

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

3 **Running Title:** Novel SCC-CI element in ST5 MRSA

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26 **Abstract**

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

33

34 **Main text**

35 Methicillin resistance in *Staphylococcus aureus* (MRSA) is due to the acquisition of
36 an alternative penicillin-binding protein PBP2a which has a low affinity for β -lactam
37 antibiotics (1). This enzyme, encoded by the *mecA* gene, is horizontally transferred
38 within staphylococci by a large mobile genetic element named the “staphylococcal
39 cassette chromosome *mec*” (SCC_{mec}) (2). SCC_{mec} elements integrate into the
40 genome of *S. aureus* within the *orfX/rlmH* gene, via recombination events mediated
41 by the site-specific recombinases (*ccr* complex) encoded by the SCC_{mec} element
42 itself (3). Majority of MRSA clones detected worldwide belongs to a few common
43 clonal complexes (CCs): 1, 5, 8, 22, 30 and 45 (4, 5). The CC5 is one of the CCs
44 clustering the higher number of MRSA clones that have a worldwide distribution.
45 Each of these MRSA clones harbors distinct SCC_{mec} elements such as the ones
46 found in the pandemic ST5-MRSA type II (New York/Japan clone), ST5-MRSA type
47 IV (Paediatrics clone), ST5-MRSA type VI (the New Paediatrics clone), ST5-MRSA
48 type I (EMRSA-3) and ST5-MRSA type I (Geraldine clone) (6–9).

49 In July 2014, a 90 year-old man was admitted at the Limoges University Hospital
50 Center, France, for a sepsis whose origin was a heel bed sore. Blood cultures were
51 positive with Gram-positive cocci in clusters. They were directly tested for the
52 presence of *S. aureus*/MRSA with the Xpert[®] MRSA/SA Blood Culture kit (Cepheid),
53 as recommended by the manufacturer. The presence of *S. aureus* was confirmed by
54 *spa* gene detection, but the presence of a MRSA was not retained as positive,
55 despite a positive *mecA* PCR result, due to the absence of amplification for the *orfX*-
56 SCCmec junction. The day after, antimicrobial susceptibility testing revealed a MRSA
57 profile (strain LIM88). Whole-genome sequencing (WGS) of LIM88 was performed
58 using the Ion Proton[™] system (ThermoFisher Scientific), according to the
59 manufacturer's instructions. The reads were assembled using MIRA (Mimicking
60 Intelligent Read Assembly). WGS analysis, using both the SRST2 v0.2.0 (10) and
61 RidomsStaphType v2.0 (Ridom GmbH), revealed that LIM88 belonged to the ST5
62 genetic background and that it had a *spa*-type t777. Search for site-specific insertion
63 sequences (ISS) characteristic of SCC elements (11) revealed the presence of 4 ISS
64 comprising direct repeat (DR) sequences typical of SCC-like cassettes. Thus, LIM88
65 carries a SCC composite island of 39.6 kb with 3 SCC elements in tandem: a
66 Ψ SCC_{*cop/ars*} element (without recombinase genes), a SCC element with a *ccrC*
67 recombinase (called SCC_{LIM88A}) and a SCCmec element with a type 4 *ccrAB* complex
68 (Figure 1). The nucleotide sequence of this novel SCC composite island was
69 deposited in the NCBI database under the accession number GenBank KX646745.

70 Comparison of the 16.6 kb SCCmec (ORF25-40, Table S1) element including 16
71 ORFs found in strain LIM88 with the 23kb type VI SCCmec element of the New
72 Paediatric ST5, t777 MRSA clone (strain HDE288) (9) reveals a high nucleotide

72 similarity (>89%) of the “non-essential” region J3, the type B1 *mec* complex (IS431-
73 *mecA*-*deltamecR*-IS1272), the “non-essential” region J2 and the *ccrAB* (type 4)
74 complex found in both *SCCmec* elements (Figure 2A). Only the J1 regions are
75 distinct (Figure 2A). Interestingly, the J1 region of LIM88 *SCCmec* type VI element
76 shows an overwhelming similarity (more than 90% nucleotide Identity) with a region
77 of a *SCC* element found in *S. epidermidis* strain ATCC12228 (Figure 2B), which
78 encompasses the *ccrA4B4* complex and a *copA/cadA* resistance gene. Based on
79 these data, the *SCCmec* element found in strain LIM88 can be classified as a novel
80 subtype of *SCCmec* type VI element, named *SCCmecVIb* with the agreement
81 obtained from the International Working Group on the Classification of
82 Staphylococcal Cassette Chromosome Elements (IWG-SCC).

83 Immediately downstream from the *SCCmec* element, a 11.6 kb *SCC* element
84 (ORF13-24, Table S1) carrying 12 ORFs, among which a *ccrC* recombinase (allele 7)
85 and no known specific virulence or resistance markers, was identified. This element
86 was named *SCC_{Lim88A}*. Genomic comparison reveals that this region is highly similar
87 to the *rlmH* proximal J3 region of *SCCmec_{ZH47}*, a *SCCmec* element previously
88 described in a CC5 strain isolated from drug-users in Switzerland (12) but, also, to
89 the J3 region found in a composite island of *SCC* elements (*SCC-CI*) of the *S.*
90 *haemolyticus* NW19A (GenBank KM369884) (13), a strain isolated from bovine milk
91 (Figure 2C). This mosaic structure is even more surprising when we look at the
92 *SCCmec* element inserted immediately downstream *SCC_{Lim88A}*. The J3 and *mecA*
93 complex itself of the *SCCmec* element are highly similar (90 % nucleotide identity) to
94 the *mecA* complex and its proximal J3 region as found in 3 different *SCCmec*
95 elements: i) the *SCCmec_{ZH47}* (*SCCmec* new subtype IV) but without the

96 aminoglycoside resistance transposon Tn4001 inserted in the *mecR* gene (Figure
97 2C); ii) the SCC-CI of *S. haemolyticus* NW19A (Figure 2C) and the SCC*mec* type VIa
98 element of strain HDE288 of the ST5-MRSA New Paediatrics clone, as presented in
99 Figure 2A.

100 Finally, the third SCC element is, in fact, a pseudo-SCC, called Ψ SCC_{cop/ars}, as it does
101 not carry any recombinase. This 11.1 kb region, located immediately downstream
102 from the *rlmH* gene, harbors 12 ORFS (ORF 1-12, Table S1). Almost all of them
103 encode for heavy metal detoxification functions: two copper related resistance genes
104 (ORF1 and ORF2) and a complete arsenic resistance operon (*ars* operon, ORF4-8,
105 ORF10). As presented in Figures 2B and 2C, this detoxification cluster is present in
106 SCC elements found in other staphylococci species, such as the SCC composite
107 island (SCC-CI) found in the SCC*mec* element of a *S. epidermidis* strain M13/0453
108 (GenBank MF062491.1) (14) or the SCC-CI of *S. haemolyticus* strain NW19A
109 (GenBank KM369884). The comparison with other simple or composite SCC
110 elements does not help to clarify the origin of this heavy metal resistance cluster as
111 this element is part of canonical SCC elements (carrying within them a cassette
112 chromosomal recombinase) (Figure 2B) but also is part of longer pseudo-SCC
113 elements (SCC elements without the site specific recombinases) (Figure 2C).
114 Nonetheless, the closeness of both functional and truncated IS nearby this heavy
115 metal cluster in almost all SCC elements suggest that they may play a role in the
116 transfer and insertion of this resistance cluster.

117 No intermediate SCC elements were found in public databases including NCBI or
118 EMBL. The shared similarity with different SCC elements from different
119 staphylococcal species suggests an array of recombination events before the final

120 structure of the SCC-CI, as found in strain LIM88, with no certainty as to the
121 ancestral donor-SCC elements. The presence of this atypical composite island
122 $\Psi\text{SCC}_{\text{cop/ars}}\text{-SCC}_{\text{Lim88A}}\text{-SCC}_{\text{mecVIb}}$ composed by 3 distinct SCC elements, with a non-
123 *SCCmec* cassette inserted at the end of the *rlmH* gene, is likely the reason for the
124 absence of amplification of the *orfX-SCCmec* junction when using the Xpert®
125 MRSA/SA Blood Culture kit.

126 We used Kondo's PCR (15) primers targeting the different recombinases complexes,
127 *ccrC* and *ccrAB* type 4, of this SCC composite island to perform a preliminary
128 screening of 190 strains of *S. aureus* isolated from blood culture during 2015 in the
129 teaching hospital of Limoges (CHU Limoges), France. The PCR screening suggests
130 that the new ST5-MRSA lineage, carrying the novel SCC-CI $\Psi\text{SCC}_{\text{cop/ars}}\text{-SCC}_{\text{Lim88A}}\text{-}$
131 *SCCmecVIb* element, represents more than 15% of blood culture MRSA's isolated in
132 the CHU Limoges. Of note, the positive isolates harbored an atypical antimicrobial
133 phenotypic profile including methicillin and fluoroquinolones resistance and
134 aminoglycoside susceptibility.

135 In conclusion, we report an original mosaic structure of the staphylococcal
136 chromosome cassette composite island $\Psi\text{SCC}_{\text{cop/ars}}\text{-SCC}_{\text{Lim88A}}\text{-SCC}_{\text{mecVIb}}$ element
137 carried by a ST5 MRSA (strain LIM88) isolated in Limoges, France. Investigations are
138 now ongoing to accurately determine the prevalence and the regional *versus* national
139 distribution of this emerging ST5 lineage in France.

140

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144 technical assistance in WGS.

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146 **Conflict of interests**

147 None to declare.

148

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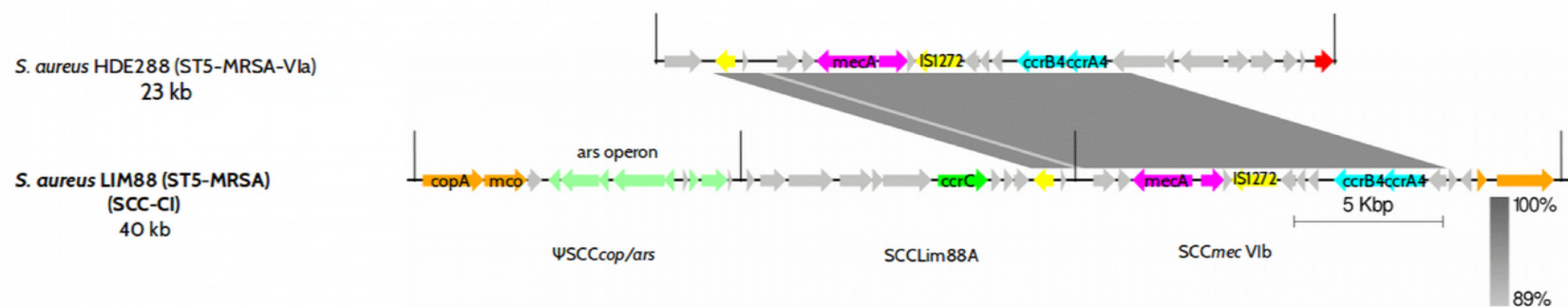


205 **Figure 1. Schematic representation of the SCC composite island Ψ SCC_{cop/ars}-SCC_{Lim88A}-SCC_{mec VIb} element in ST5 MRSA**
 206 **LIM88.**

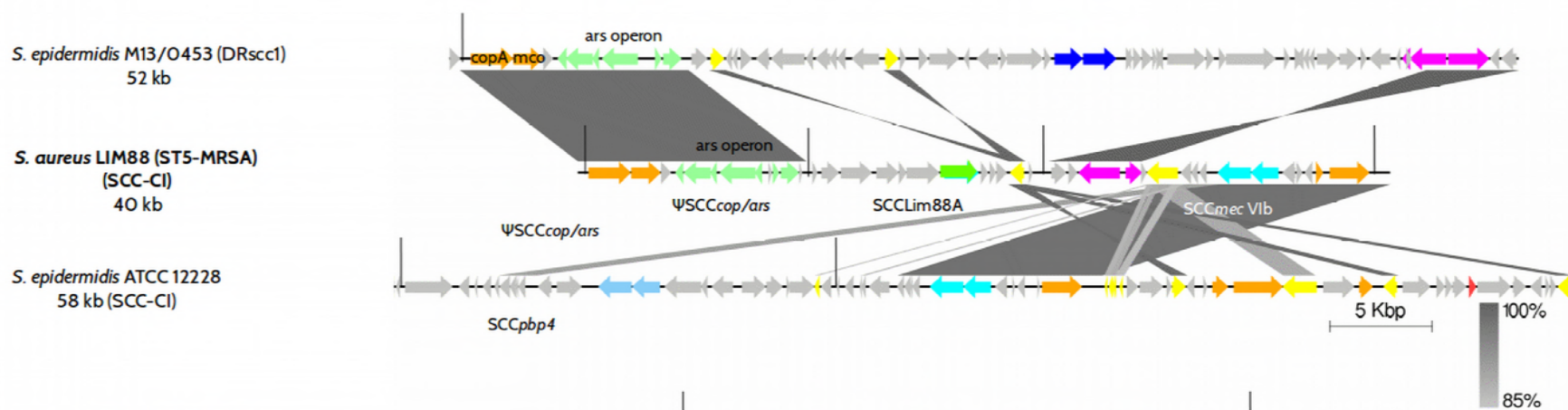
207 The sequence of the Ψ SCC_{cop/ars}-SCCLim88A-SCC_{mec} composite island of strain LIM88 is represented with open reading
 208 frames (ORFs) shown as gray arrows indicating the transcription direction. Colored arrows highlight specific genes according to the
 209 following criteria: orange = copper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

214

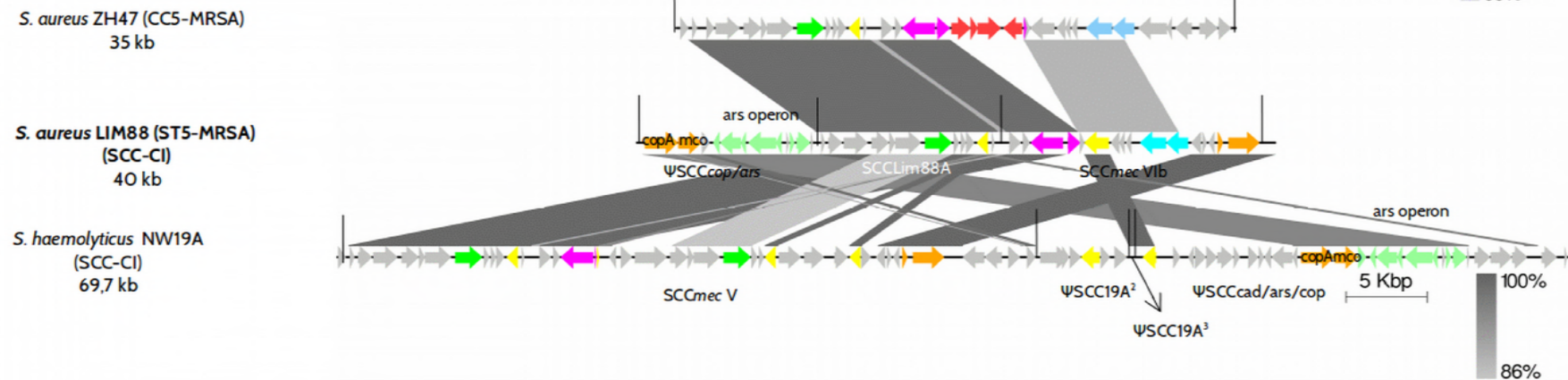
(A)



(B)



(C)



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

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

226

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