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Detection of New Methicillin-Resistant *Staphylococcus aureus* Clones Containing the Toxic Shock Syndrome Toxin 1 Gene Responsible for Hospital- and Community-Acquired Infections in France

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Methicillin-resistant *Staphylococcus aureus* (MRSA) clones harboring the toxic shock syndrome toxin 1 (*tst*) gene have been detected in France and in Switzerland since 2002. During a passive survey conducted between 2002 and 2003, we collected 103 *tst*-positive *S. aureus* isolates from 42 towns in France, of which 27 were resistant to methicillin. The *tst*-positive MRSA belonged to two clones: a major clone comprising 25 isolates of sequence type (ST) 5 and *agr* group 2 and a minor clone comprising two isolates of ST30 and *agr3*. The *tst*-positive MRSA clones were associated with both hospital-acquired (12 cases) and community-acquired (8 cases) infections. The MRSA clones were mainly isolated from children (overall median age, 3 years). They caused a variety of clinical syndromes, including toxic shock syndrome and suppurative infections. Both clones were found to harbor a type IV staphylococcal chromosomal cassette *mec* (SCC*mec*) and to have similar antibiotic resistance profiles (usually resistant to oxacillin, kanamycin, and tobramycin and with intermediate resistance to fusidic acid). The origin of these clones is unclear. The *tst*-positive *agr2* MRSA clone has the same sequence type (ST5) of two pandemic nosocomial MRSA clones, namely, the Pediatric clone and the New York/Japan clone. These findings suggest that all these clones are phylogenetically related. The pulsotype of the *tst*-positive MRSA clones differed from that of methicillin-sensitive *S. aureus* (MSSA) clones by a single band involving the SCC*mec* element. These findings suggest that the *tst*-positive MRSA clones may have emerged from their respective MSSA counterparts.

Staphylococcus aureus is an important human pathogen in both hospitals and the community. The first methicillin-resistant *S. aureus* (MRSA) isolates were detected in the hospital setting in the early 1960s. A number of pandemic nosocomial clones have been characterized by molecular methods (3, 24, 25, 34). These epidemic MRSA strains of hospital origin have also been detected in the community, infecting patients with risk factors associated with hospital-acquired MRSA infection (H-MRSA), such as recent hospitalization. The epidemiology of MRSA has changed radically since 1999; in particular, true community-acquired MRSA (C-MRSA) infections have been reported in patients with no clear risk factors (2). These C-MRSA clones predominantly infect young and previously healthy patients and have now spread throughout the world (29). They produce Panton-Valentine leucocidin (PVL) and harbor a type IV staphylococcal chromosomal cassette *mec* (SCC*mec*) element (1, 4, 5, 13, 22, 37).

Toxic shock syndrome toxin 1 (TSST-1) is a superantigenic toxin secreted by some *S. aureus* isolates. TSST-1, encoded by the *tst* gene, is a major virulence factor in toxic shock syndrome (TSS), staphylococcal scarlet fever, and neonatal toxic shock-like exanthematous diseases (NTED) recently described in Japan and France (11, 16, 38). TSS was first described in 1978 by Todd et al. as a multisystem disease characterized by rapid onset of fever, hypotension, erythematous rash, and mucosal hyperemia, followed by desquamation and multiorgan involvement. TSS was initially linked to tampon use by young women, but non-menstruation-associated TSS now predominates, occurring both in the community and in hospitals secondary to local *S. aureus* infection (10). Musser et al. showed that *tst*-positive *S. aureus* strains were clonal by comparing their isoenzymatic profiles (21), and studies based on multilocus sequence typing (MLST) have recently shown that these strains belong to sequence type (ST) 30 (27). Jarraud et al. reported that most *tst*-positive *S. aureus* strains are genetically related and have a type 3 accessory gene regulator (*agr*) allele (15). The isolates in these studies were associated with community- and hospital-acquired diseases and were all methicillin-sensitive *S. aureus* (MSSA). There have been few reports of MRSA isolates producing TSST-1 in Japan or Germany (12, 30). In 2003, we observed the first French case of NTED due to TSST-1-producing methicillin-resistant *S. aureus* (16, 38).

In order to characterize TSST-1-producing *S. aureus* isolates in France, we retrospectively typed all *tst*-positive isolates sent to the French National Reference Center for Staphylococci in

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2002 and 2003 and collected relevant epidemiological and clinical data. We observed the presence of new *tst*-positive MRSA clones responsible for both hospital- and community-acquired infections.

MATERIALS AND METHODS

Bacterial isolates. Among the 1,550 unconstrained strains sent to the French National Reference Center for Staphylococci during 2002 and 2003, 103 isolates from 42 towns were *tst* positive. As controls we used nine *tst*-positive MRSA isolates from Australia (one isolate, provided by Graeme Nimmo), Switzerland (three isolates), and Japan (five isolates causing neonatal toxic shock-like exanthematous diseases, TWCC3812, TWCC390861, TWCC4082, TWCC4382, and TWCC4410) (16). We also used an isolate representative of the Pediatric clone and an isolate representative of the New York/Japan clone.

Data collection. For each *S. aureus* strain we collected relevant clinical information (age, sex, type, and site of infection) by using a standard form provided by the French National Reference Center for Staphylococci. TSS, staphylococcal scarlet fever, and neonatal toxic shock syndrome-like NTED were diagnosed by using published criteria (11, 21, 30, 32). For this study, MRSA infection was considered to be community acquired if the specimen was obtained outside the hospital setting or less than 2 days after hospital admission of a patient with no direct or indirect exposure to the healthcare system in the previous year (2).

DNA extraction. Strains were grown on brain heart infusion agar or in brain heart infusion broth at 37°C overnight. Genomic DNA was extracted with a standard procedure, and its concentration was estimated spectrophotometrically (18). Amplification of *gyrA* was used to confirm the quality of each DNA extract and the absence of PCR inhibitors. All PCR products were analyzed by electrophoresis on ethidium bromide-stained 1% agarose gels (Sigma, France).

Identification of *agr* alleles. The *agr* group (*agr1* to *-4*) was determined by PCR as previously described (15).

Detection of the *mecA* gene and SCC*mec* typing. The *mecA* gene coding for methicillin resistance was detected by PCR as described by Murakami et al. (20). The staphylococcal chromosomal cassette *mec* (SCC*mec* I to IV) was detected by using the method of Oliveira et al. (23). The following reference strains, kindly provided by Herminia de Lencastre and Alexander Tomasz, were used as controls: COL (SCC*mec* I), BK2464 (SCC*mec* II), HU106 (SCC*mec* III), and BK2529 (SCC*mec* IV).

Detection of toxin and adhesin genes. Sequences specific for staphylococcal enterotoxin genes (*sea-e* and *seg-o*), the toxic shock syndrome toxin gene (*tst*), exfoliative toxin genes (*eta* and *etb*), PVL genes (*lukS*-PV-*lukF*-PV), the LukE-lukD leukocidin genes (*lukE-lukD*), the class F *lukM* leukocidin gene (*lukM*), and hemolysin genes (gamma [*hlg*], gamma variant [*hlgv*], and beta [*hlb*]) and for nine MSCRAMM genes (microbial surface components recognizing adhesive matrix molecules), bone sialoprotein binding protein (*bsp*), clumping factors A and B (*clfA* and -B), collagen binding protein (*cna*), elastin binding protein (*ebpS*), laminin binding protein (*eno*), fibronectin binding proteins A and B (*fnbA* and *-B*), and extracellular fibrinogen binding protein (*efb*), were detected by PCR as described elsewhere (15, 23, 26, 27, 35, 37).

Antimicrobial susceptibility testing. Susceptibility tests were performed with the ATB System (bioMérieux, France).

Capsular typing. Capsular serotyping was performed for all MRSA strains and for randomly selected MSSA strains. The strains were grown for 24 h at 37°C on Columbia agar plates containing 2% $MgCl_2$ and 0.5% $CaCl_2$. Several colonies of each strain were suspended in 0.9% saline and tested by slide agglutination with rabbit polyclonal antibodies specific for capsular polysaccharide types 5 and 8 (8, 9).

Fingerprinting by PFGE. SmaI macrorestriction patterns were obtained by using a contour-clamped homogeneous electric field DR-II apparatus (Bio-Rad), as described elsewhere (19). Strain NCTC 8325 was used as a pulsed-field gel electrophoresis (PFGE) control. Resolved macrorestriction patterns were compared as recommended by Tenover et al. (33). Isolates were assigned to a single clonal group if they differed by less than six bands. PFGE patterns with more than six band differences (<75% similarity) were considered to correspond to different types.

The *mecA* gene was tested for in one of the PFGE bands, as follows: the fragment was cut out from the agarose gel, DNA was extracted by using the MinElute gel extraction kit protocol (QIAGEN), and PCR with the *mecA* primers and multiplex PCR for SCC*mec* typing were performed on the extract as described above.

***spa* typing.** *spa* typing was performed on MRSA isolates and on *agr2* MSSA isolates, as previously described (14). The x region of the *spa* gene was amplified by PCR. *spa* types were determined with Ridom Staph Type software (Ridom GmbH, Germany), which automatically detects *spa* repeats and assigns a *spa* type.

MLST. MLST was performed on strains representative of each clonal group, as described elsewhere (6, 36). The allelic profile of each strain was obtained by sequencing internal fragments of seven housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*) and entering them on the MLST home page (http://saureus.mlst.net), where seven numbers depicting the allelic profile were assigned which defined an ST (6). To determine genetic relationships, MLST data were examined with BURST software (*b*ased *u*pon *r*elated *s*equence *t*ypes; details are available from http://www.mlst.net/BURST/burst.htm). The algorithm places STs that share five out of seven MLST alleles in a common clonal complex (7).

TABLE 1. Distribution of the *mecA* gene and *agr* alleles among 103 French *S. aureus* isolates containing the *tst* gene collected between 2002 and 2003

agr allele type	No. (%) of isolates		
	mecA$^+$ (n = 27)	*mecA* deficient (n = 76)	Total
1	0 (0)	1 (1)	1
2	25 (93)	5 (7)	30
3	2 (7)	70 (92)	72
4	0 (0)	0 (0)	0

RESULTS

Distribution of isolates according to methicillin resistance and *agr* group. Among the 103 *tst*-positive *S. aureus* isolates, 27 were methicillin resistant (*mecA*$^+$), and 76 were methicillin susceptible (*mecA* deficient) (Table 1). Twenty-five *tst*-positive MRSA isolates had *agr* allele type 2, and two had *agr* allele type 3. Seventy *tst*-positive MSSA isolates had *agr* allele type 3, five isolates had *agr* allele type 2, and one isolate had *agr* allele type 1.

Clinical characteristics of *tst*-positive MRSA infections. The median age of the 27 patients with *tst*-positive MRSA infections was 3 years (range, <1 month to 84 years), and the sex ratio was 1. Five patients had toxic shock syndrome, two had NTED (31), and one had staphylococcal scarlet fever; nine patients had toxic shock syndrome but did not fulfill all the criteria of a TSST-1-mediated syndrome (i.e., fever and rash without shock) (Table 2). Five skin infections occurred in patients with varicella. Eight patients had deep-seated infections (pneumonia or osteoarthritis), and no clinical information was available for two other patients. Two deaths occurred. The isolates were recovered from skin and soft tissues (14 isolates), blood (7 isolates), the umbilicus (2 isolates from cases of NTED), bronchopulmonary secretions (2 isolates), a prosthesis (1 isolate), and a ligament (1 isolate).

Information on the hospital or community acquisition of the infection was available for 20 MRSA infections and 51 MSSA infections. The origin of MRSA infection was unknown in seven cases. Eight of the 27 patients with *tst*-positive MRSA isolates had no known link to healthcare facilities and no known risk factors for MRSA acquisition; these cases were considered to be community acquired. Twelve cases were hospital acquired.

Microbiological characteristics of *tst*-positive MRSA isolates. The 25 *tst*-positive *agr2* MRSA strains all harbored the *sec*, *sed*, *sel*, *sem*, *seo*, *lukDE*, and *hlgv* toxin genes and the *clfA-B*, *ebpS*, *eno*, and *efb* adhesin genes (Table 3). These

TABLE 2. Clinical data on *tst*-positive MRSA infections

Clinical presentation	Patient and isolate no.[a]	City of isolation	Age (yrs)	Sex[b]	Samples	Site of acquisition	*agr* type	PFGE type
True TSS[c]	1, HT20020212	Lyon	2	M	Skin	Community	2	A1
	2, HT20020255	Bordeaux	42	F	Blood	Nosocomial	2	A1
	3, HT20020256	Bordeaux	0	F	Blood	Nosocomial	2	A1
	4, HT20030603	Lyon	25	F	Blood	Nosocomial	2	A1
	5, HT20030159	Geneva	0	ND[e]	Skin	Unknown	2	A3
Possible TSS[d]	6, HT20030119	Marseille	3	F	Skin	Nosocomial	2	A1
	7, HT20030369	Lyon	0	M	Skin	Unknown	2	D
	8, HT20030416	Lyon	9	F	Skin	Community	2	A7
	9, HT20030618	Lyon	8	F	Skin	Unknown	2	A2
	10, HT20030434	Marseille	0	F	Blood	Unknown	2	A1
	11, HT20030727	Lyon	84	M	Skin	Community	2	A1
	12, HT20030769	Fréjus	2	F	Skin	Unknown	2	A3
	13, HT20030695	Lausanne	2	ND	Skin	Unknown	2	A10
	14, HT20030849	Geneva	2	ND	Skin	Community	2	A9
Superinfection of varicella	15, HT20020188	Lyon	1	M	Skin	Community	2	A1
	16, HT20020277	Paris	1	M	Skin	Community	2	A8
	17, HT20020369	Lyon	7	M	Skin	Community	2	E
	18, HT20030228	Lyon	0	M	Skin	Community	2	A3
	19, HT20030651	Lyon	4	M	Skin	Community	2	A12
NTED	20, HT20020780	Tours	0	ND	Umbilicus	Nosocomial	2	A3
	21, HT20020781	Tours	0	ND	Umbilicus	Nosocomial	2	A3
Staphylococcal scarlet fever	22, HT20030157	Lille	0	M	Skin	Unknown	2	A3
Pneumonia	23, HT20020132	Boulogne	27	F	Skin	Nosocomial	2	A5
	24, HT20020417	Annecy	1	M	Bronchopulmonary secretion	Unknown	2	A12
	25, HT20030216	Lyon	65	M	Blood	Nosocomial	2	A1
	26, HT20030639	Lyon	31	M	Blood	Nosocomial	2	A7
	27, HT20020459	Rouen	1	F	Bronchopulmonary secretion	Nosocomial	3	G2
Osteoarthritis	28, HT20030749	Bondy	0	F	Blood	Unknown	2	A4
	29, HT20030095	Lyon	28	M	Ligament	Nosocomial	2	A3
	30, HT20020665	Marseille	14	F	Prosthesis	Nosocomial	3	G3

[a] Isolates 5, 13, and 14 from Switzerland were not among the 27 French *tst*-positive MRSA.
[b] M, male; F, female.
[c] Cases associated with TSS according to the reference criteria.
[d] Cases associated with TSS but that did not fulfill all criteria of TSST-1-mediated syndrome.
[e] ND, no data.

isolates were of capsular type 5, except for two isolates which could not be typed with this method. All but one were resistant to penicillin, oxacillin, kanamycin, and tobramycin and had intermediate resistance to fusidic acid; the remaining isolate was susceptible to kanamycin and tobramycin (Table 3). Seven isolates were resistant to other antimicrobial agents such as erythromycin, lincomycin, or tetracycline. All had an SCC*mec* element type IV, except for one isolate which had an SCC*mec* element type IVA and two isolates which were nontypeable (possibly new SCC*mec* variants). PFGE gave more diverse results: all but two of the isolates belonged to PFGE type A (14 subtypes), while the remaining isolates were of types D and E (Fig. 1). The main *spa* type was *spa* 2 (21 isolates). The other isolates had a related *spa* type that differed by one (*spa* 10 and *spa* 242) or two (*spa* 568) repeats. These isolates were all of ST5, as determined by MLST. Overall, the 25 *tst*-positive *agr2* MRSA isolates were highly clonally related. This clone was detected in 12 towns in France, and three isolates from Switzerland had similar characteristics. Five Japanese *tst*-positive *agr2* MRSA isolates from patients with NTED were related to this clone (Table 3).

Two *tst*-positive *agr3* MRSA isolates were identified. They possessed the *sea*, *sem*, *seo*, *hlg, clfA-B*, *cna*, and *ebpS* genes and were of capsular type 8. These two isolates were resistant to penicillin, oxacillin, kanamycin, tobramycin, and erythromycin and had intermediate resistance to fusidic acid. One isolate had the SCC*mec* IV element, whereas the other had the SCC*mec* IVA element. Their PFGE patterns differed by three bands, and both isolates belonged to PFGE type G (Fig. 1). Their *spa* types differed by only four repeats (*spa* 638 and *spa* 584), and both isolates were ST30, as determined by MLST. These two isolates were considered to be clonally related.

Comparison of the *tst*-positive *agr2* isolates with the New York/Japan clone and the Pediatric clone. The 25 *tst*-positive *agr2* MRSA isolates were ST5 and belonged to capsular type 5, like the New York/Japan and Pediatric clones. The New York/Japan clone contained the *tst* toxin gene, contrary to the Pediatric clone. The New York/Japan clone also did not contain the toxin gene (*sed*), contrary to the 25 *tst*-positive *agr2* MRSA isolates. The 25 *tst*-positive *agr2* MRSA isolates and the Pediatric clone harbored SCC*mec* element IV, whereas the New York/Japan clone harbored SCC*mec* element type II. The 25 *tst*-positive *agr2* MRSA isolates and the New York/Japan clone were *spa* type 2, while the Pediatric clone was *spa* type 311 (diverging by only one repeat).

Comparison of *tst*-positive MRSA isolates with MSSA isolates. The 25 *tst*-positive *agr2* MRSA isolates and the 5 *tst*-positive *agr2* MSSA isolates had similar virulence determinants, an identical capsular type (type 5) and sequence type (ST5), and a common PFGE type (A) which differed by a

TABLE 3. Microbiological characteristics of *tst*-positive *Staphylococcus aureus* isolates

Resistance group, country of origin, and no. of isolates	ST[a]	CC[b]	*spa* type	Capsular type	*tst* gene	Toxin genes	Adhesin genes	SCC*mec* type	Antibiotic resistance[c]	PFGE type(s)
MRSA isolates *agr2*										
France (n = 27)										
13	5	5	2	5	+	*sec*, *sed*, *sel*, *sem*, *seo*, *lukED*, *hlgv*	*clfA-B*, *ebpS*, *eno*, *efb*	IV	P, OX, K, T, FU	A1, A2, A3, A7, E
1	5	5	568	5	+	*sec*, *sed*, *sel*, *sem*, *seo*, *lukED*, *hlgv*	*clfA-B*, *ebpS*, *eno*, *efb*	IV	P, OX, K, T, FU	A3
1	5	5	242	5	+	*sec*, *sed*, *sel*, *sem*, *seo*, *lukED*, *hlgv*	*clfA-B*, *ebpS*, *eno*, *efb*	IV	P, OX, K, T, FU	A4
4	5	5	2	5	+	*sec*, *sed*, *sel*, *sem*, *seo*, *lukED*, *hlgv*	*clfA-B*, *ebpS*, *eno*, *efb*	IV	P, OX, K, T, E, FU	A3
1	5	5	2	5	+	*sec*, *sed*, *sel*, *sem*, *seo*, *lukED*, *hlgv*	*clfA-B*, *ebpS*, *eno*, *efb*	IV	P, OX, K, T, E, L, FU	A8
1	5	5	2	NT[d]	+	*sec*, *sed*, *sel*, *sem*, *seo*, *lukED*, *hlgv*	*clfA-B*, *ebpS*, *eno*, *efb*	NT	P, OX, K, T, TE, FU	A12
1	5	5	2	5	+	*sec*, *sed*, *sel*, *sem*, *seo*, *lukED*, *hlgv*	*clfA-B*, *ebpS*, *eno*, *efb*	IV	P, OX, K, T, E, TE, FU	A3
1	5	5	10	5	+	*sec*, *sed*, *sel*, *sem*, *seo*, *lukED*, *hlgv*	*clfA-B*, *ebpS*, *eno*, *efb*	IV	P, OX, FU	A7
1	5	5	10	5	+	*sec*, *sed*, *sel*, *sem*, *seo*, *lukED*, *hlgv*	*clfA-B*, *ebpS*, *eno*, *efb*	IVA	P, OX, K, T, FU	A1
1	5	5	2	5	+	*sec*, *sed*, *sel*, *sem*, *seo*, *lukED*, *hlgv*	*clfA-B*, *ebpS*, *eno*, *efb*	NT	P, OX, K, T, FU	A12
Switzerland (n = 3)										
3	5	5	ND[e]	5	+	*sec*, *sed*, *sel*, *sem*, *seo*, *lukED*, *hlgv*	*clfA-B*, *ebpS*, *eno*, *efb*	IV	P, OX, K, T, FU	A3, A9, A10
Japan (n = 5)										
3	5	5	ND	5	+	*sec*, *sel*, *sem*, *seo*, *lukED*, *hlgv*	*clfA-B*, *ebpS*, *eno*, *efb*	II	P, OX, K, T, G, E, L, TE, F, PE	C4, C5, C2
1	5	5	ND	5	+	*sec*, *sel*, *sem*, *seo*, *lukED*, *hlgv*	*clfA-B*, *ebpS*, *eno*, *efb*	II	P, OX, K, T, G, E, L, TE, FU	C1
1	5	5	ND	5	+	*sec*, *sel*, *sem*, *seo*, *lukED*, *hlgv*	*clfA-B*, *ebpS*, *eno*, *efb*	II	P, OX, K, T, E, L, TE, F, PE	C3
Pediatric clone	5	5	311	5	−	*sem*, *seo*, *lukED*, *hlgv*	*clfA-B*, *ebpS*, *eno*, *efb*	IV	P, OX, K, T, G, E	B
New York/Japan clone	5	5	2	5	+	*sec*, *sel*, *sem*, *seo*, *lukED*, *hlgv*	*clfA-B*, *ebpS*, *eno*, *efb*	II	P, OX, K, T, G, E, L, TE, F, PE	A6
MSSA isolates *agr2*										
France (n = 5)										
1	5	5	105	5	+	*sec*, *sed*, *sel*, *seo*, *sem*, *lukED*, *hlgv*	ND	NA[f]	ND	A13
1	5	5	88	5	+	*sec*, *sed*, *sel*, *seo*, *sem*, *lukDE*, *hlgv*	ND	NA	ND	A11
1	5	5	572	5	+	*sec*, *sed*, *sel*, *seo*, *sem*, *lukDE*, *hlgv*	ND	NA	ND	A14
1	5	5	570	5	+	*sem*, *seo*, *lukDE*, *hlgv*	ND	NA	ND	A13
1	5	5	548	5	+	*sed*, *sel*, *seo*, *sem*, *lukDE*, *hlgv*	ND	NA	ND	A11
MRSA isolates *agr3*										
France (n = 2)										
1	30	30	584	8	+	*sea*, *seo*, *sem*, *hlg*	*clfA-B*, *cna*, *ebpS*	IV	P, OX, K, T, E, FU	G2
1	30	30	638	8	+	*sea*, *seo*, *sem*, *hlg*	*clfA-B*, *cna*, *ebpS*	IVA	P, OX, K, T, E, FU	G3
MSSA isolates *agr3*										
France (n = 70)										
15	30	30	ND	8	+	*sem*, *seo*, *hlg*	ND	NA	ND	ND
6	30	30	ND	8	+	*sem*, *seo*, *hlb*, *hlg*	ND	NA	ND	ND
15	34	30	ND	8	+	*seh*, *sem*, *seo*, *hlg*	ND	NA	ND	ND
3	34	30	ND	8	+	*seh*, *sem*, *seo*, *hlb*, *hlg*	ND	NA	ND	ND
28	30	30	17, 12[g]	8	+	*sea*, *sem*, *seo*, *hlg*	ND	NA	ND	G1
1	30	30	ND	8	+	*sea*, *sel*, *sem*, *seo*, *hlg*	ND	NA	ND	ND
1	1	Singleton	ND	8	+	*lukPV*, *sea*, *seh*, *sek*, *lukDE*, *hlgv*	ND	NA	ND	ND
1	30	30	ND	8	+	*lukPV*, *sec*, *sel*, *sem*, *seo*, *lukDE*, *hlgv*	ND	NA	ND	ND

[a] ST, sequence type.
[b] CC, clonal complex.
[c] P, penicillin; OX, oxacillin; K, kanamycin; T, tobramycin; G, gentamicin; E, erythromycin; L, lincomycin; TE, tetracycline; F, fosfomycin; PE, pefloxacin; Fu, fusidic acid.
[d] NT, nontypeable.
[e] ND, not determined.
[f] NA, not applicable.
[g] Determined on two isolates only.

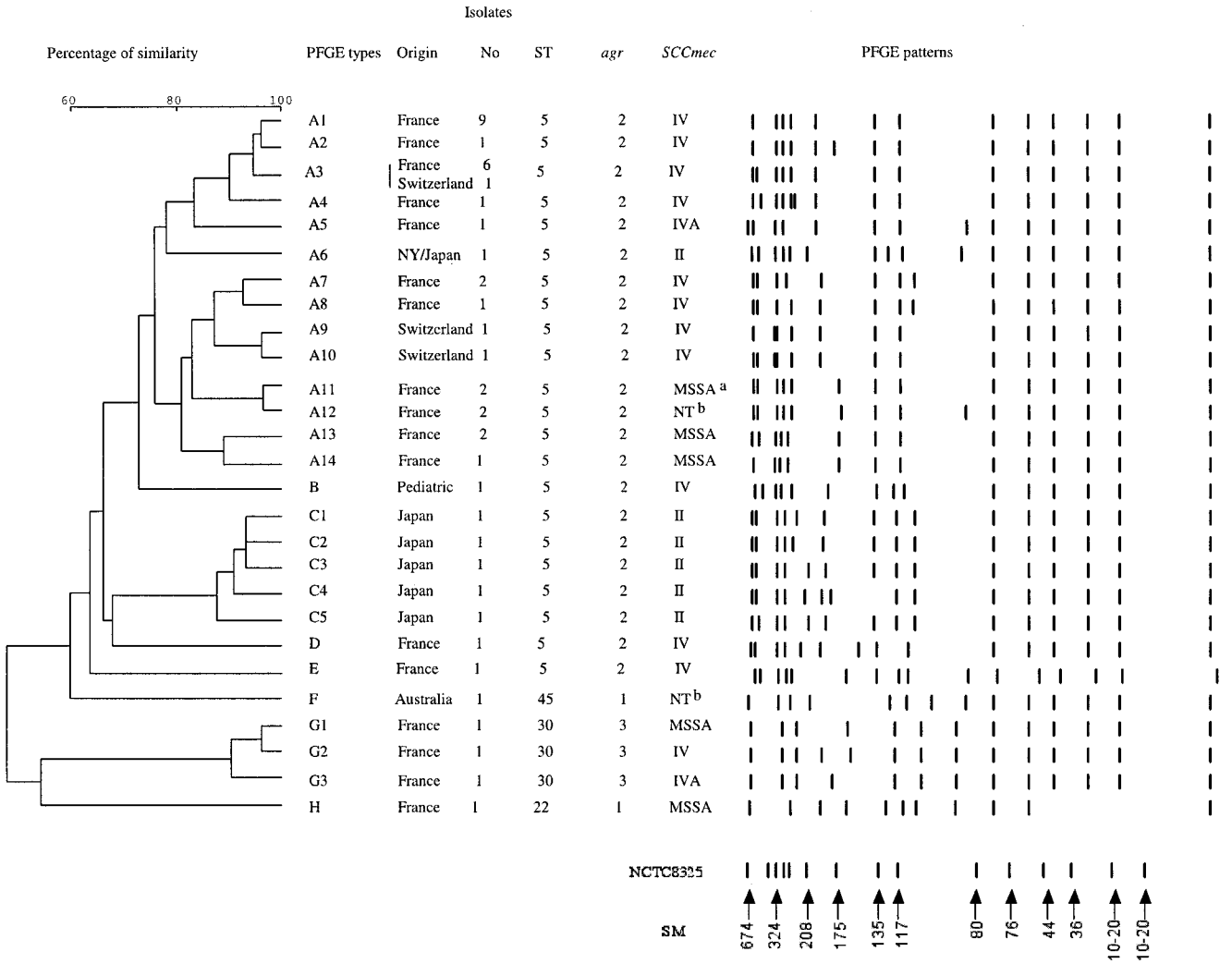

FIG. 1. Unweighted pair group method with averages dendrogram of PFGE results based on the Dice matrix and schematic representation of the pulsotype (SmaI restriction enzyme) of *tst*-positive MRSA isolates; *S. aureus* NCTC 8325 is the reference strain for the size marker (SM), expressed in kilobases. Isolates differing by more than six fragments were considered to be subtypes of a given clonal type. The *agr2* MRSA clone belonged to the PFGE type A except two isolates of PFGE types D and E. NT[b], nontypeable.

single band between the MRSA isolates (192 kb) and the MSSA isolates (151 kb). We concluded that the *tst*-positive *agr2* MRSA and MSSA clones were closely related. The presence of the SCC*mec* IV element in the 192-kb band of MRSA isolates was demonstrated after excising this band from the gel and amplifying *mecA* and the elements characteristic of SCC*mec* type IV. *mecA* and SCC*mec* PCR were negative for the 151-kb band.

The *spa* types of the MSSA isolates (*spa* 88, *spa* 105, *spa* 548, *spa* 570, and *spa* 572) were very similar to the *spa* type 2 of the MRSA isolates, diverging by only one or three repeats.

The two *tst*-positive *agr3* MRSA isolates shared characteristics with 25 of the 70 *tst*-positive *agr3* MSSA isolates; their PFGE patterns differed by a single band containing *mecA* in MRSA isolates. The *spa* types of two *tst*-positive *agr3* MSSA isolates (*spa* 12 and *spa* 17) were closely related to *spa* types 638 and 584 of the *tst*-positive *agr3* MRSA strain.

DISCUSSION

A passive survey of *S. aureus* infections in France during 2002 and 2003 identified *tst*-positive MRSA clones. We identified a major ST5 *agr2* clone which included 25 of the 27 *tst*-positive MRSA isolates, and a minor ST30 *agr3* clone accounted for the remaining two isolates. Both clones mainly caused hospital-acquired infections (12 cases), but the ST5 *agr2* clone was also sometimes acquired in the community (8 cases). These infections mainly affected children (overall median age, 3 years) and corresponded to both TSS and suppurative infections. The clones were widely disseminated throughout France, as *tst*-positive MRSA isolates were recovered from 12 French towns between 2002 and 2003. In our database, this clone was also detected in 2000 (one case) and in 2001 (two cases); these three cases were hospital acquired. The ST5 *agr2* clone was also detected in Switzerland.

The two French *tst*-positive MRSA clones had similar antibiotic resistance profiles: they were usually resistant to oxacillin, kanamycin, and tobramycin and had intermediate resistance to fusidic acid, while resistance to erythromycin was more variable. This antibiotic resistance profile is uncommon among French hospital MRSA isolates. Intermediate fusidic acid resistance is rare in French hospital MRSA clones, which are usually susceptible to fusidic acid and resistant to quinolones (39). It is noteworthy that the antibiotic resistance profiles of the two French *tst*-positive MRSA clones are very similar to that of the major community-acquired ST80 MRSA clone harboring the PVL genes, which is currently spreading throughout Europe (37). For instance, the ST80 clone is also resistant to oxacillin and kanamycin and has intermediate resistance to fusidic acid, whereas it is susceptible to tobramycin and resistant to tetracycline. These differences in antibiotic resistance profiles may help to identify the PVL-positive clone ST80 and the *tst*-positive clones ST5 and ST30 in the clinical setting. It is surprising that these emerging clones, which are either *tst* or PVL positive, share certain genetic determinants encoding resistance to antibiotics despite their very different genetic backgrounds. This may reflect a peculiar pattern of antibiotic usage in France, notably in the community.

Two categories of MRSA had previously been recognized in France. The first comprises hospital strains (H-MRSA) that can potentially spread into the community, giving rise to infections in patients with risk factors such as recent hospitalization or surgery, chronic underlying diseases, immunosuppression, or intravenous drug use. The second category corresponds to MRSA strains arising de novo in the community (C-MRSA), which infect patients with no established risk factors. H-MRSA infections differ from C-MRSA infections in their epidemiological, clinical, and microbiological characteristics: C-MRSA infects younger subjects and mainly causes skin infections, whereas H-MRSA is associated with a wider range of infections (urinary tract, respiratory tract, skin, etc.). C-MRSA usually harbors the PVL genes, which are associated with skin and soft tissue infections (37), and occasionally the exfoliative toxin genes (17). The epidemiology of the *tst*-positive MRSA clones is atypical. Like C-MRSA, *tst*-positive MRSA generally infects children in the community, but 12 of our cases were strictly hospital acquired. However, it is not known whether the patients with "hospital-acquired" infections were nasal carriers of *tst*-positive MRSA or whether they actually acquired the strain in the hospital. None of the hospital-acquired *tst*-positive MRSA infections was associated with hospital outbreaks or with documented horizontal transmission. The known prevalence of H-MRSA in French pediatric units is low (9.8% in our hospital in Lyon [J. Etienne, personal communication]), as is the overall prevalence of *tst*-positive MRSA in France (27 isolates from 12 different hospitals in a 2-year period). This suggests that these *tst*-positive MRSA strains are being imported into hospitals from the community. *tst*-positive MRSA strains appear to be highly virulent and to cause a variety of illnesses, ranging from toxic shock syndrome to various suppurative infections.

The two *tst*-positive MRSA clones, with *agr2* or *agr3* genetic backgrounds, seem to be clonally related to their respective *agr2* or *agr3* *tst*-positive MSSA counterparts. A single PFGE band difference, corresponding to an SCC*mec* IV element, distinguished the MRSA isolates from the MSSA isolates. It is unclear whether insertion of a *mecA* element can occur in such MSSA strains. The *tst*-positive *agr2* MSSA clone has rarely been detected in France (only 5 isolates in our collection), contrary to the *tst*-positive *agr3* MSSA clone (70 isolates in our collection). It is surprising that the major *tst*-positive MRSA clone (*agr2*) should have emerged from an infrequently detected *tst*-positive MSSA background. We compared our *tst*-positive *agr2* MRSA clone with the well-described New York/Japan and Pediatric MRSA clones that have spread worldwide. Our *tst*-positive *agr2* MRSA clone has the same genetic background as the New York/Japan clone. Even if SCC*mec* acquisition by MSSA clones was four times more common than the replacement of one SCC*mec* by another, we cannot exclude the possibility that our clone arose from the New York/Japan clone through SCC*mec* II substitution by SCC*mec* IV (28). Further phylogenetic studies are needed to determine the precise origin of our clone, and these studies may help to identify factors that tend to promote the spread of *tst*-positive *agr2* MRSA rather than *tst*-positive *agr3* MRSA.

Most emerging C-MRSA isolates with heightened virulence have been found to harbor the PVL genes and, less frequently, exfoliative toxin genes (17). The emergence and spread of virulent C-MRSA isolates harboring the *tst* gene is of major concern, as they appear to share certain characteristics with PVL-positive C-MRSA, including a predilection for children. Prospective studies are needed to determine the incidence of infections due to these different clones, in order to bolster measures aimed at limiting the spread of C-MRSA.

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