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Epidemiology of Invasive Methicillin-Resistant *Staphylococcus aureus* Clones Collected in France in 2006 and 2007▽

Olivier Dauwalder,[1,2,3] Gérard Lina,[1,2,3] Géraldine Durand,[1,2,3] Michèle Bes,[1,2,3] Hélène Meugnier,[1,2,3] Vincent Jarlier,[4] Bruno Coignard,[5] François Vandenesch,[1,2,3] Jerome Etienne,[1,2,3] and Frédéric Laurent[1,2,3]*

Université Lyon, Centre National de Référence des Staphylocoques, F-69622, Lyon, France[1]; INSERM, U851, F-69370, Lyon, France[2]; Hospices Civils de Lyon, F-69004, Lyon, France[3]; Laboratoire de Bactériologie-Hygiène, UFR de Médecine Pierre et Marie Curie Paris VI, Paris, France[4]; and Département des Maladies Infectieuses, Institut de Veille Sanitaire, F-95000, Saint-Maurice, France[5]

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We conducted a prospective multicenter study of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates, including the first five consecutive clinical isolates, collected between September 2006 and February 2007 in 23 hospitals located throughout France (Fig. 1). The 111 isolates were tested for their antibiotic susceptibility patterns and were extensively characterized by screening for drug resistance and *agr* alleles, multilocus sequence typing (ST), staphylococcal cassette chromosome *mec* (SCC*mec*) typing, *spa* typing, and PCR profiling of 21 toxin genes. Clones were designated by their ST followed by their SCC*mec* type (I to VI). The Lyon clone ST8-IV or ST8-IV$_{variant}$ ($n = 77$; 69.4%) was widely distributed. Four minor clones were also detected, namely, the "classical" Pediatric clone ST5-IV ($n = 9$; 8.1%), the "new" Pediatric clone ST5-VI ($n = 8$; 7.2%), the clone Geraldine ST5-I$_{truncated}$ ($n = 7$; 6.3%), and the European clone ST80-IV ($n = 4$; 3.6%). The six other isolates were related to five rare clones. Relative to that of other European countries, the situation in France is marked by the predominance of a specific major clone and the worrying emergence of minor clones with enhanced virulence and new antibiotic susceptibility profiles.

A thorough knowledge of the epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates is needed to prevent their spread, to determine the clinical spectrum of MRSA disease, and to optimize treatment. Many molecular techniques have been developed to allow the unambiguous assignment of each isolate to previously described or new MRSA clones (8, 15, 26, 29), and these techniques have helped to monitor the evolutionary process of the emergence and temporal and geographical spread of pandemic clones (1, 8, 38).

Several epidemic healthcare-associated MRSA clones (HA-MRSA) have emerged since the 1970s. During the last decade, five major pandemic clones, named Iberian, Brazilian, Hungarian, New York/Japan, and Pediatric, have been identified (1, 35, 36), while other new or preexisting clones emerged in some specific areas. The recent worldwide spread of several community-acquired MRSA (CA-MRSA) clones made even more complex the understanding of this epidemiology (42).

In France, several new MRSA clones have been described in the last decade (5, 16, 28), but the current epidemiology of MRSA is poorly documented. The aim of this study was to characterize MRSA isolates collected during a 6-month prospective multicenter study in representative French hospitals. The isolates were extensively typed by accessory gene regulator (*agr*) allele determination, multilocus sequence typing (MLST), *spa* typing, structural analysis of the staphylococcal cassette chromosome *mec* (SCC*mec*) element, toxin profiling, and antimicrobial resistance phenotyping.

MATERIALS AND METHODS

We conducted a prospective multicenter study of MRSA isolates collected between September 2006 and February 2007 by 23 representative randomly selected French hospital laboratories. The first five isolates, obtained from different patients by invasive sampling (blood or fluid from a normally sterile site; one isolate per patient) were sent to the French National Reference Center for Staphylococci (Lyon, France). Isolates were considered to be community acquired if a sample obtained within 48 h after admission was culture positive and hospital acquired if obtained later. Antimicrobial susceptibility was determined by each participating laboratory as recommended by the French Society for Microbiology (17).

The identification of the 111 isolates was confirmed by multiplex PCR amplification of the *agr* (25) and by determining the *agr* allelic group. The isolates were fully typed by the French National Reference Center for Staphylococci (Lyon, France) and were screened for genes encoding methicillin resistance (*mecA*), staphylococcal enterotoxins (*se*) A, B, C, D, H, K, L, M, O, P, Q, and R (*sea* to *sed*, *seh*, *sek* to *sem*, and *seo* to *ser*), toxic shock syndrome toxin 1 (*tst*), exfoliative toxins A, B, and D (*eta*, *etb*, and *etd*), Panton-Valentine leukocidin (PVL; *lukPV*), class F LukM leukocidin (*lukM*), beta-hemolysin (*hlb*), and epidermal cell differentiation inhibitor (*edinA*, *edinB*, and *edinC*), as previously described (25, 43). *spa* typing was performed with the Ridom Staph Type standard protocol (www.ridom.com) and by using the Ridom *Spa*Server, which automatically analyses *spa* repeats and assigns *spa* types (http://*spa*.ridom.de/index.shtml). MLST was performed as described elsewhere (14) for all isolates carrying *agr-2* or *agr-3* and for 54 of 81 *agr-1* isolates representative of all toxin types, susceptibility profiles, and SCC*mec* types detected within this subgroup. SCC*mec* types were determined by PCR with a simplified version of Kondo's typing system, including M-PCR-1 and M-PCR-2, without determining differences in the junkyard region (26). In specific cases, SCC*mec* typing was also performed as recommended by Oliveira et al. (33) and *ccrB* gene sequencing was performed as previously described (33). We use the classification of Ito et al. (24) and Ma et al. (30) for SCC*mec* elements.

* Corresponding author. Mailing address: Centre National de Référence des Staphylocoques, Faculté Laennec, Université Lyon, Lyon F-69372, France. Phone: 33478778657. Fax: 33478778658. E-mail: frederic.laurent@chu-lyon.fr.

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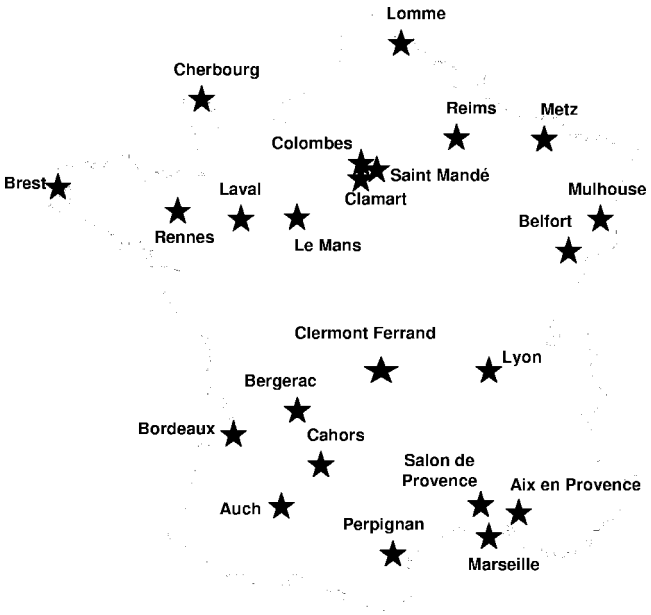

FIG. 1. Geographical distribution of the 23 French hospitals that participated in this study.

RESULTS AND DISCUSSION

The results of molecular typing methods indicated that one major clone and four minor clones of MRSA are currently predominant in France. A total of 111 different MRSA isolates were examined. They were isolated by culture of blood (n = 82; 74%), puncture fluid from a sterile cavity (n = 28; 25%), or cerebrospinal fluid (one case). The patients were hospitalized in intensive care units (n = 26, 23.4%), daycare units (n = 62; 55.9%), or outpatient departments (n = 23; 20.7%). Sixty-seven isolates (60.3%) were hospital acquired. All isolates with the same ST and identical or variant SCC*mec* elements were considered to belong to the same clone.

A first major clone, comprising 77 strains (69.4% of all isolates), was related to the Lyon clone (16). Since the 1990s, the Lyon clone has gradually replaced the gentamicin-resistant Iberian clone (ST247; SCC*mec* type I) that predominated in France in the 1990s (28). In our study, the Lyon clone was isolated in 22 of the 23 participating hospitals, confirming its wide dissemination all over France, and was mainly hospital acquired (48 hospital-acquired and 31 community-acquired isolates). The characteristics shared by these isolates corresponded largely to those reported by Ferry et al. (16), namely, *agr* allele 1, ST8, *spa* type t008 and related, SCC*mec* type IV (n = 70) or type $IV_{variant}$ (n = 7) with *mec* gene complex class B, and *ccrAB* allotype 2, associated with a supplementary *ccr* gene in variants. Nevertheless, the strains were more diverse than initially reported (16). The *sea* gene was found in only 88% of isolates (68 out of 77), *sed* in 49% (38 out of 77), *ser* in 49% (38 out of 77), and *hlb* in 12.7% (10 out of 77 isolates). The most-common toxin types were *sea* alone (43.6%) and *sea* plus *sed* and *ser* (41%). Although these isolates were consistently resistant to methicillin and fluoroquinolones, the antimicrobial susceptibility profiles were highly variable (more than 20 antibiotypes). This points to frequent acquisition and loss of toxin-carrying and resistance-carrying mobile genetic elements, such as bacteriophages, plasmids, or transposons, even if some changes in antibiotic susceptibility are due to point mutations. This confirms that the horizontal mobility of such genetic elements in MRSA is probably greater than previously believed (23, 37). Moreover, in addition to the classical *ccrAB* allotype 2 of SCC*mec* type IV, a supplementary recombinase locus (*ccrC* in two cases and *ccrAB4* in five cases) was detected in seven isolates belonging to the Lyon clone and confirmed in single isolated colonies. Except in strains harboring SCC*mec* type III (with *ccrAB3*), which is always associated with SCC*mercury* (with *ccrC*) (8), the combination of two *ccr* genes (*ccrAB* with *ccrC* or two *ccrC* genes) has rarely been reported (8, 21, 41). To our knowledge, the combination of two *ccrAB* genes (*ccrAB2* and *ccrAB4*) has not previously been reported in an *S. aureus* strain and has only been reported once in a *Staphylococcus epidermidis* strain (20). It remains to be determined whether the different *ccr*s are located within the SCC*mec* element or outside the cassette in the chromosome, as previously described for *mecA*-negative strains. Further structural studies of these SCC*mec* elements are under way. Full sequencing of the cassette, cohybridization, or artificial excision with a high-copy-number plasmid harboring an excisionase will probably help to resolve this issue. Finally, this clone shared some features (ST8 and SCC*mec* IV) with USA300, an epidemic CA-MRSA clone in the United States (42), but these two clones are very different according to their resistance profiles and their toxin profiles, and no relation has been established between these two clones. Moreover, USA300 was initially described as a CA-MRSA clone (even if this clone has now invaded U.S. hospitals), while the Lyon clone is typically a French HA-MRSA clone.

The first minor clone found here (n = 7), the Geraldine clone, had already been identified during a passive French survey of *S. aureus* infections in 2002 and 2003 (12). This *tst*-MRSA clone caused both toxic shock syndrome and suppurative infections. It was described as *agr-2*, ST5, *spa* type t002 or related, and SCC*mec* type IV. In our study, seven isolates (6.3%) from six hospitals located throughout France matched these molecular characteristics. As previously reported, the infections were both community acquired (n = 3) and hospital acquired (n = 4). However, our patients had a median age of 74 years, compared to only 3 years in Durand's study (12). As also described by Durand et al., we observed a specific toxinotype in which the *tst*, *sec*, *sed*, *sel*, *sem*, *seo*, and *ser* genes were always present, with the exception of one strain that was negative for *tst*, *sec*, and *sel* (Table 1). This latter strain probably arose through the loss of the *SaPI* mobile genetic element known to harbor these three toxin genes. The antibiotic susceptibility pattern of this clone was characteristic, with resistance to methicillin and fusidic acid in every case and to kanamycin and tobramycin in five of the seven isolates. The main difference relative to the previous description of this clone concerned the type of SCC*mec* cassette. It was initially identified as type IV in Oliveira's typing system (33), because PCR was positive for locus D but negative for locus A (corresponding to the *pls* fragment). The use of Kondo's typing system (26) revealed that it displayed features associated with SCC*mec* type I (*mec* gene complex class B and *ccrAB* allotype 1) as previously reported (29). We therefore assume that this

TABLE 1. Characteristics of the most frequent MRSA clones isolated in France[a]

Characteristic or parameter	Lyon clone	Geraldine Clone	Pediatric clone		European ST80 clone
			"Classical" subtype	"New" subtype	
No. of isolates (%)	77 (69.4)	7 (6.3)	9 (8.1)	8 (7.2)	4 (3.6)
No. of health care-associated isolates (%)	47 (61)	4 (57)	6 (66)	6 (75)	3 (75)
No. of hospitals (out of 23)	22	6	5	5	3
Molecular characteristics					
agr allelic group	1	2	2	2	3
ST	8	5	5	5	80
Clonal complex ST	8	5	5	5	80
spa type	t008 and related	t002 and related	t002 and related	t777	t044
SCC*mec* type	IV (n = 70) or $IV_{variant}$ (n = 7)	$I_{truncated}$	IV	VI	IV
mec complex	B	A	B	B	B
ccr allotype	AB2 with or without AB4 (n = 5) or C (n = 2)	AB1	AB2	AB4	AB2
Toxin genes					
Always positive		*tst*, *sec*, *sed*, *sel*, *sem*, *seo*, *ser*[b]	*sem*, *seo*	*sed*, *sem*, *seo*, *ser*	*luk-PV*, *etd*, *edin*
Always negative	*seb*, *sec*, *seh*, *sek*, *sel*, *sem*, *seo*, *tst*, *eta*, *etb*, *etd*, *luk-PV*, *lukM*, *edin*	*sea*, *seb*, *seh*, *sek*, *sel*, *sep*, *eta*, *etb*, *etd*, *luk-PV*, *lukM*, *edin*, *hlb*	*sea*, *sec*, *seh*, *sek*, *seq*, *tst*, *eta*, *etb*, *etd*, *luk-PV*, *lukM*, *edin*	*sea*, *seb*, *sec*, *sek*, *sel*, *eta*, *etb*, *luk-PV*, *lukM*, *edin*	*sea*, *seb*, *sec*, *sed*, *seh*, *sek*, *sel*, *sem*, *seo*, *sep*, *seq*, *tst*, *eta*, *etb*, *lukM*, *edin*
Variably positive (%)	*sea* (86), *sed* (49), *ser* (49), *hlb* (13)		*seb* (22), *sed* (66), *sep* (44), *ser* (66), *hlb* (11)	*sep* (87), *hlb* (25)	
Susceptibility pattern					
Always resistant	Pen, Met, Flq	Pen, Met, Fus	Pen, Met, Flq	Pen, Met, Flq	Pen, Met, Kan, Fus
Variably resistant (% resistant)	Kan (75.3), Tob (75.3), Gen (2.6), Ery (50.6), Lin (53.2), Pri (7.8), Rif (1.3), Fos (9.1), Fus (9.1), Sxt (96.1)	Kan (28), Tob (28), Ery (14)	Kan(33), Tob (33), Ery (11)	Ery (12), Lin (12)	Ery (25)
Always susceptible	Tec, Van	Gen, Flq, Lin, Pri, Rif, Fos, Sxt, Tec, Van	Gen, Lin, Pri, Fus, Rif, Fos, Sxt, Tec, Van	Kan, Tob, Gen, Pri, Fus, Rif, Fos, Sxt, Tec, Van	Tob, Gen, Flq, Lin, Pri, Rif, Fos, Sxt, Tec, Van

[a] *spa*, staphylococcal protein A; *ccr*, cassette chromosome recombinase; *tst*, toxic shock syndrome toxin 1 gene; *luk*, staphylococcal leukocidin; *hlb*, beta-hemolysin; *edin*, epidermal cell differentiation inhibitor; *eta*, exfoliatin A; *etb*, exfoliatin B; *etd*, exfoliatin D; Pen, penicillin; Met, methicillin; Kan, kanamycin; Tob, tobramycin; Gen, gentamicin; Ery, erythromycin; Lin, lincomycin; Pri, pristinamycin; Rif, rifampin; Fos, fosfomycin; Van, vancomycin; Tec, teicoplanin; Sxt, trimethoprim-sulfamethoxazole; Fus, fusidic acid; Flq, fluoroquinolone.

[b] One Geraldine clone isolate was negative for *tst*, *sec*, and *sel*.

clone harbors a new subtype of the SCC*mec* type I element, characterized by deletion of the *pls* fragment, and therefore propose to name it "truncated SCC*mec* type I".

Another minor clone was the Pediatric clone (n = 17), first identified in a pediatric hospital in Portugal in 1992 (40) and subsequently detected in Poland, Argentina, Colombia, and the United States (19, 32, 40). This clone was recently subdivided into two clones on the basis of a different *ccrAB* allotype and J1 region of the genetic SCC*mec* element (34). The first, referred to here as the "classical" Pediatric clone, had SCC*mec* type IV with *ccrAB2* and represented most strains belonging to the original Pediatric clone identified in Portugal and internationally (40). The second, referred to here as the "new" Pediatric clone, had SCC*mec* type VI with *ccrAB4* and was restricted to a few Portuguese hospitals (34). We detected both these clones in this study. Nine isolates (8.1% of all MRSA isolates) collected from five hospitals matched the characteristics of the "classical" Pediatric clone, with the *agr-2* allele, ST5, *spa* type related to t311 (t045, t067, t509, t2173, or t1818), and SCC*mec* type IV (*mec* gene complex class B and *ccrAB* allotype 2). These isolates were also characterized by the presence of *sem* and *seo* and by resistance to methicillin and fluoroquinolones (Table 1). Eight other isolates (7.2%) were related to the "new" Pediatric clone. They possessed the *agr-2* allele, ST5, a unique *spa* type t777, and SCC*mec* type VI (*mec* gene complex class B and *ccrAB* type 4). The *sed*, *sem*, *seo*, and *ser* genes were always present, and the isolates were resistant to methicillin and fluoroquinolones and susceptible to all aminoglycosides. This is the first description of the "new" Pediatric clone outside of Portugal. Interestingly, it was as frequent as the "classical" Pediatric clone that represents a specific and unusual epidemiology.

The last minor clone was the European ST80 clone, which is one of the CA-MRSA clones most widespread in Europe (10, 22, 31, 42, 45). It expresses PVL and can cause severe skin and soft-tissue infections, as well as rapidly fatal pneumonia and extensive bone and joint infections (11, 18). The prevalence of this clone in France was estimated at less than 1% in 2004 (39). In the present study, four isolates (3.6%), three of which were community acquired, presented the specific profile of this clone, namely the *agr-3* allele, ST80, and SCC*mec* type IV (*mec* gene complex class B and *ccrAB* type 2), that is associated with a specific antibiotic-susceptibility pattern, including resistance to methicillin, kanamycin, and fusidic acid. All the isolates had the same *spa* type (t044) and the same toxinotype (*luk-PV*, *etd*, and *edin*). They were collected from three hospitals that are distant from one another, highlighting the widespread dissemination of this clone. Our results indicate a slow but worrying emergence of this clone (39) that should be watched closely in coming years.

Finally, we collected six sporadic isolates: one *agr-1*, ST1041 with SCC*mec* type V harboring *luk-PV*; one *agr-1*, ST1042 with SCC*mec* type III; two *agr-1*, ST247 with SSC*mec* type I; one *agr-2*, ST5 with SCC*mec* type I; and one *agr-3*, ST88 with SCC*mec* type I.

This study provides an overview of the invasive MRSA clones currently circulating in France. The pattern of MRSA clonal lineages is very different from that observed in neighboring countries, where, according to the most-recent data, the predominant clones are ST45-SCC*mec* type IV and ST8-SCC*mec* type IV in Belgium (9), ST22-SCC*mec* type IV in the United Kingdom (13), ST225-SCC*mec* type I and ST22-SCC*mec* type IV in Germany (27), ST228-SCC*mec* type I in Italy (7), and ST125-SCC*mec* type IV in Spain (4). No isolates belonging to these different clones were isolated in our study, and conversely, the Lyon clone seems to be infrequent in these countries (except in Belgium, where the Lyon clone shared the first place with ST45-SCC*mec* type IV (37% and 34% of all MRSA isolates, respectively) (9). Possible explanations are geographic segregation and/or differences in antimicrobial chemotherapy or antisepsis. In the same way, we confirm the complete disappearance of the gentamicin-resistant Iberian clone (ST247-SCC*mec* type I, *spa* t008, and related) that was by far the most-frequent French clone in the 1990s (5, 28) and its replacement by the Lyon clone. Several similar reports have been published, with the Iberian clone in a hospital setting in two cases (2, 38) or with other epidemic clones (3, 6). Various genetic factors have been proposed to explain such rapid evolution and the dissemination of particular epidemic clones. One characteristic of the Lyon clone is the presence of *sea* (87% of the isolates), and it was recently demonstrated that this gene is part of an immune evasion cluster carried by a phage and harboring a collection of virulence factors able to modulate the human immune system, namely SEA, SCIN (staphylococcal complement inhibitor), CHIPS (chemotaxis inhibitory protein of *S. aureus*), and SAK (staphylokinase) (44). This pathogenicity island could be involved in the success of the Lyon clone and in its ability to cause invasive infections. Finally, it must be noted that the two major clones isolated during this study, the Lyon clone and the "classical subtype" of the Pediatric clone, are both typically HA-MRSA (16, 34, 40), although they harbor SCC*mec* type IV that is usually considered a CA-MRSA marker.

We found that variable traits, such as toxin production and antibiotic susceptibility encoded by mobile genetic elements, were more variable than expected. Care should thus be taken when using such characteristics to define or even to identify MRSA clones circulating in a given area. However, they could be useful for monitoring the evolution of these clones and their potent pathogenicity profiles and for adapting empirical antimicrobial chemotherapy.

Finally, the results of this study indicate the emergence and spread of new MRSA clones in France, namely, the Geraldine clone, the European ST80 clone, and the "new" Pediatric clone. The dissemination of clones producing PVL has been extensively reported, but the emergence of a *tst*-positive MRSA clone is new. The spread of such clones with potent superantigenic activity (TSST-1) or the capacity to cause invasive disease (through PVL production) is of major concern. Even if their prevalence is currently low, the possibility of rapid spread, as recently reported for the USA300 clone, should be kept in mind. These clones with enhanced virulence thus represent a new threat in terms of pathogenicity, treatment, and the prevention of transmission and should be closely monitored in coming years.

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