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Partial Excision of the Chromosomal Cassette Containing the Methicillin Resistance Determinant Results in Methicillin-Susceptible *Staphylococcus aureus*

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We report a detailed characterization of methicillin-susceptible *Staphylococcus aureus* isolates from five French hospitals negative for both the *mecA* and the *ccrAB* loci but positive for the IS*431*::pUB110::IS*431*::*dcs* structure, present in some *Staphylococcus* cassette chromosome *mec* (SCC*mec*) types. The presence of SCC*mec*-associated elements suggests that this unusual resistant phenotype is due to a partial excision of SCC*mec* from epidemic methicillin-resistant *S. aureus*. The hypothesis of a genetic relatedness is strengthened by common sequence and *spa* types and similar susceptibility patterns.

Methicillin-resistant *Staphylococcus aureus* (MRSA) organisms are among the most important nosocomial pathogens, being responsible for a wide range of infections, some of which are associated with high mortality (2). Although the first MRSA strain was isolated in 1961, MRSA is still considered as an emerging pathogen, and public health threats result from the spread of hospital-acquired as well as community-acquired MRSA (1, 17, 20). Thus, important efforts have been made during the past decades in order to get a detailed knowledge of MRSA epidemiology and to improve infection control strategies (9). For instance, the enigma of acquisition of methicillin resistance has been partially solved with the discovery of the *Staphylococcus aureus* cassette chromosome *mec* (SCC*mec*): susceptible staphylococci acquire methicillin resistance by means of transfer of these mobile elements carrying *mecA*, the central element of methicillin resistance in staphylococci, and the *ccrAB* locus, which encodes its integration and excision (13).

In French hospitals, the MRSA isolation rate in blood cultures reached 33% in 2002, and this value has remained stable over the past years (25). Nevertheless, since 1992 MRSA epidemiology in France has changed with the emergence of gentamicin-susceptible MRSA strains, which are also more susceptible to other antibiotic classes when compared to previous dominant strains (4). These strains belong to clonal complex 8, which comprises most of the epidemic MRSA strains in Europe since 1960, and they derive directly from the methicillin-susceptible *Staphylococcus aureus* (MSSA) with sequence type 8 (ST8) after the acquisition of SCC*mec* type IV (22). An intriguing feature of these strains is the high frequency of the loss-of-methicillin-resistance phenotype due to the excision of SCC*mec*. This excision phenomenon originates in MSSA strains that have no SCC*mec* but that are still resistant to erythromycin, lincomycin, and fluoroquinolones (3). However, between 1999 and 2002, we have collected MSSA isolates from several French hospitals with a similar resistance profile except for the unusual addition of the resistance to tobramycin. Since resistance to tobramycin in French MRSA clone isolates is due to the *aadD* gene localized within the SCC*mec* type IV (linearized pUB110 in the *mecA* downstream vicinity) and encoding a nucleotidyl transferase (20), it was of interest to check for the presence of *mecA* and other SCC*mec*-associated elements in these isolates.

The relevant characteristics of the nine strains used in this study are summarized in Table 1 together with results of the detailed PCR analysis of the SCC structures. All primers used for PCRs were described elsewhere (12, 13, 16, 17, 18, 19, 20). Amplification of the *attB* site, the site of integration of the SCC*mec* into the *S. aureus* chromosome, was performed using cL1 and cR2 primers as described by Katayama et al. (13) and using the MSSA strain RO791 as a positive control. The allelic characterization of the *ccrAB* locus by PCR was performed as described by Ma et al. (16) with four sets of primers: (i) αc/βc, control for the presence of the *ccrAB* locus; (ii) α1/βc, specific for *ccrAB* allele 1; (iii) α2/βc, specific for *ccrAB* allele 2; and (iv) α3/βc, specific for *ccrAB* allele 3. The presumptive assignment of SCC*mec* types was performed by a multiplex PCR strategy according the method of Oliveira and de Lencastre (20). MRSA strains used as controls were COL for type I, PM64 (EMRSA-16) for type II, AR1239 for type III, and PM60 (EMRSA-15) for type IV. Then, six "uniplex" PCRs were performed as previously described (18, 19) to scan for the presence of the structure HVR-IS*431*-pUB110-IS*431*-*dcs*-*orfx*, which may be found in SCC*mec* type II and in some variants of SCC*mec* types I and IV (subtypes I-A and IV-A, respectively)

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TABLE 1. Origins and characteristics of *Staphylococcus aureus* strains used in this study[a]

Strain	Isolation			Typing		*attB* PCR result	SCC*mec* typing			SCC*mec* uniplex PCR result for:						
	Hospital	Yr	Resistance pattern	Sequence type by MLST	*spa* type		SCC*mec* multiplex PCR result	*ccrAB* type	SCC*mec* type	HVR::IS*431*	IS*431*::pUB110	pUB110 (3F02/3R01)	pUB110 (3F10/3LR10)	pUB110::IS*431*	IS*431*::*dcs*	dcs::*orfX*
660804	Rennes	1999	ERY, LIN, PEF, SPE	ND	ND	+	No band	NA	NA	ND	ND	ND	ND	ND	ND	ND
311563	Lannion	2002	TOB, ERY, LIN, PEF, SPE	ND	YHGFMBQBLO	–	*dcs*, IS*431*::pUB110	NA	NA	+ (ΔHVR)	+	+	+	+	+	+
8134	Cahors	2000	TOB, ERY, LIN, PEF, SPE	ND	ND	–	*dcs*, IS*431*::pUB110	NA	NA	–	+	+	+	+	+	+
1060728	St Brieuc	2002	TOB, ERY, LIN, PEF, SPE	ND	ND	–	*dcs*, IS*431*::pUB110	NA	NA	–	+	+	+	+	+	–
270617	St Brieuc	2002	TOB, ERY, LIN, PEF, SPE	ND	ND	–	*dcs*, IS*431*::pUB110	NA	NA	–	+	–	+	+	+	+
479968	Rennes	2002	TOB, ERY, LIN, PEF, SPE	8	YHGFMBQBLO	–	*dcs*, IS*431*::pUB110	NA	NA	–	+	+	+	+	+	+
MAQ	Vannes	2002	TOB, ERY, LIN, PEF, SPE	ND	ND	–	*dcs*, IS*431*::pUB110	NA	NA	–	+	+	+	+	+	+
761641	Rennes	1998	OXA, TOB, ERY, LIN, PEF, SPE	8	YHGFMBQBLO	–	*mecA*, *dcs*, IS*431*::pUB110	2	IV variant	ND	ND	ND	ND	ND	ND	ND
856985	Rennes	2002	OXA, TOB, ERY, LIN, PEF, SPE	ND	YHGFMBQBLO	–	*mecA*, *dcs*, IS*431*::pUB110	2	IV variant	ND	ND	ND	ND	ND	ND	ND

[a] OXA, oxacillin; TOB, tobramycin; ERY, erythromycin; LIN, lincomycin; PEF, pefloxacin; SPE, spectinomycin; ND, not determined; NA, not applicable.

FIG. 1. Putative organization of the IS*431*::pUB110::IS*431*::*dcs* structure. The relative positions of the primers used in the confirmation PCRs are indicated; the arrowheads indicate orientation (5′ to 3′).

(Fig. 1). Strain N315, which is the prototype strain for SCC*mec* type II and positive for the HVR-IS*431*-pUB110-IS*431*-*dcs*-*orfx* structure, was used as positive control. Finally, *spa* typing and multilocus sequence typing (MLST) were performed as previously described (6, 14, 23).

By SCC*mec* multiplex PCR all these isolates were negative for *mecA*. Nevertheless, positive signals for the *dcs* and the IS*431*::pUB110 junction were detected for tobramycin-resistant MSSA isolates but not for the tobramycin-susceptible strain 660804. To investigate if SCC*mec* derivative elements were still integrated at the *attB* site (the conserved insertion site for SCC*mec* within the putative open reading frame *orfX*), a PCR was performed. A positive result was obtained only for MSSA strain 660804 but not for any of the tobramycin-resistant MSSA isolates. This suggests that SCC*mec* element derivatives are integrated at the *attB* site like the complete SCC*mec* in MRSA. By "uniplex" PCR, all tobramycin-resistant isolates were confirmed to be positive for the IS*431*::pUB110::IS*431*::*dcs* structure, which, except for isolate 1060728, closely maps to the *orfX* (Fig. 1). The more parsimonious hypothesis is that these MSSA strains are derived from a French MRSA clone which is characterized by a SCC*mec* type IV variant (subtype A) harboring the HVR::IS*431*::pUB110::IS*431*::*dcs* structure at its 3′ end (19). The likelihood of this hypothesis is strengthened by the fact that both MSSA 479968 and MRSA 761641 have the same sequence type, ST8, by MLST and the same *spaA* type, YHGFMBQBLO. Since all these MSSA strains were found to be negative by PCR for the *ccrAB* locus, the presence of a SCC-like structure may be excluded. Therefore, the presence of the IS*431*::pUB110::IS*431*::*dcs* structure is likely due to a partial excision of the SCC*mec* from MRSA, which involved the deletion of the *mecA* gene and its upstream vicinity. Like the diversity in geographic origin, the variability found in the organization of the SCC*mec* element derivatives argues strongly for frequent and independent excision events rather than dissemination of an epidemic MSSA strain due to the clonal expansion of a single isolate. Nevertheless, since isolates have been collected over a 3-year period, we cannot exclude rearrangements of SCC*mec* occurring in an epidemic strain.

Recovery of methicillin susceptibility in resistant isolates has been described a few years after emergence of MRSA, first in laboratory studies (5, 8, 13) and then in clinical strains (3, 11, 15). The recent description of the staphylococcal cassettes allows a better understanding of this phenomenon. SCC*mec* carries not only the *mecA* gene but also *ccrAB* genes, which encode recombinases acting for the integration into or the excision from the chromosome (13). There are four main types of SCC*mec* and probably many variants, especially for type IV (12, 24). This last type is present in many more genetic backgrounds, including hospital-acquired and community-acquired

MRSA epidemic lineages, than other types, suggesting an enhanced mobility (4, 7, 19, 21, 22). An indirect argument for this mobility would be the high frequency of excision observed among isolates belonging to the French MRSA clone.

To our knowledge this is the first detailed report of clinical MSSA isolates containing SCC*mec* elements. Recently Hutlesky et al. have reported amplification of the junction SCC*mec-orfX* in 4.6% of isolates from a worldwide collection of MSSA strains, but, as stated by the authors, it was not clear if these isolates retained SCC*mec* elements after excision or possessed another staphylococcal cassette (10). Recognition of these MSSA isolates may pose some problems in clinical microbiology laboratories which perform detection of MRSA carriage by real-time amplification of the *dcs*::*orfX* junction for SCC*mec* (10, 26). The presence of *dcs* in MSSA strains, as described in this study, might compromise the efficiency of this test.

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