following criteria: orange = copper resistance related genes (*copA* and *mco*,

235 *copA/cadA*); light green = arsenic resistance related genes (*ars* operon); green =
 236 staphylococcal cassette recombinase type C gene (*ccrC*); cyan = staphylococcal
 237 cassette recombinase type AB genes (*ccrA4* and *ccrB4*); yellow = transposases (type
 238 IS431 and IS1272); magenta = *mecA* complex genes (*mecA* and truncated *mecR1*).
 239 The *SSCmec* element architecture with the “non-essential” J1, J2, J3 regions, *mec*
 240 complex and *ccr* complex are indicated above the *SSCmec* VIb element of strain
 241 LIM88.

242

243

244 **Figure 2. Comparison of the SCC composite island Ψ SCC_{*cop/ars*}-SCC_{LIM88A}-**
 245 **SCC*mec* in ST5 MRSA LIM88 with other SCC elements or SCC composite islands**
 246 **(SCC-CI) found in staphylococci.**

247 The SCC composite islands or SCC elements are represented with open reading
 248 frames (ORFs) shown as gray arrows indicating the transcription direction. Colored
 249 arrows highlight specific genes according to the following criteria: orange = copper
 250 resistance related genes; light green = arsenic resistance related genes; green =
 251 staphylococcal cassette recombinase type C gene (*ccrC*); cyan = staphylococcal
 252 cassette recombinase AB (*ccrA4B4*) genes; light blue = staphylococcal cassette
 253 recombinase AB (*ccrA2B2*) genes; dark blue = staphylococcal cassette recombinase
 254 AB (*ccrA3B3*) genes; yellow = transposases (type IS431 and IS1272); magenta =
 255 *mecA* complex genes and red = fusidic acid resistance gene (*fus*) in panel 2A or
 256 aminoglycoside resistance transposon Tn4001 in panel 2B. Homologous gene clusters
 257 between SCC elements are indicated by gray diamond areas. The percentage of
 258 homology is coded by a gray gradient as indicated in the figure.

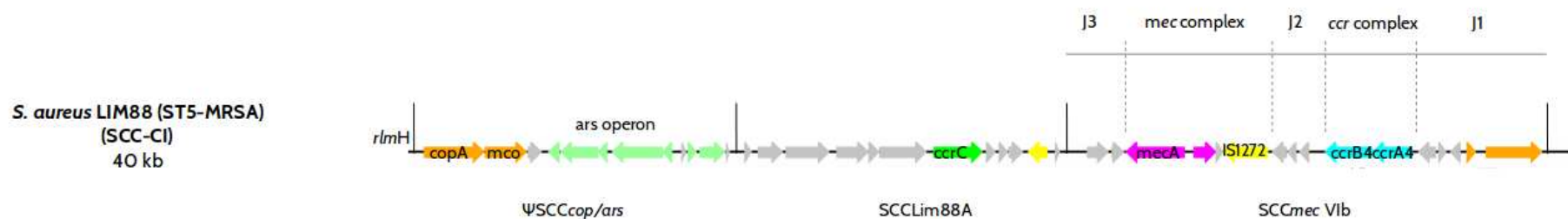
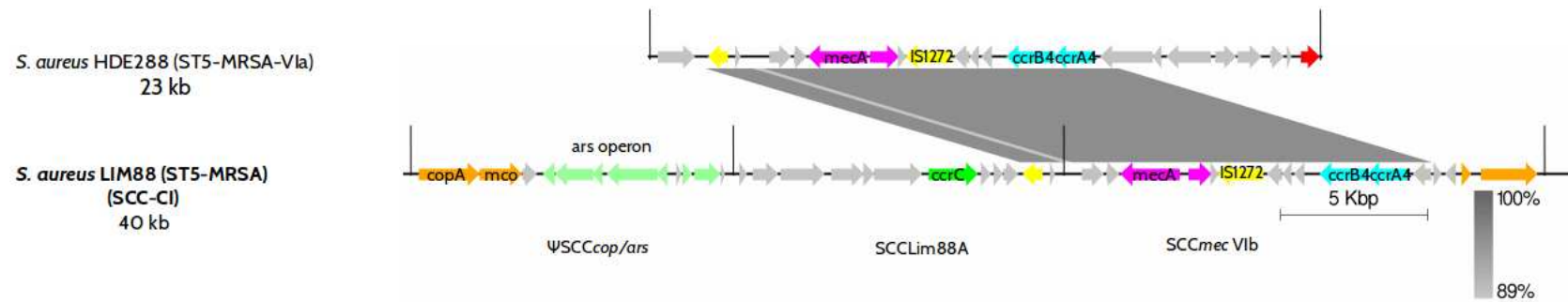


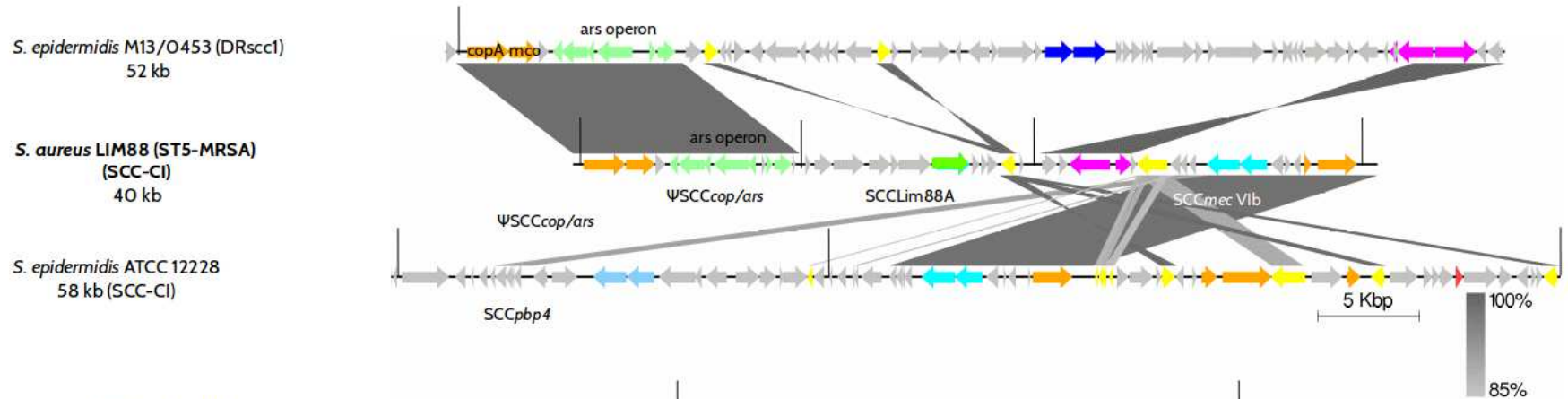
Figure 1. Schematic representation of the SCC composite island Ψ SCC_{cop/ars}-SCCLim88A-SCCmec VIb element in ST5 MRSA LIM88.

The sequence of the Ψ SCC_{cop/ars}-SCCLim88A-SCCmec composite island of strain LIM88 is represented with open reading frames (ORFs) shown as gray arrows indicating the transcription direction. Colored arrows highlight specific genes according to the following criteria: orange = copper resistance related genes (*copA* and *mco*, *copA/cadA*); light green = arsenic resistance related genes (*ars* operon); green = staphylococcal cassette recombinase type C gene (*ccrC*); cyan = staphylococcal cassette recombinase type AB genes (*ccrA4* and *ccrB4*); yellow = transposases (type *IS431* and *IS1272*); magenta = *mecA* complex genes (*mecA* and truncated *mecR1*). The SSCmec element architecture with the “non-essential” J1, J2, J3 regions, *mec* complex and *ccr* complex are indicated above the SCCmec VIb element of strain LIM88.

(A)



(B)



(C)

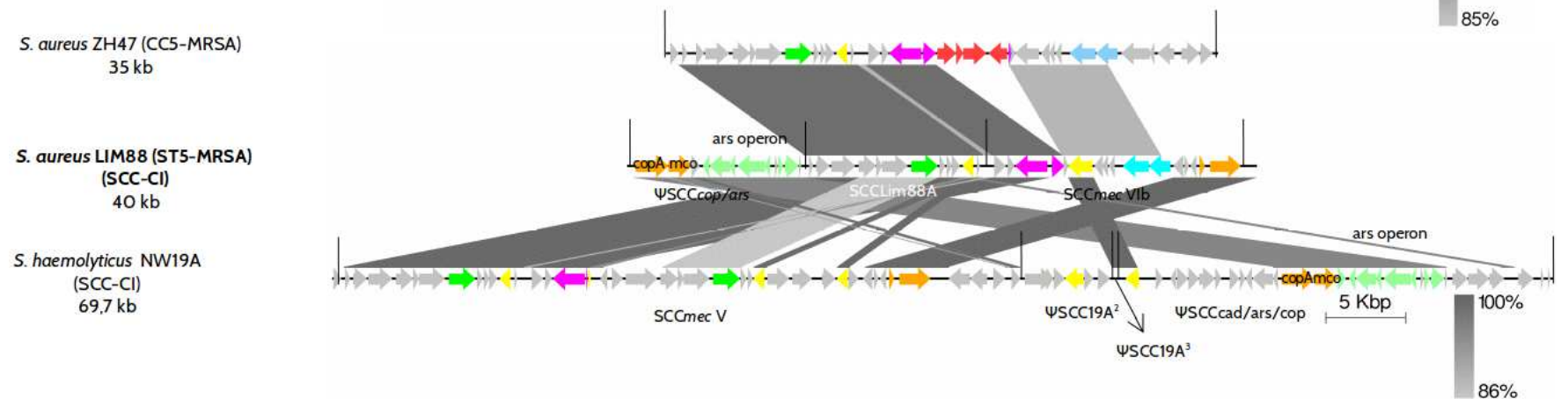


Figure 2. Comparison of the SCC composite island Ψ SCC_{cop/ars}-SCC_{LIM88A}-SCC_{mec} in ST5 MRSA LIM88 with other SCC elements or SCC composite islands (SCC-CI) found in staphylococci.

The SCC composite islands or SCC elements are represented with open reading frames (ORFs) shown as gray arrows indicating the transcription direction. Colored arrows highlight specific genes according to the following criteria: orange = copper resistance related genes; light green = arsenic resistance related genes; green = staphylococcal cassette recombinase type C gene (*ccrC*); cyan = staphylococcal cassette recombinase AB (*ccrA4B4*) genes; light blue = staphylococcal cassette recombinase AB (*ccrA2B2*) genes; dark blue = staphylococcal cassette recombinase AB (*ccrA3B3*) genes; yellow = transposases (type IS431 and IS1272); magenta = *mecA* complex genes and red = fusidic acid resistance gene (*fus*) in panel 2A or aminoglycoside resistance transposon Tn4001 in panel 2B. Homologous gene clusters between SCC elements are indicated by gray diamond areas. The percentage of homology is coded by a gray gradient as indicated in the figure.