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

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25 **Abstract**

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

32

33 **Keywords:** *Staphylococcus aureus*, Antimicrobial resistance, MRSA, *mecA*, novel
34 SCC*mec*

35

36 **1. Introduction**

37 Methicillin resistance in *Staphylococcus aureus* (MRSA) is due to the acquisition of an
38 alternative penicillin-binding protein PBP2a which has a low affinity for β -lactam
39 antibiotics [1]. This enzyme, encoded by the *mecA* gene, is horizontally transferred
40 within staphylococci by a large mobile genetic element named the “staphylococcal
41 cassette chromosome *mec*” (SCC*mec*) [2]. SCC*mec* elements integrate into the
42 genome of *S. aureus* within the *orfX/rlmH* gene, via recombination events mediated by
43 the site-specific recombinases (*ccr* complex) encoded by the SCC*mec* element itself
44 [3]. Majority of MRSA clones detected worldwide belongs to a few common clonal
45 complexes (CCs): 1, 5, 8, 22, 30 and 45 [4,5]. The CC5 is one of the CCs clustering
46 the higher number of MRSA clones that have a worldwide distribution. Each of these
47 MRSA clones harbors distinct SCC*mec* elements such as the ones found in the
48 pandemic ST5-MRSA type II (New York/Japan clone), ST5-MRSA type IV (Paediatrics

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

51 **2. Materials and Methods**

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

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

73 Screening for the new *SCCmec* 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 *SCCmec* 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 *SCCmec* (ORF25-40, Table S1) element including 16 ORFs
86 found in strain LIM88 with the 23kb type VI *SCCmec* 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 *SCCmec* elements (Figure 2A). Only the J1 regions are distinct (Figure 2A).
91 Interestingly, the J1 region of LIM88 *SCCmec* 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 *SCCmec*
95 element found in strain LIM88 can be classified as a novel subtype of *SCCmec* type
96 VI element, named *SCCmecVIb* 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 *SCCmec* 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 *SCCmec_{ZH47}*, a *SCCmec* 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 *SCCmec* element
108 inserted immediately downstream *SCC_{Lim88A}*. The J3 and *mecA* complex itself of the
109 *SCCmec* element are highly similar (90 % nucleotide identity) to the *mecA* complex
110 and its proximal J3 region as found in 3 different *SCCmec* elements: i) the *SCCmec_{ZH47}*
111 (*SCCmec* 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 *SCCmec* 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

121 found in other staphylococci species, such as the SCC composite island (SCC-CI)
122 found in the SCC*mec* element of a *S. epidermidis* strain M13/0453 (GenBank
123 MF062491.1) [18] or the SCC-CI of *S. haemolyticus* strain NW19A (GenBank
124 KM369884). The comparison with other simple or composite SCC elements does not
125 help to clarify the origin of this heavy metal resistance cluster as this element is part of
126 canonical SCC elements (carrying within them a cassette chromosomal recombinase)
127 (Figure 2B) but also is part of longer pseudo-SCC elements (SCC elements without
128 the site specific recombinases) (Figure 2C). Nonetheless, the closeness of both
129 functional and truncated IS nearby this heavy metal cluster in almost all SCC elements
130 suggest that they may play a role in the transfer and insertion of this resistance cluster.
131 No intermediate SCC elements were found in public databases including NCBI or
132 EMBL. The shared similarity with different SCC elements from different staphylococcal
133 species suggests an array of recombination events before the final structure of the
134 SCC-CI, as found in strain LIM88, with no certainty as to the ancestral donor-SCC
135 elements. The presence of this atypical composite island Ψ SCC_{*cop/ars*}-SCC_{*Lim88A*}-
136 SCC*mecVIb* composed by 3 distinct SCC elements, with a non-SCC*mec* cassette
137 inserted at the end of the *rlmH* gene, is likely the reason for the absence of amplification
138 of the *orfX*-SCC*mec* junction when using the Xpert[®] MRSA/SA Blood Culture kit.
139 Phylogenetic analysis of LIM88 and other CC5 MRSA strains, based on 2288 core
140 SNP, suggests the emergence of a novel clonal ST5 MRSA population harboring this
141 novel composite SCC-CI (Figure S1).

142 A preliminary screening of 40 MRSA isolated from blood culture during 2015,
143 suggests that the new ST5-MRSA lineage, carrying the novel SCC-CI Ψ SCC_{*cop/ars*}-
144 SCC_{*Lim88A*}-SCC*mecVIb* element, represents 15% (n=6 isolates) of blood culture

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

148

149 **4. Conclusion:**

150 We report an original mosaic structure of the staphylococcal chromosome cassette
151 composite island Ψ SCC_{cop/ars}-SCC_{Lim88A}-SCC_{mecVIb} element carried by a ST5 MRSA
152 (strain LIM88) isolated in Limoges, France. Investigations are now ongoing to
153 accurately characterize this novel lineage and to determine the prevalence and the
154 regional *versus* national distribution of this emerging ST5 lineage in France.

155

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158 USA), Poster Saturday-417. We thank the Limoges University BISCEm platform for
159 technical assistance in WGS and the French “Groupe de Microbiologie Clinique”
160 (GMC).

161

162 **Declarations**

163 **Funding** : No funding

164 **Competing Interests** : No conflict of Interest for all authors

165 **Ethical Approval** : Not required

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- 228 doi:10.1128/AAC.00165-06.

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 SSC*mec* element architecture with the “non-essential” J1, J2, J3 regions, *mec*
240 complex and *ccr* complex are indicated above the SCC*mec* 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.

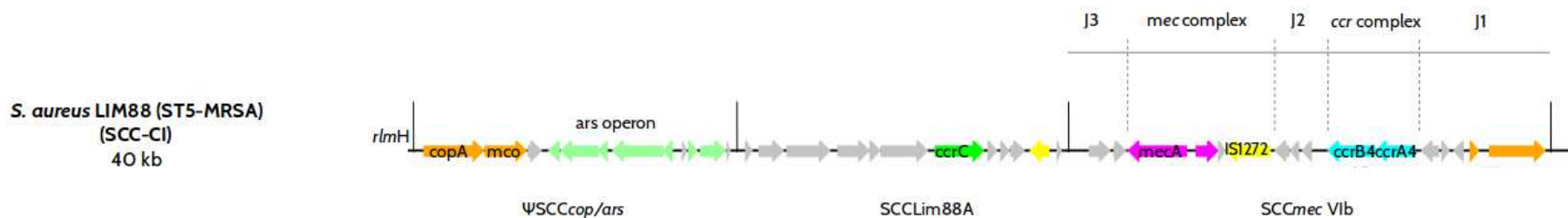
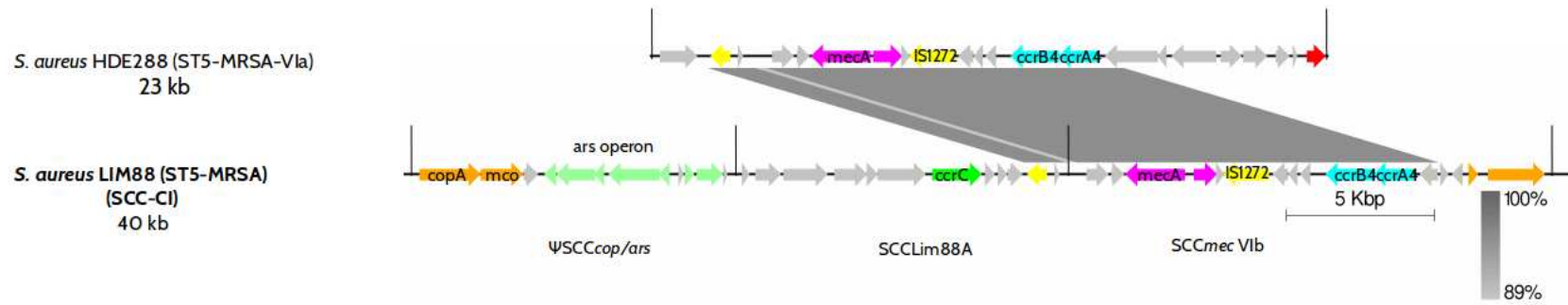


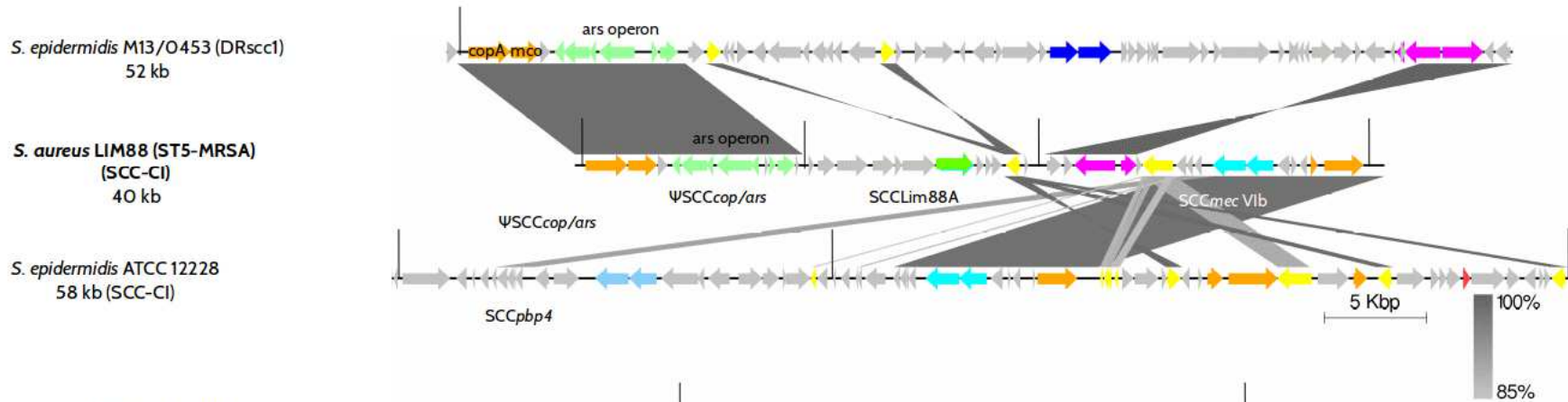
Figure 1. Schematic representation of the SCC composite island Ψ SCC_{cop/ars}-SCCLim88A-SCCmec VIb element in ST5 MRSA LIM88.

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 *SCCmec* 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)

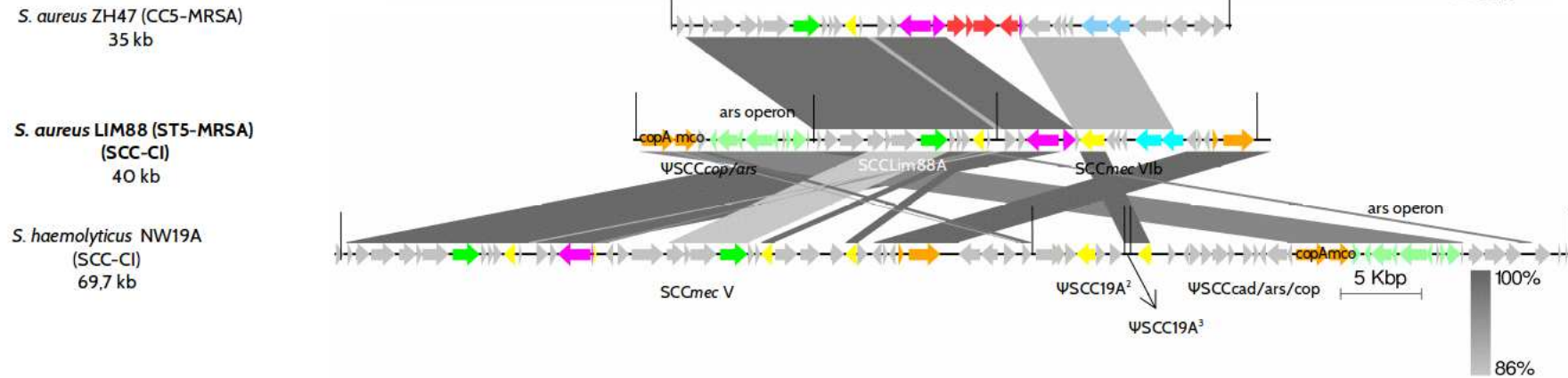


Figure 2. Comparison of the SCC composite island Ψ SCC_{cop/ars}-SCC_{Lim88A}-SCC_{mec} in ST5 MRSA LIM88 with other SCC elements or SCC composite islands (SCC-CI) found in staphylococci.

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.