Variable-Number Tandem Repeat Analysis and Multilocus Sequence Typing Data Confirm the Epidemiological Changes Observed with *Staphylococcus aureus* Strains Isolated from Bloodstream Infections[▽]

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Since 2000, our geographical region in France systematically surveys bloodstream infections (BSI) due to *Staphylococcus aureus*. This survey involves 39 health care institutions (HCIs) encompassing 6,888 short-stay beds and was performed during two 3-month periods during 2007 and 2008. The study periods of this survey identified 292 *S. aureus* isolates causing BSI. Extensive molecular characterization, including genotyping as well as toxin, *agr*, and staphylococcal cassette chromosome content determinations, allowed us to describe epidemiological evolution in comparison to that discussed in our previous study. Our main epidemiological observation shows that the incidence of BSI remained constant but that methicillin (meticillin)-resistant *S. aureus* strains with a wider variety of genetic backgrounds now harbor *pyl*, as has already been reported in different European countries. We noticed stable numbers of BSI episodes involving community-acquired methicillin-sensitive *S. aureus* (MSSA), whereas a drastic increase in the number of strains harboring the *tst* gene was recorded. The increase in the number of *tst* gene-harboring strains is related to known hospital-acquired MSSA isolates and appears related to epidemic episodes in specific HCIs. Monitoring the increase in prevalence of specific strains helps us understand where the standard precautions are not satisfactorily applied or do not efficiently prevent the spread of epidemic MSSA strains in these HCIs. The recent increases in incidence of these strains call for particular vigilance to avoid the spread of potentially virulent MSSA strains harboring the *tst* gene and for continuance of this strategy of BSI surveillance.

Reporting of methicillin-resistant *Staphylococcus aureus* (MRSA) bloodstream infections (BSI) is often mandatory, and reduction of BSI rates is a performance target (2, 15, 16, 19, 22, 23). Since 2000, a prospective, longitudinal survey of BSI has been under way at a regional level in France. Results obtained during the first 7 years of surveillance have been reported (26, 27). After sustained and decreasing incidence rates of *S. aureus* BSI attributed to successful infection control efforts in participating health care institutions (HCIs), we reported in 2006 a sudden increase in incidence that involved two populations of *S. aureus* strains: one of methicillin-sensitive *S. aureus* (MSSA) strains and one of MRSA isolates.

First, an increasing incidence of BSI was observed due to MSSA strains, including (i) strains associated with epidemic phenomena in HCIs and (ii) a genetically homogeneous population of *tst*-positive MSSA isolates, mostly associated with community-acquired (CA) BSI, suggesting clonal spread at a regional level.

Second, we observed the emergence of BSI associated with genetically diverse nonmultiresistant staphylococcal cassette chromosome *mec* type IV (SCC*mec* IV) MRSA strains (named NORSA) that could not be related to any local outbreak in the participating HCIs.

We report here data collected in 2007 and 2008 using exactly the same study design. Again, a large *S. aureus* population genetic study, including MSSA and MRSA isolates in parallel, was conducted. Strain characterization included antimicrobial susceptibility profiles; *luk*, *tst*, *eta*, and *etb* gene detection; and SCC*mec* and pulsed-field gel electrophoresis (PFGE) typing. In addition, and to better understand how populations evolved and interacted, characterization of the *S. aureus* strains was completed by accessory global regulator (*agr*) typing and multiple-locus variable-number tandem-repeat analysis (MLVA) and multilocus sequence typing (MLST) analysis.

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MATERIALS AND METHODS

BSI epidemiological survey method. A BSI surveillance program and a microbiological study of *S. aureus* strains isolated from BSI cases have been conducted since 2000 in the central region of France (2.5 million inhabitants) (Fig. 1) (26, 27). In 2007 and 2008, 39 HCIs, comprising a total of 6,888 short-stay beds, participated in this program, which involves an annual 3-month survey of all

FIG. 1. Map of France and the central region.

cases of BSI. The survey covered 1,518,475 patient days (PD). The methods, study design, and data for the years 2000 to 2006 have been reported previously (26, 27). Briefly, BSI is defined as a positive (not contaminated) blood culture from a patient with clinical or laboratory evidence of infection. A blood culture was considered to be contaminated when only one blood culture was positive for a bacterium that is frequently found as a contaminant (i.e., coagulase-negative staphylococci, *Corynebacterium* spp., or *Propionibacterium* spp.) and when the clinician did not prescribe any antibiotic treatment against the bacterium. The variables studied included patient age and sex, portal of entry (skin [primitive cutaneous form or superinfection of a skin breach], surgical site, lungs, urine, intravascular device, or digestion), community-associated or hospital-acquired (HA) BSI, death within 7 days of BSI diagnosis, and duration of hospital stay. The data were analyzed with Epi Info version 6 software. Ethical approval of the BSI surveillance program was obtained at the national level from the Réseau Alerte Investigation Surveillance des Infections Nosocomiales (RAISIN).

Microbiological methods. (i) Bacteriology. BSI-associated *S. aureus* strains were collected during the survey period. The strains were sent to the laboratory of the Réseau des Hygiénistes du Centre. The isolates were identified as *S. aureus* on the basis of their characteristic growth pattern, colony morphology, Gram staining result, catalase test result, coagulase production (rabbit plasma; Difco Laboratories, Elancourt, France), slide test result for clumping factor (Pastorex Plus-Staph; Bio-Rad, Ivry-sur-Seine, France), and results obtained with ID 32 Staph cards (VITEK2 system; bioMérieux, Lyon, France).

(ii) Antimicrobial susceptibility testing. We used the disk diffusion method (Bio-Rad, France) to test the antibiotic susceptibilities of *S. aureus* strains. According to the French National Committee recommendations (25), the antibiotics tested were penicillin G, cefoxitin, erythromycin, lincomycin, pristinamycin, tetracycline, kanamycin, tobramycin, gentamicin, rifampin (rifampicin), fusidic acid, fosfomycin, pefloxacin, cotrimoxazole, vancomycin, and teicoplanin. The cefinase test (bioMérieux, Lyon, France) was used to detect β-lactamase production. The MRSA-Screen latex agglutination assay was performed in accordance with the manufacturer's protocol (bioMérieux, Lyon, France). Agglutination was assessed visually within 3 min (18). The *mecA* gene was detected by PCR according to the previously described procedure (27). MRSA strains displaying typical resistance to multiple antibiotics were referred to as CMRSA (classic multiresistant MRSA) strains, whereas MRSA strains resistant to no more than three antimicrobial agents (excluding penicillin and methicillin) were referred to as NORSA.

(iii) Multiplex PCR assay for typing of SCC*mec* elements. Multiplex PCR was used to type the SCC*mec* element in MRSA strains as described by Oliveira and de Lencastre (21).

(iv) VNTR analysis. All *S. aureus* strains were analyzed by VNTR analysis according to François et al. (11). Multiplex PCR amplifications with eight primer pairs that target gene regions with variable numbers of tandem repeats were resolved by microcapillary electrophoresis and automatically assessed by cluster analysis.

(v) DNA macrorestriction and PFGE. PFGE was used as a typing technique according to the previously described procedure (27). PFGE patterns (pulsotypes) were analyzed with the Taxotron package (Taxolab, Institut Pasteur, Paris, France). The images were transferred to a microcomputer, and the distances migrated by each band in each lane were determined in pixel units by RestrictoScan. The molecular size of each fragment was calculated from the distance migrated using cubic Schaffer and Sederoff method algorithms with RestrictoTyper. The relationships between pulsotypes were determined by the unweighted-pair group method using arithmetic means and the Adanson pulsogrouping program (dissimilarity). A dendrogram was drawn with Dendrograf.

(vi) agr typing. The *agr* gene is a crucial regulatory component of *Staphylococcus aureus* involved in the control of bacterial virulence factor expression. The *agr* types 1 to 4 were determined for *S. aureus* strains using a real-time multiplex quantitative PCR assay (13).

(vii) MLST. MLST is a highly discriminatory method of characterizing bacterial isolates on the basis of the sequences of ~450-bp internal fragments of seven housekeeping genes. For each gene fragment, the different sequences are assigned as distinct alleles, and each isolate is defined by the alleles at each of the seven housekeeping loci (the allelic profile or sequence type [ST]). *S. aureus* strains ($n = 120$) representative of the genetic diversity of the panel of studied strains were analyzed by MLST according to the procedure described by Enright et al. (9).

(viii) Virulence factors. Given the diffusion of Panton-Valentine leukocidin- and toxic shock syndrome toxin-1-producing MRSA strains in France since 2002, we sought sequences corresponding to *lukS-PV* and *lukF-PV*, encoding Panton-Valentine leukocidin, to *tst*, encoding toxic shock syndrome toxin-1, and to the *eta* and *etb* genes, encoding exfoliatins A and B, respectively. Genomic DNA was extracted from staphylococcal cultures and used as the template for PCR amplification using a procedure previously described by Jarraud et al. (16).

(ix) Statistical data. Chi-square tests and Fisher's exact test (two-tailed) were used to test associations, and a *P* value of 0.050 was considered significant.

RESULTS

***S. aureus* BSI incidence.** During the study period, 292 cases of *S. aureus* BSI were identified in 188 males and 104 females (median age, 68 years). The mean incidence of *S. aureus* BSI was 0.192 per 1,000 PD (Table 1).

All *S. aureus* strains were submitted to a cefoxitin disk diffusion test and MRSA-Screen latex agglutination assay. According to the French National Committee recommendations (25), an *S. aureus* strain was defined as a MRSA strain when the cefoxitin disk diffusion test was positive (diameter < 25 mm) or when the MRSA-Screen latex agglutination assay was positive. Also, strains negative by the cefoxitin disk diffusion test and MRSA-Screen latex agglutination assay but resistant to one antibiotic were furthermore studied by PCR for *mecA* gene detection. Among these 292 *S. aureus* strains, 208 (71%) were MSSA and 84 (29%) were MRSA strains (Table 2). The mean incidence of MSSA BSI was 0.137 per 1,000 PD. Thirteen of the 208 MSSA strains (6.3%) displayed fluoroquinolone (FQ) resistance. The mean incidence of MRSA BSI was 0.055 per 1,000 PD. The mean incidence of NORSA BSI was 0.022 per 1,000 PD (Table 1). Twenty-five of the 33 NORSA strains (76%) were resistant only to FQs and methicillin (designated NORSA FQ strains) (Table 2).

CA and HA *S. aureus* BSI. According to the study design, BSI cases were defined as either CA or HA. The mean incidences of CA BSI did not vary significantly according to the year of survey and whether the strains were MSSA or MRSA (Table 1). For HA BSI, the mean incidences varied distinctly according to the strains, as incidence decreased with MRSA and increased with MSSA.

SCC*mec* typing. Sixty-four of the 78 MRSA strains were of SCC*mec* type IV (82%). Four strains were of SCC*mec* type I,

TABLE 1. BSI incidence per 1,000 PD according to the annual 3-month survey, with epidemiological and strain characteristics[a]

Yr	No. of PD	No. of patient admissions	S. aureus	MRSA	CMRSA	HA CMRSA	CA CMRSA	NORSA	HA NORSA	CA NORSA	MSSA	EMSSA	HA MSSA	CA MSSA	tst gene + MRSA	tst gene + MSSA	luk gene + MRSA	luk gene + MSSA	eta gene + MSSA
						BSI incidence per 1,000 PD according to the annual 3-month survey													
2002	604,850	89,959	0.202	0.055	0.046			0.008			0.147	0.015			NK	NK	NK	NK	NK
2003	662,555	86,530	0.178	0.047	0.047			0.006			0.125	0.023			NK	NK	NK	NK	NK
2004	729,013	81,867	0.167	0.059	0.045			0.014			0.108	0.008			0.012	NK	NK	NK	NK
2005	607,300	71,579	0.13	0.038	0.03	0.023	0.006	0.008	0.007	0.001	0.092	0.005	0.048	0.042	0.008	0.004			NK
2006	671,368	80,499	0.234	0.068	0.04	0.03	0.009	0.028	0.022	0.005	0.165	0.016	0.068	0.078	0.030	0.008		0.001	NK
2007	759,214	86,938	0.175	0.053	0.038	0.024	0.008	0.014	0.013	0.001	0.122	0.01	0.074	0.037	0.018	0.001	0.001	0.001	0.001
2008	759,261	86,876	0.209	0.058	0.029	0.018	0.008	0.029	0.018	0.008	0.151	0.01	0.086	0.056	0.024	0.005	0.001*	0.001	0.001

[a] Results obtained before 2007 were described previously (26, 27) and are reported to show the changes through time affecting the different groups of strains. An incidence of 0.001 represents one BSI case. NK, not known; EMSSA, MSSA with resistance to FQs or at least two antimicrobial agents.

TABLE 2. Distribution of *S. aureus* strains associated with BSI according to the annual 3-month survey and the antibiogroup

Antibiogroup	No. (%) of strains, according to annual 3-month survey period, in[a]:						
	2002	2003	2004	2005	2006	2007	2008
MRSA	33 (27)	35 (30)	43 (35)	23 (29)	46 (29)	40 (30)	44 (28)
CMRSA	28 (85)	31 (89)	33 (77)	18 (78)	27 (59)	29 (72)	22 (50)
NORSA	5 (15)	4 (11)	10 (23)	5 (22)	19 (41)	11 (28)	22 (50)
NORSA FQ[b]	1	4	7	4	16	9	16
Other NORSA	4		3	1	3	2	6
FQ resistance	31 (94)	35 (100)	43 (100)	23 (100)	46 (100)	38 (97)	38 (89)
MSSA	89 (73)	83 (70)	79 (65)	56 (71)	111 (71)	93 (70)	115 (72)
EMSSA[c]	9	15	6	3	11	8	8
EMSSA FQ[d]					4	7	6
FQ resistance	6 (7)	14 (17)	8 (8)	4 (7)	9 (8)	7 (8)	5 (4)
Total	122	118	122	79	157	133	159

[a] Results obtained before 2007 were described previously (26, 27) and are reported to show the changes through time affecting the different groups of strains. Percentages indicate the proportions of *S. aureus* strains that are of the indicated antibiogroup.
[b] NORSA strains resistant only to FQs.
[c] EMSSA, MSSA strains with resistance to FQs or at least two antibiotics.
[d] EMSSA FQ, MSSA strains with resistance to FQs only.

and no SCC*mec* fragment could be amplified by PCR from the remaining 10 strains (13%), which were therefore designated nontypeable.

***luk*, *tst*, *eta*, and *etb* genes.** Thirty-seven BSI cases, identified in 24 males and 13 females (median age, 70 years), were associated with strains scoring positive for the *tst* gene. The strains that scored positive for the *tst* gene included 32 MSSA strains (32/200 [16%]) and five MRSA strains (5/78 [6.4%]). One MSSA strain and two MRSA strains, the first ever since 2000, scored positive for *luk* genes. Two MSSA strains were positive for the *eta* gene.

VNTR typing. VNRT typing was conducted with 275 of the 292 *S. aureus* BSI strains. VNTR typing revealed seven major divisions (designated V1 to V7) (Fig. 2). Divisions V1, V4, and V7 were composed exclusively of MSSA strains, and MSSA strains were predominant in the divisions V3, V5, and V6. MRSA strains were predominant only in division V2. The CMRSA strains predominated in divisions V2 (39 CMRSA and 11 NORSA strains) and V6 (5 CMRSA strains and 1 NORSA strain). In contrast, NORSA strains were predominant in divisions V3 (17 NORSA and 2 CMRSA strains) and V5, where the 3 MRSA strains were all NORSA strains. The NORSA FQ strains distributed among divisions V2, V3, and V6. The two *luk*-positive MRSA strains were observed in V5, whereas the *luk*-positive MSSA strains clustered in V7. Division V1 contained the most *tst*-positive strains (26/32 [81%]). The five *tst*-positive MRSA strains were in V2 (*n* = 3) and V3 (*n* = 2).

SmaI macrorestriction. PFGE has been conducted with 267 of the 292 *S. aureus* BSI strains. PFGE revealed 184 different pulsotypes across the studied strains. After performance of an analysis by the unweighted-pair group method using average linkages, the strains were distributed into 15 major divisions (P1 to P15) (Fig. 3). Divisions P3, P10, P12, and P15 were exclusively composed of MSSA strains, and MSSA strains were also predominant in divisions P1, P6, P7, P9, P11, and P14. MRSA strains were distributed across 11 divisions. CMRSA strains were predominant in divisions P5 and P6; in contrast, NORSA strains were predominant in P4. The two *luk*-positive

MRSA strains were in P8, and the *luk*-positive MSSA strain was in P11. Division P9 contained most of the *tst*-positive strains (23/32 [72%]), and the five *tst*-positive MRSA strains were in P4 (*n* = 1), P5 (*n* = 1), P11 (*n* = 2), and P13 (*n* = 1). A significant link was observed between VNTR divisions and the major PFGE divisions (Table 3): V1 was associated with P9 (*P* < 0.001), V2 with P5 (*P* < 0.001), V3 with P1 (*P* < 0.001), V5 with P1 (*P* = 0.002), and V7 with P11 (*P* < 0.001). To evaluate the likelihood of epidemic phenomena, we studied the occurrence, in the same HCI, of at least two BSI cases with strains presenting similar pulsotypes. Here, 80 of the 200 MSSA strains (40%) and 14 of the 78 MRSA strains (18%) (*P* < 0.001) were involved in such clusters of strains.

MLST. MLST was performed with 120 strains representative of the genetic diversity of the studied strains according to PFGE results and included 44 MRSA and 76 MSSA strains (Fig. 2 and 3). The 44 MRSA strains were distributed into four clonal complexes (CCs) or STs: 18 strains were of CC5, 22 of CC8, 3 of CC80, and 1 of ST247, the gentamicin-resistant Iberian clone that was the most frequent clone in France in the 1990s. Among the 29 NORSA strains studied, 16 were of CC5, 10 were of CC8, and 3 were of CC80. The five *tst*-positive MRSA strains were distributed into CC8 (*n* = 3) and CC5 (*n* = 2). Both of the two *luk*-positive MRSA strains were found in CC80.

The 76 MSSA strains studied were distributed into 17 CCs or STs, and at least 4 MSSA strains were clustered in 7 CCs or STs: 23 strains were clustered in CC30, 11 in CC5, 8 in CC8, 6 in CC45, 5 in CC1, 4 in CC22, and 4 in ST15. The 25 *tst*-positive MSSA strains were distributed among five CCs or STs, but most (20/25 [80%]) were observed in CC30. The *luk*-positive MSSA strain was found in ST45. The two *eta*-positive MSSA strains were in ST1 and ST6.

Again, a significant link was observed between the major VNTR divisions and the major CCs and STs (Fig. 1): V1 was associated with CC30 (*P* < 0.001), V2 with CC8 (*P* < 0.001), V3 with CC5 (*P* < 0.001), V7 with CC45 (*P* < 0.001), and V5 with CC80 (*P* < 0.001).
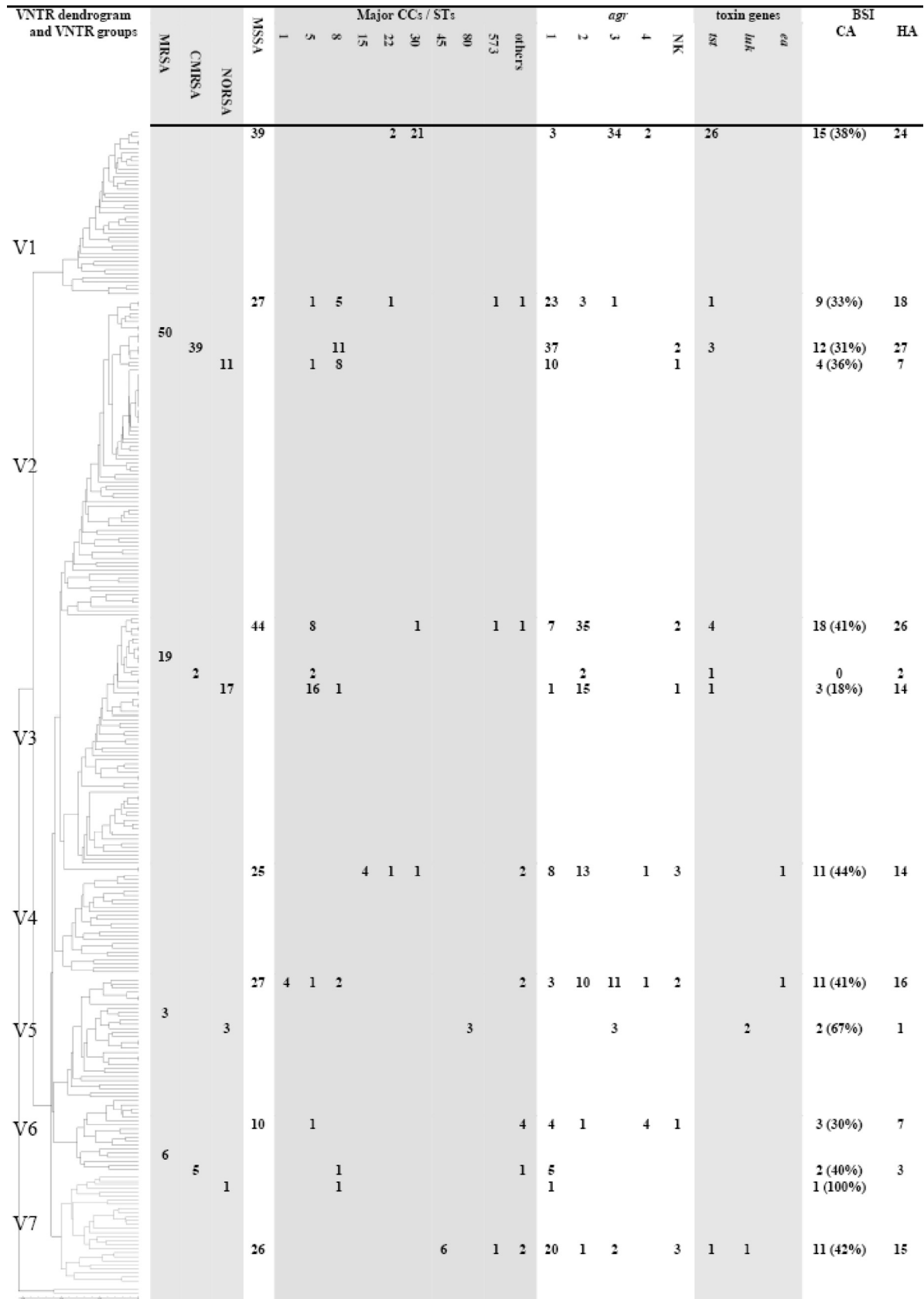
FIG. 2. Characteristics of strains and BSI according to VNTR groups. The dendrogram was calculated using the MLVA data. On the basis of their MLVA fingerprints, the isolates segregated into different clusters, showing a distance limit of 0.1 for clonality and a distance limit of 0.2 for related isolates, as previously described (10). Results of other molecular tests, including *agr* and SCC*mec* typing as well as determination of toxin content, are also indicated. Based on previous determinations, the origins of isolates composing each cluster (either HA or CA strains) appear in the figure. NK, not known.

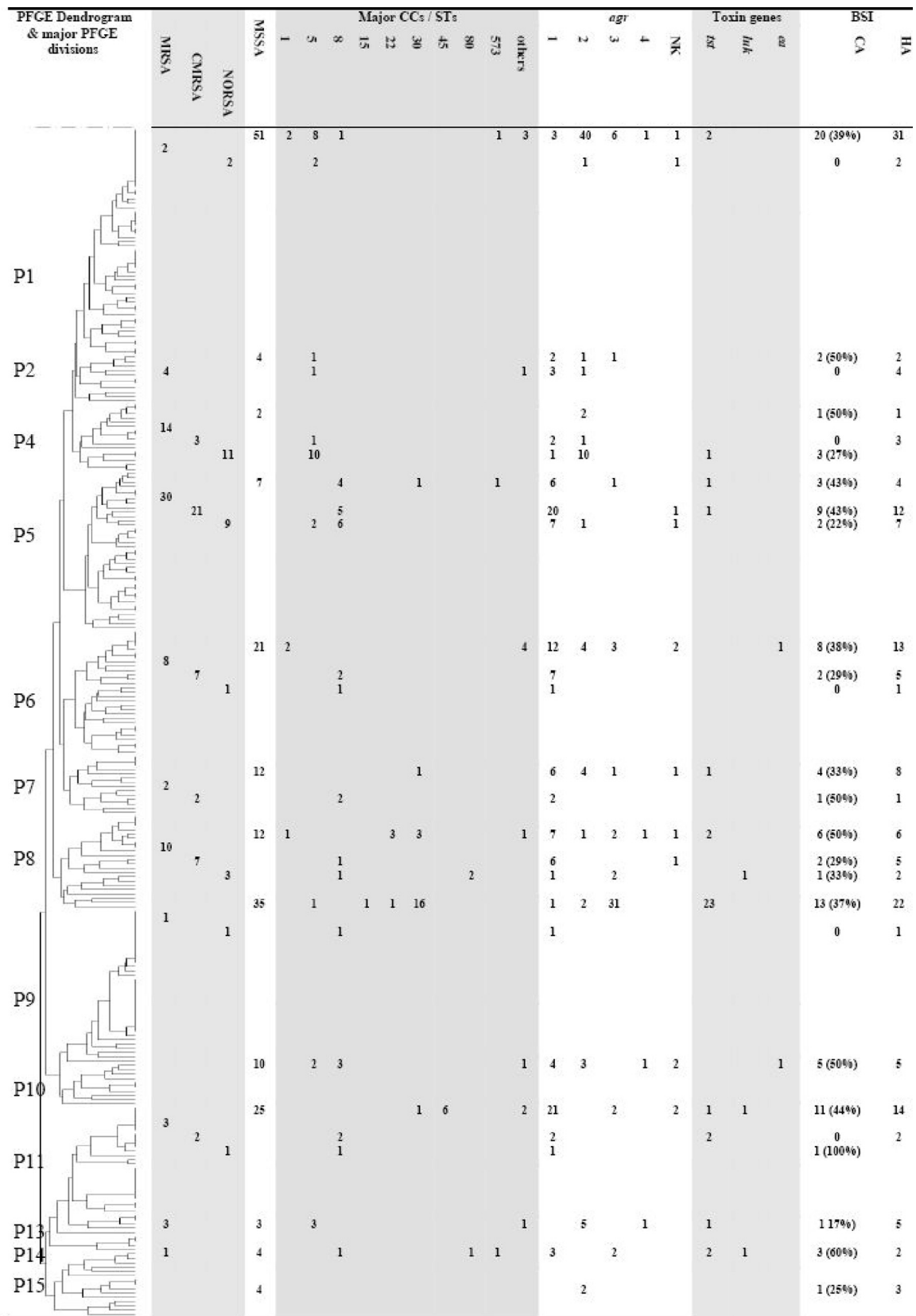| VNTR group	MRSA	CMRSA	NORSA	MSSA	1	5	8	15	22	30	45	80	573	others	agr 1	agr 2	agr 3	agr 4	NK	tst	luk	ea	BSI CA	BSI HA
V1				39						2	21				3	34	2			26			15 (38%)	24
V2				27	1	5		1					1	1	23	3	1			1			9 (33%)	18
	50	39					11								37			2		3			12 (31%)	27
			11		1	8									10				1				4 (36%)	7
V3				44		8			1				1	1	7	35		2		4			18 (41%)	26
	19	2				2										2		1		1	1		0	2
			17		16	1									1	15		1		1	1		3 (18%)	14
V4				25				4	1	1				2	8	13		1	3			1	11 (44%)	14
V5				27	4	1	2							2	3	10	11	1	2			1	11 (41%)	16
	3		3									3					3				2		2 (67%)	1
V6				10		1								4	4	1		4	1				3 (30%)	7
	6	5					1							1	5								2 (40%)	3
			1				1								1								1 (100%)	
V7				26								6	1	2	20	1	2		3	1	1		11 (42%)	15

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FIG. 3. Characteristics of strains and BSI according to PFGE groups. The dendrogram was calculated using the relationships between pulsotypes determined by the unweighted-pair group method using arithmetic means and the Adanson pulsogrouping program (dissimilarity). Results of other molecular tests, including *agr* and SCC*mec* typing as well as determination of toxin content, are also indicated. Based on previous determinations, the origins of isolates composing each cluster (either HA or CA strains) appear in the figure. NK, not known.

PFGE division	MRSA	CMRSA	NORSA	MSSA	1	5	8	15	22	30	45	80	573	others	agr 1	agr 2	agr 3	agr 4	NK	tst	luk	eta	CA	HA
	2			51	2	8	1						1	3	3	40	6	1	1	2			20 (39%)	31
			2			2										1			1				0	2
P2				4		1									2	1	1						2 (50%)	2
	4					1								1	3	1							0	4
P4				2												2							1 (50%)	1
	14	3				1									2	1							0	3
			11				10								1	10					1		3 (27%)	
P5				7			4			1				1	6		1			1			3 (43%)	4
	30	21					5								20				1	1			9 (43%)	12
			9			2	6								7	1			1	1			2 (22%)	7
P6				21	2									4	12	4	3		2			1	8 (38%)	13
	8	7								2					7								2 (29%)	5
			1							1					1								0	1
P7				12							1				6	4	1		1	1			4 (33%)	8
	2	2								2					2								1 (50%)	1
P8				12	1				3	3				1	7	1	2	1	1	2			6 (50%)	6
	10	7								1					6				1				2 (29%)	5
			3							1		2			1		2				1		1 (33%)	2
	1			35		1		1	1	16					1	2	31			23			13 (37%)	22
			1				1								1								0	1
P10				10	2		3							1	4	3		1	2			1	5 (50%)	5
				25						1	6			2	21	2			2	1	1		11 (44%)	14
P11	3	2					2								2					2			0	2
			1				1								1								1 (100%)	
P13	3			3	3									1		5		1		1			1 (17%)	5
P14	1			4			1					1	1		3		2			2	1		3 (60%)	2
P15				4												2							1 (25%)	3

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TABLE 3. Distribution of strains among major PFGE divisions
according to the VNTR groups

VNTR group	No. of strains in indicated PFGE division						Total
	P1	P5	P6	P9	P11	Other PFGE divisions	
1		1		30	1	7	39
2	3	31	14	2	3	24	77
3	33	1	2	1	1	25	63
4	2		4	1	3	15	25
5	12	1	5	1	1	10	30
6	1	3	2			9	15
7	2		1		20	2	25
Singletons		1				4	5
Total	53	37	29	35	29	96	279

Determination of the *agr* groups. The *agr* groups was determined for 266 strains (Fig. 2 and 3). The MSSA strains were rather equally distributed among *agr* group 1 (68/189 [36%]), *agr* group 2 (63/189 [33%]), and *agr* group 3 (49/189 [26%]). In contrast, the MRSA strains were mostly of *agr* group 1 (56/77 [73%]), while *agr* groups 2 and 3 were found, respectively, in 17 (22%) and 3 (3.9%) MRSA strains. The six *agr* group 4 strains were all MSSA strains. The distributions of strains across the various *agr* groups differed significantly between MSSA and MRSA strains ($P = 0.010$). *agr* genes of group 3 were mostly recovered among *tst* gene-positive MSSA isolates (25/31 [81%]). In contrast, *tst*-positive MRSA strains were of *agr* groups 1 ($n = 3$) and 2 ($n = 2$). The two *luk*-positive MRSA strains were of *agr* group 3, and the *luk*-positive MSSA strain was placed into *agr* groups 1 and 3. A significant link was found between the major VNTR divisions and the various *agr* types (Table 3): type V1 was associated with *agr* group 3 ($P < 0.001$), V2 with *agr* group 1 ($P < 0.001$), V3 with *agr* group 2 ($P < 0.001$), and V6 with *agr* group 4 ($P = 0.035$).

DISCUSSION

In our reporting of *S. aureus* BSI since 2000 (26, 27), we observed a regular decrease in incidence, followed in 2006 by a sudden increase. The data obtained during the years 2007 and 2008 according to the same study design (26) confirmed the changes observed in 2006. The surge in *S. aureus* BSI incidence was first associated with the diffusion of epidemic MSSA strains responsible for outbreaks in participating HCIs (27). The new data indicate (i) a high level of MSSA BSI incidence, (ii) a stable CA-MSSA BSI incidence, (iii) an increase in HA-MSSA BSI incidence, and (iv) the persistence of MSSA epidemic phenomena at the HCI level. This indicates that the standard precautions may not be satisfactorily applied or that they are not sufficient to prevent the diffusion of the MSSA strains that are spreading today. These data must prompt us to have particular vigilance against the spread of these strains and especially against the *tst*-positive MSSA strains. Second, the surge of the *S. aureus* BSI incidence was also linked to NORSA strains. The new data indicate (i) a stable level of MRSA BSI incidence, (ii) stable CA- and HA-MRSA BSI incidences, (iii) a progressive replacement of the CMRSA by NORSA strains, (iv) the preponderance of MRSA

strains resistant only to FQs, and (v) limited MRSA epidemic phenomena. Despite infection control efforts conducted in the HCIs to prevent transmission of MRSA strains, the MRSA BSI incidence did not decrease significantly. This suggests that measures to prevent the diffusion of MRSA strains either are not satisfactorily applied or are not efficient at preventing the diffusion of these strains. As most MRSA strains harbor type IV SCC*mec*, the small SCC*mec* element that may enable more-efficient horizontal gene transfer and permit potentially higher growth rates (8), we cannot exclude the possibility that these factors may be related to the spread of such strains and thus concur with the limited impact of the measures conducted.

MLST allows us to better characterize *S. aureus* strains spreading in our region. The MSSA and MRSA clones belonging to CC5, CC8, CC22, CC30, and CC45 are pandemic lineages (3, 4, 9, 12, 17, 19, 20, 24). MSSA strains were highly diverse and rather equally distributed across such lineages. In contrast, MRSA isolates were much more homogenous and mostly distributed with the pandemic Lyon clone (ST8-IV-agr1) and the pediatric clone (ST5-IV-agr2) (6, 7).

Nevertheless, while CMRSA strains were homogenous and mostly of the CC8 background, NORSA strains revealed a more diverse group of backgrounds; they distributed with CC8 and CC5 strains and with the European clone ST80-IV-agrIII (V5) (5). No isolate was characterized as one of the emerging clones observed in neighboring European countries, such as ST22-IV, ST45-IV, ST125-IV, and ST225-I (1, 10, 14, 20).

The first two *luk*-positive MRSA strains identified since 2000 differed according to their antimicrobial susceptibility profiles and PFGE patterns but belonged to the European or the Mediterranean clone CC80-IV-agr3 (5). This suggests that the current emergence of *luk*-positive strains in France is not associated with an epidemic spread such as that reported in Greece (28). In contrast, three of the five *tst*-positive MRSA strains identified in 2007 to 2008 were all ST8-IV-agr1 and shared similar PFGE patterns, suggesting the installation of these strains at a regional level.

Using a highly diverse set of MSSA and MRSA strains, VNTR analysis provided highly congruent results with data obtained by MLST, as all major pandemic lineages were clustered into distinct VNTR groups. These results support the use of these methods to easily genetically characterize MSSA and MRSA strains. VNTR data confirmed (i) the genetic diversity of NORSA strains, (ii) the homogenous population of *tst*-positive MSSA strains, and (iii) the homogenous population of *luk*-positive MRSA strains.

Conclusions. Data reported here encourage us to continue the implementation of active control measures with all *S. aureus* BSI cases and raise questions about the efficiency of the measures currently applied in our HCIs facing the clonal spread of *tst*-harboring *S. aureus* strains. We confirm the trends observed in 2006 and emphasize the need to reinforce the prevention of transmission of bacteria, especially that of MSSA strains. Our findings demonstrate that our current infection control practices, focusing on the prevention of infection spreading from patients with symptomatic disease, mainly restricted to the hospital setting, may not be sufficient.

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REFERENCES

1. **Aires-de-Sousa, M., B. Correia, H. de Lencastre, and the Multilaboratory Project Collaborators.** 2008 Changing patterns in frequency of recovery of five methicillin-resistant *Staphylococcus aureus* clones in Portuguese hospitals: surveillance over a 16-year period. J. Clin. Microbiol. **46:**2912–2917.

2. **Comité Technique des Infections Nosocomiales.** 1999. 100 recommandations pour la surveillance et la prévention des infections nosocomiales, 2nd ed. Ministère de l'Emploi et de la Solidarité, Secrétariat d'Etat à la Santé et à l'Action Sociale, Paris, France.

3. **Crisóstomo, M. I., H. Westh, A. Tomasz, M. Chung, D. C. Oliveira, and H. de Lencastre.** 2001. The evolution of methicillin resistance in *Staphylococcus aureus*: similarity of genetic backgrounds in historically early methicillin-susceptible and -resistant isolates and contemporary epidemic clones. Proc. Natl. Acad. Sci. USA **98:**9865–9870.

4. **Crum, N. F., R. U. Lee, S. A. Thornton, O. C. Stine, M. R. Wallace, C. Barrozo, A. Keefer-Norris, S. Judd, and K. L. Russell.** 2006. Fifteen-year study of the changing epidemiology of methicillin-resistant *Staphylococcus aureus*. An. J. Med. **119:**943–951.

5. **Dauwalder, O., G. Lina, G. Durand, M. Bes, H. Meugnier, V. Jarlier, B. Coignard, F. Vandenesch, J. Etienne, and F. Laurent.** 2008. Epidemiology of invasive methicillin-resistant *Staphylococcus aureus* clones collected in France in 2006 and 2007. J. Clin. Microbiol. **46:**3454–3458.

6. **de Miranda, O. P., M. C. Silva-Carvalho, A. Ribeiro, F. Portela, R. P. Cordeiro, N. Caetano, C. F. Vidal, and A. M. Figueiredo.** 2007. Emergence in Brazil of methicillin-resistant *Staphylococcus aureus* isolates carrying SCCmecIV that are related genetically to the USA800 clone. Clin. Microbiol. Infect. **13:**1165–1172.

7. **Durand, G., M. Bes, H. Meugnier, M. C. Enright, F. Forey, N. Liassine, A. Wenger, K. Kikuchi, G. Lina, F. Vandenesch, and J. Etienne.** 2006. 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. J. Clin. Microbiol. **44:**847–853.

8. **Ender, M., N. McCallum, R. Adhikari, and B. Berger-Bächi.** 2004. Fitness cost of SCC*mec* and methicillin resistance levels in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **48:**2295–2297.

9. **Enright, M. C., N. P. Day, C. E. Davies, S. J. Peacock, and B. G. Spratt.** 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J. Clin. Microbiol. **38:**1008–1015.

10. **Fossum, A. E., and G. Bukholm.** 2006. Increased incidence of methicillin-resistant *Staphylococcus aureus* ST80, novel ST125 and SCCmecIV in the south-eastern part of Norway during a 12-year period. Clin. Microbiol. Infect. **12:**627–633.

11. **François, P., A. Huyghe, Y. Charbonnier, M. Bento, S. Herzig, I. Topolski, B. Fleury, D. Lew, P. Vaudaux, S. Harbarth, W. van Leeuwen, A. van Belkum, D. S. Blanc, D. Pittet, and J. Schrenzel.** 2005. Use of an automated multiple-locus, variable-number tandem repeat-based method for rapid and high-throughput genotyping of *Staphylococcus aureus* isolates. J. Clin. Microbiol. **43:**3346–3355.

12. **François, P., S. Harbarth, A. Huyghe, G. Renzi, M. Bento, A. Gervaix, D. Pittet, and J. Schrenzel.** 2008. Methicillin-resistant *Staphylococcus aureus*, Geneva, Switzerland, 1993–2005. Emerg. Infect. Dis. **14:**304–307.

13. **François, P., T. Koessler, A. Huyghe, S. Harbarth, M. Bento, D. Lew, J. Etienne, D. Pittet, and J. Schrenzel.** 2006. Rapid *Staphylococcus aureus agr* type determination by a novel multiplex real-time quantitative PCR assay. J. Clin. Microbiol. **44:**1892–1895.

14. **Ghebremedhin, B., W. König, and B. König.** 2005. Heterogeneity of methicillin-resistant *Staphylococcus aureus* strains at a German university hospital during a 1-year period. Eur. J. Clin. Microbiol. Infect. Dis. **24:**388–398.

15. **Haley, R. W., D. H. Culver, J. W. White, W. M. Morgan, T. G. Emori, and V. P. Munn.** 1985. The efficacy of infection surveillance and control programs in preventing nosocomial infections in US hospitals. Am. J. Epidemiol. **121:**182–205.

16. **Jarraud, S., C. Mougel, J. Thiioulouse, G. Lina, H. Meugnier, F. Forey, X. Nesme, J. Etienne, and F. Vandenesch.** 2002. Relationships between *Staphylococcus aureus* genetic background, virulence factors, *agr* groups (alleles), and human disease. Infect. Immun. **70:**631–641.

17. **Jenkins, T. C., B. D. McCollister, R. Sharma, K. K. McFann, N. E. Madinger, M. Barron, M. Bessesen, C. S. Price, and W. J. Burman.** 2009. Epidemiology of healthcare-associated bloodstream infection caused by USA300 strains of methicillin-resistant *Staphylococcus aureus* in 3 affiliated hospitals. Infect. Control Hosp. Epidemiol. **30:**233–241.

18. **Lee, J. H., J. M. Jeong, Y. H. Park, S. S. Choi, Y. H. Kim, J. S. Chae, J. S. Moon, H. Park, S. Kim, and S. K. Eo.** 2004. Evaluation of the methicillin-resistant *Staphylococcus aureus* (MRSA)-Screen latex agglutination test for detection of MRSA of animal origin. J. Clin. Microbiol. **42:**2780–2782.

19. **Nulens, E., E. E. Stobberingh, H. van Dessel, S. Sebastian, F. H. van Tiel, P. S. Beisser, and R. H. Deurenberg.** 2008. Molecular characterization of *Staphylococcus aureus* bloodstream isolates collected in a Dutch university hospital between 1999 and 2006. J. Clin. Microbiol. **46:**2438–2441.

20. **Nulens, E., E. E. Stobberingh, E. Smeets, H. van Dessel, M. A. Welling, S. Sebastian, F. H. van Tiel, P. S. Beisser, and R. H. Deurenberg.** 2009. Genetic diversity of methicillin-resistant *Staphylococcus aureus* in a tertiary hospital in The Netherlands between 2002 and 2006. Eur. J. Clin. Microbiol. Infect. Dis. **28:**631–639.

21. **Oliveira, D. C., and H. de Lencastre.** 2002. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **46:**2155–2161.

22. **Paul, J.** 2006. Surveillance and management of all types of *Staphylococcus aureus* bacteraemia. BMJ **333:**269–270.

23. **Popovich, K. J., R. A. Weinstein, and B. Hota.** 2008. Are community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) strains replacing traditional nosocomial MRSA strains. Clin. Infect. Dis. **46:**787–794.

24. **Rodriguez-Bano, J., M. Angeles Dominguez, A. Blas Millan, C. Borraz, M. Pau Gonzales, B. Almirante, E. Cercenado, B. Padilla, and M. Pujol on behalf of GEIH/GEMARA (SEIMC), REIPI.** 15 May 2009. Clinical and molecular epidemiology of community-acquired, healthcare-associated and nosocomial methicillin-resistant *Staphylococcus aureus* in Spain. Clin. Microbiol. Infect. [Epub ahead of print.] doi:10.1111/j.1469-0691.2009.02717.x.

25. **Société Française de Microbiologie.** 2009. Recommandations du Comité de l'Antibiogramme de la Société Française de Microbiologie. Société Française de Microbiologie, Paris, France. www.sfm.asso.fr.

26. **van der Mee-Marquet, N., A. S. Domelier, N. Girard, R. Quentin, and the Bloodstream Infection Study Group of the Relais d'Hygiene du Centre.** 2004. Epidemiology and typing of *Staphylococcus aureus* strains isolated from bloodstream infections. J. Clin. Microbiol. **42:**5650–5657.

27. **van der Mee-Marquet, N., C. Epinette, J. Loyau, L. Arnault, A. S. Domelier, B. Losfelt, N. Girard, R. Quentin, and the Bloodstream Infection Study Group of the Relais d'Hygiene du Centre.** 2007. *Staphylococcus aureus* strains isolated from bloodstream infections changed significantly in 2006. J. Clin. Microbiol. **45:**851–857.

28. **Vourli, S., H. Vagiakou, G. Ganteris, M. Orfanidou, M. Polemis, A. Vatopoulos, and H. Malamou-Ladas.** 15 January 2009. High rates of community-acquired, Panton-Valentine leukocidin (PVL)-positive methicillin-resistant *S. aureus* (MRSA) infections in adult outpatients in Greece. Euro Surveill. vol. 14, issue 2, article 5.