International Journal of Antimicrobial Agents 53 (2019) 694–697

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Short Communication

Novel staphylococcal cassette chromosome composite island (SCC-CI) with a new subtype of *SCCmec*VI cassette found in ST5 methicillin-resistant *Staphylococcus aureus* in France

Olivier Barraud[a,b,c], Frederic Laurent[d,e,f], Virginie Dyon-Tafani[d], Celine Dupieux-Chabert[d,e], Michele Bes[d,e], Marie-Cecile Ploy[a,b,c], Fabien Garnier[a,b,c], Patricia Martins Simoes[d,e,f,*]

[a] *CHU de Limoges, Laboratoire de Bactériologie-Virologie-Hygiène, Limoges, France*
[b] *Inserm, U1092, Limoges, France*
[c] *University of Limoges, UMR-S1092, Limoges, France*
[d] *National Reference Center for Staphylococci, Hospices Civils de Lyon, Lyon, France*
[e] *Department of Clinical Microbiology, Northern Hospital Group, Hospices Civils de Lyon, Lyon, France*
[f] *International Centre for Research in Infectious diseases, INSERM U1111, University of Lyon, Lyon, France*

ARTICLE INFO

Article history:
Received 13 July 2018
Accepted 9 March 2019

Editor: Mr Marc Stegger

Keywords:
Staphylococcus aureus
Antimicrobial resistance
MRSA
mecA
Novel SCC*mec*

ABSTRACT

An emergent kanamycin-susceptible ST5 methicillin-resistant *Staphylococcus aureus* (MRSA) lineage has been identified in France. Whole-genome sequencing revealed a 40-kb staphylococcal cassette chromosome (SCC) composite island with a mosaic structure including three SCC elements: a $\Psi SCC_{cop/ars}$, a SCC_{Lim88A} with a *ccr*C 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.

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 (SCCmec) [2]. SCC*mec* elements integrate into the genome of *S. aureus* within the orf*X*/*rl*mH g*ene*, via recombination events mediated by the site-specific recombinases (*ccr* complex) encoded by the SCC*mec* element itself [3]. The majority of MRSA clones detected worldwide belong 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 to 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* polymerase chain reaction (PCR) result, due to the absence of amplification for the *orfX*-SCC*mec* junction. The day after, antimicrobial susceptibility testing revealed an 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),

* Corresponding author.
E-mail address: patricia.martins-simoes01@chu-lyon.fr (P. Martins Simoes).

https://doi.org/10.1016/j.ijantimicag.2019.03.015

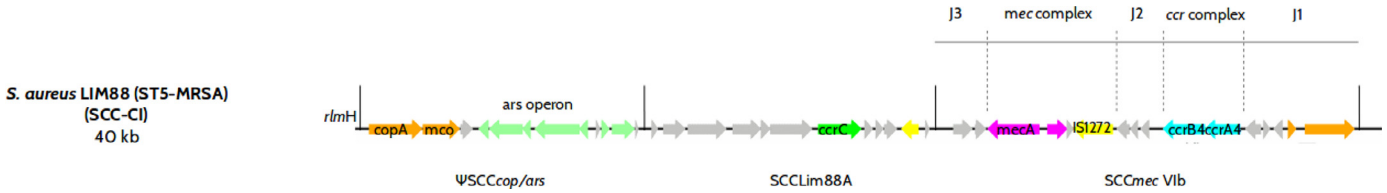

Fig. 1. Schematic representation of the staphylococcal cassette chromosome (SCC) composite island ΨSCC$_{cop/ars}$-SCC$_{Lim88A}$-SCC*mec* VIb element in ST5 methicillin-resistant *Staphylococcus aureus* (MRSA) LIM88. The sequence of the ΨSCC*cop*/*ars*-SCCLim88A-SCC*mec* composite island of strain LIM88 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 (*cop*A and *mco, copA*/*cad*A); light green = arsenic-resistance-related genes (*ars* operon); green = staphylococcal cassette recombinase type C gene (*ccr*C); cyan = staphylococcal cassette recombinase type AB genes (*ccr*A4 and *ccr*B4); yellow = transposases (type IS*431* and IS*1272*); magenta = *mec*A complex genes (*mec*A and truncated *mec*R1). The SSC*mec* element architecture with the 'non-essential' J1, J2, J3 regions, *mec* complex and *ccr* complex are indicated above the SCC*mec* VIb element of strain LIM88.

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 (ISSs) 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 non-redundant 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].

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

3. Results and Discussion

We identified the presence of four ISSs comprising direct-repeat (DR) sequences typical of SCC-like cassettes. Thus, LIM88 carries an SCC composite island (CI) of 39.6 kb with three SCC elements in tandem: an ΨSCC$_{cop/ars}$ element (without recombinase genes), an SCC element with a *ccr*C recombinase (called SCC$_{Lim88A}$) and an SCC*mec* element with a type 4 *ccr*AB complex (Fig. 1). The nucleotide sequence of this novel SCC-CI was deposited in the NCBI database under the accession number GenBank KX646745.

Comparison of the 16.6-kb SCC*mec* (ORF25-40, Supplementary Table S1) element including 16 ORFs found in strain LIM88 with the 23-kb type VI SCC*mec* element of the New Paediatric ST5, t777 MRSA clone (strain HDE288) [9] reveals a high nucleotide similarity (>89%) of the 'non-essential' region J3, the type B1 *mec* complex (IS*431*-*mec*A-delta*mec*R-IS*1272*), the 'non-essential' region J2 and the *ccr*AB (type 4) complex found in both SCC*mec* elements (Fig. 2A). Only the J1 regions are distinct (Fig. 2A). Interestingly, the J1 region of LIM88 SCC*mec* type VI element shows an overwhelming similarity (more than 90% nucleotide Identity) with a region of an SCC element found in *S. epidermidis* strain ATCC12228 (Fig. 2B), which encompasses the *ccr*A4B4 complex and a *cop*A/*cadA* resistance gene. Based on these data, the SCC*mec* element found in strain LIM88 can be classified as a novel subtype of SCC*mec* type VI element, named SCCmecVIb after consulting and with the agreement of the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC).

Immediately downstream from the SCC*mec* element, a 11.6-kb SCC element (ORF13–24, Supplementary Table S1) carrying 12 ORFs, among which a *ccr*C recombinase (allele 7) and no known specific virulence or resistance markers, was identified. This element was named SCC$_{Lim88A}$. Genomic comparison reveals that this region is highly similar to the *rlm*H proximal J3 region of SCC*mec*$_{ZH47}$, an SCC*mec* element previously described in a CC5 strain isolated from drug users in Switzerland [16] but, also, to the J3 region found in an SCC-CI of the *S. haemolyticus* NW19A (GenBank KM369884) [17], a strain isolated from bovine milk (Fig. 2C). This mosaic structure is even more surprising when we look at the SCC*mec* element inserted immediately downstream SCC$_{Lim88A}$. The J3 and *mecA* complex itself of the SCC*mec* element are highly similar (90% nucleotide identity) to the *mec*A complex and its proximal J3 region as found in three different *SCCmec* elements: (i) the SCC*mec*$_{ZH47}$ (SCC*mec* new subtype IV) but without the aminoglycoside resistance transposon Tn*4001* inserted in the *mec*R gene (Fig. 2C); (ii) the SCC-CI of *S. haemolyticus* NW19A (Fig. 2C) and the SCC*mec* type VIa element of strain HDE288 of the ST5-MRSA New Paediatrics clone, as presented in Fig. 2A.

Finally, the third SCC element is, in fact, a pseudo-SCC, called ΨSCC$_{cop/ars}$, as it does not carry any recombinase. This 11.1-kb region, located immediately downstream from the *rlm*H gene, harbors 12 ORFS (ORF 1–12, Supplementary Table S1). Almost all of them encode for heavy metal detoxification functions: two copper-related resistance genes (ORF1 and ORF2) and a complete arsenic resistance operon (*ars* operon, ORF4-8, ORF10). As presented in Fig. 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 SCC*mec* 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) (Fig. 2B) but is also part of longer pseudo-SCC elements (SCC elements without the site-specific recombinases) (Fig. 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 ΨSCC$_{cop/ars}$-SCC$_{Lim88A}$-SCC*mec*VIb composed of three distinct SCC elements, with a non-SCC*mec* cassette inserted at the end of the *rlm*H gene, is likely the reason for the absence of amplification of the *orfX*-SCC*mec* 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 (Supplementary Fig. S1).

A preliminary screening of 40 MRSAs isolated from blood culture during 2015 suggests that the new ST5-MRSA lineage,

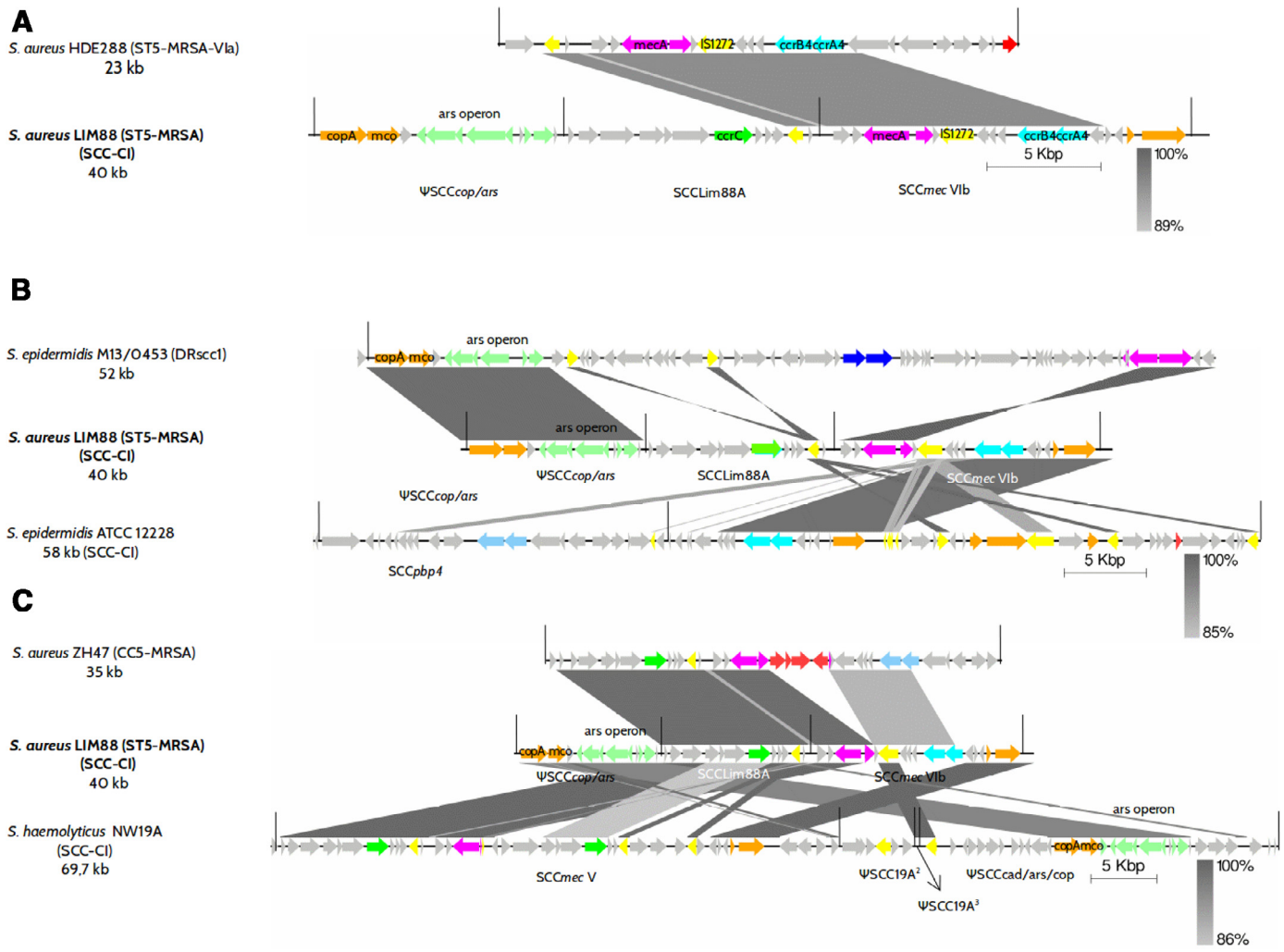

Fig. 2. Comparison of the staphylococcal cassette chromosome (SCC) composite island ΨSCC$_{cop/ars}$-SCC$_{Lim88A}$-SCC*mec* in ST5 methicillin-resistant *Staphylococcus aureus* (MRSA) LIM88 with other SCC elements or SCC composite islands (SCC-CIs) found in staphylococci. The SCC-CIs or SCC elements 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 (*ccr*C); cyan = staphylococcal cassette recombinase AB (*ccr*A4B4) genes; light blue = staphylococcal cassette recombinase AB (*ccr*A2B2) genes; dark blue = staphylococcal cassette recombinase AB (*ccr*A3B3) genes; yellow = transposases (type IS*431* and IS*1272*); 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.

carrying the novel SCC-CI ΨSCC$_{cop/ars}$-SCC$_{Lim88A}$-SCC*mec*VIb element, represents 15% (n=6 isolates) of blood culture MRSAs isolated in the CHU Limoges. LIM88 and the other six MRSA strains were co-resistant to fluoroquinolones but susceptible to all other antibiotic classes, notably to kanamycin. One out of the other six strains was also resistant to fosfomycin.

4. Conclusion

We report an original mosaic structure of the staphylococcal chromosome cassette composite island ΨSCC$_{cop/ars}$-SCC$_{Lim88A}$-SCC*mec*VIb element carried by an 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.

Acknowledgments

This work was presented at the 2016 ASM Microbe congress in June 2016 (Boston, USA), Poster Saturday-417. We thank the Limoges University BISCEm platform for technical assistance in WGS and the French Groupe de Microbiologie Clinique (GMC).

Funding

No funding.

Competing interests

No conflict of Interest for all authors.

Ethical approval

Not required.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.03.015.

References

[1] Utsui Y, Yokota T. Role of an altered penicillin-binding protein in methicillin- and cephem-resistant Staphylococcus aureus. Antimicrob Agents Chemother 1985;28:397–403.

[2] Katayama Y, Ito T, Hiramatsu K. Genetic organization of the chromosome region surrounding mecA in clinical Staphylococcal strains: role of IS431-mediated mecI deletion in expression of resistance in mecA-carrying, low-level methicillin- resistant *Staphylococcus haemolyticus*. Antimicrob Agents Chemother 2001;45:1955–63. doi:10.1128/AAC.45.7.1955-1963.2001.

[3] Hiramatsu K. Vancomycin-resistant Staphylococcus aureus: a new model of antibiotic resistance. Lancet Infect Dis 2001;1:147–55.

[4] Nübel U, Roumagnac P, Feldkamp M, Song J-H, Ko KS, Huang Y-C, et al. Frequent emergence and limited geographic dispersal of methicillin-resistant *Staphylococcus aureus*. Proc Natl Acad Sci U S A 2008;105:14130–5. doi:10.1073/pnas.0804178105.

[5] Chambers HF, Deleo FR. Waves of resistance: Staphylococcus aureus in the antibiotic era. Nat Rev Microbiol 2009;7:629–41. doi:10.1038/nrmicro2200.

[6] Monecke S, Müller E, Schwarz S, Hotzel H, Ehricht R. Rapid microarray-based identification of different mecA alleles in Staphylococci. Antimicrob Agents Chemother 2012;56:5547–54. doi:10.1128/AAC.00574-12.

[7] 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;43:1449–58.

[8] Sá-Leão R, Santos Sanches I, Dias D, Peres I, Barros RM, de Lencastre H. Detection of an archaic clone of Staphylococcus aureus with low-level resistance to methicillin in a pediatric hospital in Portugal and in international samples: relics of a formerly widely disseminated strain? J Clin Microbiol 1999;37:1913–20.

[9] Oliveira DC, Tomasz A, de Lencastre H. The evolution of pandemic clones of methicillin-resistant Staphylococcus aureus: identification of two ancestral genetic backgrounds and the associated mec elements. Microb Drug Resist Larchmt N 2001;7:349–61. doi:10.1089/10766290152773365.

[10] Inouye M, Dashnow H, Raven L-A, Schultz MB, Pope BJ, Tomita T, et al. SRST2: Rapid genomic surveillance for public health and hospital microbiology labs. Genome Med 2014;6:90. doi:10.1186/s13073-014-0090-6.

[11] Ito T, Ma XX, Takeuchi F, Okuma K YH, Hiramatsu K. Novel type V Staphylococcal cassette chromosome mec driven by a novel cassette chromosome recombinase, ccrC. Antimicrob Agents Chemother 2004;48:2637–51.

[12] Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, et al. The RAST Server: rapid annotations using subsystems technology. BMC Genomics 2008;9:75. doi:10.1186/1471-2164-9-75.

[13] Altschul S. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997;25:3389–402. doi:10.1093/nar/25.17.3389.

[14] Darling AE, Mau B, Perna NT. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One 2010;5:e11147. doi:10.1371/journal.pone.0011147.

[15] Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, Etienne J, et al. Combination of multiplex PCRs for staphylococcal cassette chromosome mec type assignment: rapid identification system for mec, ccr, and major differences in junkyard regions. Antimicrob Agents Chemother 2007;51:264–74.

[16] Heusser R, Ender M, Berger-Bächi B, McCallum N. Mosaic Staphylococcal cassette chromosome mec containing two recombinase loci and a new mec complex, B2. Antimicrob Agents Chemother 2007;51:390–3.

[17] Xue H, Wu Z, Li L, Li F, Wang Y, Zhao X. Coexistence of heavy metal and antibiotic resistance within a novel composite staphylococcal cassette chromosome in a Staphylococcus haemolyticus isolate from bovine mastitis milk. Antimicrob Agents Chemother 2015;59:5788–92. doi:10.1128/AAC.04831-14.

[18] Lazaris A, Coleman DC, Kearns AM, Pichon B, Kinnevey PM, Earls MR, et al. Novel multiresistance cfr plasmids in linezolid-resistant methicillin-resistant Staphylococcus epidermidis and vancomycin-resistant Enterococcus faecium (VRE) from a hospital outbreak: co-location of cfr and optrA in VRE. J Antimicrob Chemother 2017;72:3252–7. doi:10.1093/jac/dkx292.