Staphylococcus aureus Strains Isolated from Bloodstream Infections Changed Significantly in 2006[▽]

Nathalie van der Mee-Marquet,^{1,2*} Christophe Epinette,² Jeremy Loyau,² Laurence Arnault,² Anne-Sophie Domelier,² Barbara Losfelt,² Nicole Girard,² Roland Quentin,¹ and the Bloodstream Infection Study Group of the Relais d'Hygiène du Centre

EA 3854, IFR 136, UFR Médecine, Université François-Rabelais, 2 bis boulevard Tonnelé, 37032 Tours Cedex, France,¹ and Service de Bactériologie et d'Hygiène, Hôpital Trousseau, Centre Hospitalier Universitaire, F37044 Tours Cedex, France²

Received 25 October 2006/Returned for modification 22 November 2006/Accepted 10 January 2007

We studied 358 *Staphylococcus aureus* strains isolated from bloodstream infections (BSI) observed during an epidemiological study covering 2,007,681 days of hospitalization in 32 healthcare institutions (HCIs) between 2004 and 2006. The strains were tested for antibiotic susceptibility and characterized genetically. The incidence of *S. aureus* BSI declined regularly through 2004 and 2005 and then significantly increased in 2006 (+80%). This was largely due to an increase in BSI involving methicillin-sensitive *S. aureus* (MSSA) strains and nonmultiresistant methicillin-resistant *S. aureus* (NORSA) strains. Ninety-six percent of the NORSA strains were resistant only to methicillin and fluoroquinolones. Most of the MSSA strains belonged to a small number of pulsed-field gel electrophoresis (PFGE) divisions and were associated with epidemic phenomena in HCIs. The NORSA strains also clustered into a limited number of PFGE divisions but could not be related to any local outbreak in HCIs. In 2006, there was a significant increase in the incidence of BSI associated with *tst* gene-positive MSSA strains (+275%) and the first three BSI associated with *tst* gene-positive MRSA were observed. PFGE data revealed a limited heterogeneity among the *tst* gene-positive strains without any outbreak in the HCIs. Our study underlines the need for infection control teams to focus efforts on preventing both MRSA and MSSA BSI. As recently demonstrated *in vitro*, fluoroquinolones may enhance horizontal transfer of virulence and antibiotic resistance genes. These antibiotics are widely used in France, so our findings raise the issue of whether their use has contributed to the acquisition of *mecA* and *tst* genes by *S. aureus* strains.

Methicillin-resistant *S. aureus* (MRSA) frequently causes disease outbreaks and has become endemic in many regions, adding to the morbidity, mortality, and cost of care associated with hospital-acquired infections. Enhanced surveillance and infection control measures have been adopted by healthcare institutions (HCIs) to address this unresolved problem (5). In particular, reporting of bloodstream infections (BSI) by MRSA is often mandatory and reduction of BSI rates is a performance target (5, 12, 21).

In the Centre region of France, an extensive, prospective, longitudinal, region-wide survey of BSI has been under way since 2000. Data are collected for 3 months of each year in a large number of HCIs to establish a comprehensive picture of the epidemiology of severe hospital-acquired infections. MRSA BSI and methicillin-sensitive *S. aureus* (MSSA) BSI are extensively studied within this framework. All of the *S. aureus* strains isolated during successive study periods are sent to our central laboratory for susceptibility testing, molecular typing, and analysis of virulence genes with the aim of determining the spread and diversity of *S. aureus* strains in the region. The results obtained during the first 4 years of surveillance (2000 to 2003) of MRSA BSI have been reported previously (27).

Here we report the data from 2004 to 2006. We looked for any major changes in the epidemiology of antibiotic resistance and of virulence genes in strains of *S. aureus* responsible for BSI. We identify a need to focus efforts on preventing both MRSA and MSSA BSI infections and raise the issue of whether the use of fluoroquinolones (FQs) has contributed to the acquisition of resistance and virulence genes by *S. aureus* strains.

MATERIALS AND METHODS

BSI epidemiological survey method. A BSI surveillance program in the Centre region of France (2.5 million inhabitants) and a microbiological study of *S. aureus* strains isolated from BSI cases have been conducted since 2000. Thirty-two HCIs, comprising 6,027 short-stay beds, participated in this annual 3-month survey of all cases of BSI. Here, we report results for the years 2004 to 2006. The survey covered 2,007,681 patient days (PD). The methods, study design, and data for the years 2000 to 2003 have been reported elsewhere (27). Briefly, the variables studied included patient age and sex, portal of entry, community- or hospital-acquired BSI, occurrence of death within 7 days of BSI diagnosis, and duration of hospital stay. Data were analyzed with Epi Info v.6 software. Data were analyzed with a χ^2 test with five degrees of freedom. The incidences of community-acquired and nosocomial BSI were determined with respect to the number of PD.

Microbiological methods. (i) Bacteriology. Three hundred fifty-eight BSI-associated *S. aureus* strains were collected during the three survey periods (2004, 2005, and 2006). The strains were sent to the reference laboratory of the Relais d'Hygiène du Centre. The isolates were identified as *S. aureus* according to previously described procedures (27).

(ii) Antimicrobial susceptibility testing. We used the disk diffusion method (Bio-Rad, France) to test the antibiotic susceptibility of *S. aureus* strains. The antibiotics tested were penicillin G, oxacillin, erythromycin, lincomycin, pristinamycin, tetracycline, kanamycin, tobramycin, gentamicin, rifampin, fusidic acid,

* Corresponding author. Mailing address: Laboratoire de Bactériologie et Hygiène, Hôpital Trousseau, 37044 Tours Cedex, France. Phone: 33 234 389 430. Fax: 33 247 478 588. E-mail: n.vandermee@chu-tours.fr.

[▽] Published ahead of print on 24 January 2007.

fosfomycin, pefloxacin, cotrimoxazole, vancomycin, and teicoplanin. The cefinase test (bioMérieux, France) was used to detect β -lactamase production, and the *mecA* gene was detected by PCR. Classic multisensitive methicillin-sensitive *S. aureus* (MSSA) will be referred to as CMSSA, and methicillin-sensitive strains displaying resistance to FQs or to at least two antibiotics (not including penicillin) will be referred to as EMSSA. MRSA strains displaying typical resistance to multiple antibiotics will be referred to as CMRSA (classic multiresistant MRSA). MRSA strains resistant to no more than three antibiotics (excluding penicillin and methicillin) will be referred to as NORSA (nonmultiresistant MRSA); NORSA strains resistant only to FQs will be referred to as NORSA FQ.

(iii) **Multiplex PCR assay for typing of major staphylococcal chromosome cassette *mec* elements.** Multiplex PCR was used to type the staphylococcal chromosome cassette *mec* element (SCC*mec*) in MRSA and multiresistant oxacillin-sensitive *S. aureus* (EMSSA) as described by Oliveira and de Lencastre (20) and Zhang et al. (30).

(iv) **DNA macrorestriction and PFGE.** Pulsed-field gel electrophoresis (PFGE) was used as a typing technique. Genomic DNA was extracted from the isolates, digested with *Sma*I, and then subjected to PFGE as previously described (27). PFGE patterns were analyzed with the Taxotron package (Taxolab; Institut Pasteur, Paris, France). Images were transferred to a microcomputer, and the distance that each band migrated in each lane was determined in pixel units by RestrictoScan. The molecular size of each fragment was calculated from the distance migrated by using cubic s&s algorithms with RestrictoTyper. This software generated a schematic representation of electrophoretic patterns and produced a distance matrix. The relationships between pulsotypes were determined by the unweighted-pair group method using average linkages and the Adanson pulsogrouping program (dissimilarity). A dendrogram was drawn with Dendrograf.

(v) **MLST.** Seven MRSA strains were analyzed by multilocus sequence typing (MLST) according to the procedure described by Enright et al. (8).

Virulence factors. Given the emergence of Panton-Valentine leukocidin- and toxic shock syndrome toxin 1-producing MRSA strains worldwide (1, 9, 10, 13, 23, 25) and in France since 2002 (7, 28), we sought sequences corresponding to the *lukS-PV* and *lukF-PV* genes encoding Panton-Valentine leukocidin and to the *tst* gene encoding toxic shock syndrome toxin 1. Genomic DNA was extracted from staphylococcal cultures and used as a template for PCR amplification by using a procedure and primers previously described (16).

RESULTS

***S. aureus* BSI incidence.** During the study period, 2004 to 2006, 358 cases of *S. aureus* BSI were identified (Table 1). The mean incidence of *S. aureus* BSI was 0.178 per 1,000 PD (0.130 to 0.234, depending on the year) (Table 1; Fig. 1). The incidence of *S. aureus* BSI decreased regularly from 0.202 in 2002 to 0.130 in 2005 (Table 1) and increased substantially in 2006 to 0.234. The incidence observed in 2006 resulted from a significant increase in (i) the MSSA BSI incidence (0.092/1,000 PD in 2005 and 0.165 in 2006; $P < 0.001$) and (ii) the NORSA BSI incidence (0.008/1,000 PD in 2005 and 0.028 in 2006; $P = 0.010$).

Methicillin resistance. Of the 358 *S. aureus* strains isolated from BSI, 246 were MSSA strains (69%) and 112 were MRSA strains (31%). The MRSA BSI incidence and the proportion of *S. aureus* BSI that were due to MRSA did not vary significantly during this study (Table 1; Fig. 1). Nevertheless, the prevalence of NORSA strains among MRSA strains and the NORSA BSI incidence increased between 2004 and 2006; in 2004, 10 of the 43 MRSA strains were NORSA strains (23%, 0.014/1,000 PD), and in 2006, 19 of the 46 MRSA strains were NORSA strains (41%, 0.028/1,000 PD; $P < 0.001$) (Table 1). Most (27/34, 79%) of these 34 NORSA strains were resistant only to FQs and methicillin (designated NORSA FQ) (Table 1).

Twenty of the 256 MSSA strains were EMSSA strains (8%). The incidence of MSSA BSI increased during the study period,

TABLE 1. Distribution of *S. aureus* strains associated with BSI and incidence according to survey period and antibiogram

Antibiogram	No. (%) of strains according to survey period ^a				
	2002	2003	2004	2005	2006
MRSA ^a	33 (27)	35 (30)	43 (35)	23 (29)	46 (29)
CMRSA ^b	28 (85)	31 (89)	33 (77)	18 (78)	27 (59)
NORSA ^c	5 (15)	4 (11)	10 (23)	5 (22)	19 (41)
NORSA FQ ^d	1	4	7	4	16
Other	4		3	1	3
FQ resistance	31 (94)	35 (100)	43 (100)	23 (100)	46 (100)
MSSA ^e	89 (73)	83 (70)	79 (65)	56 (71)	111 (71)
EMSSA ^f	9	15	6	3	11
EMSSA FQ ^g					4
FQ resistance	6 (7)	14 (17)	8 (8)	4 (7)	9 (8)
Total	122	118	122	79	157

^a Percentage indicates the proportion of *S. aureus* strains that are MRSA.

^b Percentage indicates the proportion of MRSA strains that are CMRSA.

^c Percentage indicates the proportion of MRSA strains that are NORSA.

^d NORSA FQ strains resistant only to FQs.

^e Percentage indicates the proportion of *S. aureus* strains that are MSSA.

^f MSSA with resistance to FQs or to at least two antibiotics.

^g MSSA with resistance to FQs only.

^h Results obtained in 2002 and 2003 were described previously (27) and are reported to show the changes through time affecting the different groups of strains.

but the incidence of EMSSA BSI did not. Six percent (15/256) of the MSSA strains displayed FQ resistance, and this proportion remained stable during the study period. Note that the four EMSSA strains resistant only to FQs (designated EMSSA FQ) were all isolated in 2006.

SCC*mec* typing. Only 108 of the 112 MRSA strains isolated during the study period were SCC*mec* typed because 4 strains could not be cultivated from storage medium (Table 2). Most of the 108 MRSA strains were of SCC*mec* type I (13%), IVA (52%), or IV (12%). No SCC*mec* fragment could be amplified by PCR from 24 of the 108 strains (22%), and these strains were therefore designated nontypeable. They were also typed by the procedure described by Zhang et al. (30) to test whether they were of recently described type V (15). No PCR SCC*mec* fragment was obtained for 22 strains by this procedure, and two strains were scored as polytypeable because PCR SCC*mec* fragments for both types IV and IA were obtained. Most of the CMRSA strains were of SCC*mec* types I and IVA (66/74, 89%). Four (12%) of the 33 NORSA strains were of SCC*mec* type IV, and 24 were not typeable (24/33, 73%). Nontypeable strains were significantly associated with NORSA (24/33 NORSA versus 0/74 CMRSA, $P < 0.001$). SCC*mec* PCR testing was negative for all of the 20 EMSSA strains.

MLST. MLST was conducted with three MRSA SCC*mec* type IVA strains and four NORSA FQ SCC*mec* nontypeable strains. The three MRSA SCC*mec* type IVA strains were of ST8 and were assigned to CC8. One of the NORSA FQ SCC*mec* nontypeable strains was of ST683 and was assigned to CC8. The remaining three NORSA FQ SCC*mec* nontypeable strains were of ST5 and were assigned to CC5.

***tst*, *lukS-PV*, and *lukF-PV* genes.** Thirty-eight (11%) of the 358 *S. aureus* strains scored positive for *tst* or the *luk* genes. One MSSA strain (1/246, <1%) scored positive for the *luk*

	Incidence /1000 patient days according to the year				
	2002 ⁴	2003 ⁴	2004	2005	2006
Patient days	604,850	662,555	729,013	607,300	671,368
Patient admissions	89,949	86,530	81,867	71,579	80,499
<i>S. aureus</i> BSI	0.202	0.178	0.167	0.130	0.234
MRSA BSI	0.055	0.047	0.059	0.038	0.068
C-MRSA BSI ¹	0.046	0.047	0.045	0.030	0.040
NORSA BSI ²	0.008	0.006	0.014	0.008	0.028
MSSA BSI	0.147	0.125	0.108	0.092	0.165
EMSSA BSI ³	0.015	0.023	0.008	0.005	0.016
<i>tst</i> gene + MRSA BSI					0.004
<i>tst</i> gene + MSSA BSI	NK ⁵	NK	0.012	0.008	0.030
<i>Luk</i> genes + MRSA BSI					
<i>Luk</i> genes + MSSA BSI	NK	NK			0.001

FIG. 1. BSI incidence (per 1,000 days of hospitalization) according to year of survey, antibiogram, and virulence genes. Superscript numbers: 1, CMRSA, classical multiresistant MRSA; 2, NORSA, nonmultiresistant MRSA; 3, MSSA with resistance to quinolones or to at least two antibiotics; 4, results for 2002 and 2003 described previously (27) are reported to illustrate the changes through time affecting the different groups of strains; 5, NK, not known.

genes. Thirty-seven strains, including 34 MSSA strains (34/246, 14%) and 3 MRSA strains (3/112, 3%), were positive for the *tst* gene. The three *tst* gene-positive MRSA strains were all CMRSA SCCmec type IVA strains isolated during 2006. The incidence of *tst* gene-positive MSSA strains increased significantly between 2004 to 2005 and 2006 (Table 1; Fig. 1) ($P =$

0.007). None of the *tst* gene-positive MSSA strains were EMSSA strains.

Genotyping. PFGE revealed 283 different pulsotypes among the 358 strains. Analysis by the unweighted-pair group method using average linkages distributed the strains into 13 major divisions (designated I to XIII; Fig. 2 contains a dendrogram). MRSA strains were predominant in divisions I, II, IV, and V (75/112, 67%). Three divisions (VI, VIII, and XII) were exclusively composed of MSSA strains, and MSSA strains were predominant in divisions III, VII, IX, X, and XI. These eight divisions contained 85% of the MSSA strains (210/246). The remaining division, XIII, contained 8 MRSA strains and 14 MSSA strains. The population of 34 NORSA strains was distributed across nine of the PFGE divisions (Fig. 2). Nevertheless, 17 NORSA strains (50%) were found in only two divisions: 12 strains in division III and 5 in division XIII. Nineteen (70%) of the 27 NORSA FQ strains were clustered in three divisions (I, III, and XIII, Fig. 2). Each of these three divisions

TABLE 2. SCCmec typing results for 108 MRSA strains according to antibiogram

Group of strains	No. (%) of strains according to SCCmec type					
	I	II	IIIx	IV	IVA	NT
CMRSA ($n = 75$)	12 (16)	1 (1)	1 (1)	9 (12)	54 (72)	
NORSA ($n = 33$)	2 (6)		1	4 (12)	2 (6)	24 (73)
NORSA FQ ($n = 25$)			1	2		22
Total	14 (13)	1 (1)	2 (2)	13 (12)	56 (52)	24 (22)

PFGE Type	Div	Number of strains	MRSA			MSSA			PCR (%)		Death at day 7 (%)
			Total	NORSA (%)	NORSA FQ*	Total	EMSSA (%)	EMSSA FQ*	tst	Luk	
	I	25	19 (76)	4 (16)	4 (16)	6 (24)	1 (4)		1 (4)		8 (32)
	II	26	21 (81)	4 (15)	2 (8)	5 (19)			1 (4)		5 (19)
	III	57	13 (23)	12 (21)	11 (19)	44 (77)	5 (9)	3			8 (14)
	IV	24	17 (71)	3 (12)	2 (8)	7 (29)	2 (8)		1 (4)		2 (8)
	V	22	18 (82)	2 (9)	1 (4)	4 (18)	3 (14)				3 (14)
	VI	40				40 (100)	4 (10)		5 (12)		5 (12)
	VII	26	4 (15)	1 (4)	1 (4)	22 (85)			2 (8)		3 (11)
	VIII	6				6 (100)					1 (17)
	IX	28	5 (18)	2 (7)	1 (4)	23 (82)	3 (11)		1 (4)		4 (14)
	X	43	5 (12)			38 (88)			19 (44)	1 (10)	6 (14)
	XI	30	2 (7)	1 (3)	1 (3)	28 (93)	2 (7)	1	3 (10)		3 (10)
	XII	9				9 (100)			4 (44)		4 (44)
	XIII	22	8 (36)	5 (23)	4 (18)	14 (64)					2 (9)

FIG. 2. Characteristics of the 358 *S. aureus* strains included in this study.

TABLE 3. Distribution of the 112 MRSA and 246 MSSA strains included in this study into PFGE divisions

Survey period or parameter	No. of strains (incidence/1,000 PD) according to PFGE division													
	Total	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
All strains	358 (0.178)	25 (0.012)	26 (0.013)	44 (0.022)	24 (0.012)	22 (0.011)	40 (0.020)	26 (0.013)	6 (0.003)	28 (0.014)	43 (0.021)	30 (0.015)	9 (0.004)	22 (0.011)
2004	122 (0.167)	7 (0.010)	11 (0.015)	15 (0.021)	18 (0.025)	6 (0.008)	24 (0.033)	18 (0.025)			11 (0.015)	6 (0.008)	2 (0.003)	4 (0.005)
2005	79 (0.130)	12 (0.020)	3 (0.005)	6 (0.010)	1 (0.002)	8 (0.013)	13 (0.021)	3 (0.005)	6 (0.010)	2 (0.003)	5 (0.008)	12 (0.020)	4 (0.007)	4 (0.007)
2006	157 (0.234)	6 (0.009)	12 (0.018)	36 (0.054)	5 (0.007)	8 (0.012)	3 (0.004)	5 (0.007)		26 (0.039)	27 (0.040)	12 (0.018)	3 (0.004)	14 (0.021)
<i>P</i> value ^a	<0.001 (†)	0.096	0.036 (†)	<0.001 (†)	0.139	0.814	0.006 (‡)	0.429	0.011	<0.001 (†)	<0.001 (†)	0.773	0.436	0.035 (†)
MRSA	112 (0.056)	19 (0.009)	21 (0.010)	13 (0.006)	17 (0.008)	18 (0.009)		4 (0.002)		5 (0.002)	5 (0.002)	2 (0.001)		8 (0.004)
2004	43 (0.059)	5 (0.007)	11 (0.015)	3 (0.004)	15 (0.021)	5 (0.007)				1 (0.001)				3 (0.004)
2005	23 (0.038)	11 (0.018)	3 (0.005)	2 (0.003)	1 (0.002)	6 (0.010)				4 (0.006)		2 (0.003)		5 (0.007)
2006	46 (0.068)		7 (0.010)	8 (0.012)	1 (0.001)	7 (0.010)				5 (0.007)				
<i>P</i> value	0.022	<0.001 (‡)	0.224	0.078	0.720	0.947		0.078		0.042 (†)	0.078	0.280		0.042
CMRSA	78 (0.039)	15 (0.007)	17 (0.008)	1 (<0.001)	14 (0.007)	16 (0.008)		3 (0.001)		3 (0.001)	5 (0.002)	1 (<0.001)		3 (0.001)
2004	33 (0.045)	3 (0.004)	10 (0.014)	1 (0.001)	12 (0.016)	4 (0.005)					1 (0.001)			2 (0.003)
2005	18 (0.030)	9 (0.015)	2 (0.003)		1 (0.002)	6 (0.010)					4 (0.006)	1 (0.001)		1 (0.001)
2006	27 (0.040)	3 (0.004)	5 (0.007)		1 (0.001)	6 (0.009)		3 (0.004)		3 (0.004)				
<i>P</i> value	0.342	0.052	0.277		0.720	0.839		0.148		0.148	0.078	0.529		0.529
NORSA	34 (0.017)	4 (0.002)	4 (0.002)	12 (0.006)	3 (0.001)	2 (0.001)		1 (<0.001)		2 (0.001)		1 (<0.001)		5 (0.002)
2004	10 (0.014)	2 (0.003)	1 (0.001)	2 (0.003)	3 (0.004)	1 (0.001)								1 (0.001)
2005	5 (0.008)	2 (0.003)	1 (0.002)	2 (0.003)				1 (0.001)		2 (0.003)		1 (0.001)		4 (0.006)
2006	19 (0.028)		2 (0.003)	8 (0.012)		1 (0.001)								
<i>P</i> value	0.010 (†)	0.221	0.544	0.078		0.529		0.529		0.280		0.529		0.078
MSSA	246 (0.122)	6 (0.003)	5 (0.002)	44 (0.022)	7 (0.003)	4 (0.002)	40 (0.020)	22 (0.011)	6 (0.003)	23 (0.011)	38 (0.019)	28 (0.014)	9 (0.004)	14 (0.007)
2004	79 (0.108)	2 (0.003)		12 (0.016)	3 (0.004)	1 (0.001)	24 (0.033)	18 (0.025)		2 (0.003)	10 (0.014)	6 (0.008)	2 (0.003)	1 (0.001)
2005	56 (0.092)	1 (0.002)		4 (0.007)		2 (0.003)	13 (0.021)	3 (0.005)	6 (0.010)		5 (0.008)	12 (0.020)	4 (0.007)	4 (0.007)
2006	111 (0.165)	3 (0.004)	5 (0.007)	28 (0.042)	4 (0.006)	1 (0.001)	3 (0.004)	1 (0.001)		21 (0.028)	23 (0.034)	10 (0.015)	3 (0.004)	9 (0.013)
<i>P</i> value	<0.001 (†)	0.358	0.042 (†)	<0.001 (†)	0.078	0.456	0.006 (‡)	0.270	0.011 (‡)	<0.001 (†)	0.002 (†)	0.482	0.436	0.239
EMSSA	20 (0.010)	1 (<0.001)		5 (0.002)	2 (0.001)	3 (0.001)	4 (0.002)			3 (0.001)		2 (0.001)		
2004	6 (0.008)	1 (0.001)		1 (0.001)		1 (0.001)	2 (0.003)					1 (0.001)		
2005	3 (0.005)					1 (0.002)								
2006	11 (0.016)			4 (0.006)	2 (0.003)	1 (0.001)	2 (0.003)			3 (0.004)		1 (0.001)		
<i>P</i> value	0.055			0.078	0.280	0.720	0.221			0.148		0.529		

^a Chi square was calculated for data for the years 2005 and 2006. †, increase; ‡, decrease.

corresponded to strains isolated in different HCIs. The population of 20 EMSSA strains was distributed across seven PFGE divisions (Fig. 2). Three of the four EMSSA FQ strains isolated in 2006 were clustered in division III. The 37 *tst* gene-positive strains were distributed among nine PFGE divisions (Fig. 2), although division X contained 19/37 (51%) *tst* gene-positive strains, including 1 MRSA strain and 18 MSSA strains. The three MRSA *tst* gene-positive strains were each in a different division, VII, IX, or X. Notable changes in the incidence of *S. aureus* BSI belonging to several PFGE divisions were observed during this study (Table 3). The incidence of *S. aureus* BSI associated with strains belonging to PFGE divisions VI and VIII significantly decreased, whereas that of BSI associated with strains belonging to PFGE divisions II, III, IX, X, and XIII increased. The incidence of MRSA BSI remained stable, whereas the incidence of BSI associated with strains belonging to PFGE division I decreased and that of BSI associated with strains belonging to PFGE division IX increased. Also, the significant increase in the incidence of NORSA BSI was not associated with any significant variation in the distribution of the strains among the PFGE divisions.

Mortality during the 7 days following BSI diagnosis. All-cause mortality was known for all cases (Fig. 2). Fifty-four deaths were identified (54/358, 15%), and these cases included 22 of the 112 MRSA BSI cases (20%) and 32 of the 246 MSSA BSI cases (13%). The mortality rate was higher for CMRSA strains (18/78, 23%) than for NORSA strains (4/34, 12%) (no statistically significant difference). The mortality rates of the *tst*-positive strains (5/37, 13%) and the *tst*-negative strains (49/321, 15%) were similar.

DISCUSSION

We present a comprehensive picture of the epidemiology of *S. aureus* isolated from cases of BSI in the Centre region of France during the last 3 years (2004 to 2006). There were substantial and rapid changes in the epidemiology of antibiotic resistance and of virulence genes in the *S. aureus* strains responsible for BSI. The incidence of *S. aureus* BSI regularly decreased from 2002 to 2005, but in 2006 it surged (Table 1 and Fig. 1). This surge was not due to changes in MRSA BSI but was associated with an increased incidence of each MSSA BSI, NORSA FQ BSI, and *tst* gene-positive *S. aureus* BSI.

Indeed, CMRSA strains are responsible for outbreaks in HCIs. During the study period, the incidence of CMRSA BSI was low and stable. The distribution of CMRSA strains among the PFGE groups was wide and unchanging (Table 3; Fig. 2). These findings suggest that patient isolation, recommended for the prevention of nosocomial transmission of MRSA (3, 5) and applied in the HCIs for patients colonized or infected with MRSA, satisfactorily restricts the epidemic diffusion of such CMRSA strains.

Conversely, there was a significant increase in the incidence of MSSA BSI in 2006 (Fig. 1), mostly due to strains from four PFGE divisions (Fig. 2; Table 3). This finding is consistent with epidemic diffusion of particular clones of MSSA, and the geographical origin of strains of these clones supported this view; MSSA strains of PFGE division III spread (at least two strains isolated during the survey) in 6 of the 32 participating HCIs, MSSA strains of PFGE division IX spread in 4 HCIs, and

MSSA strains of PFGE division X spread in 4 HCIs. These data highlight the need to focus efforts on preventing MSSA BSI. This is in accordance with remarks published recently by J. Paul (21). Also, further work should be initiated to determine the prevalence of both patient colonization by MSSA and non-BSI MSSA infections. In addition, it would be valuable to assess the potential impact of isolation of these patients to avoid the epidemic diffusion of strains in HCIs by comparison with the consequences of application of standard precautions alone. Nevertheless, our survey is restricted to a 3-month period each year, and despite the repeatability of the period studied (3 months into the first quarter of each year), we cannot exclude the possibility that the outbreaks we studied are not representative of the year-round situation and bias the real trends in changes in *S. aureus* populations. This emphasizes the importance of continuing surveillance of MSSA BSI.

The increase in the incidence of NORSA BSI was mostly due to genetically diverse NORSA FQ strains (Tables 1 and 3; Fig. 2). While the geographical origin of the strains indicated that each NORSA FQ strain of each of the PFGE divisions originated from a different HCI, outbreaks in the participating HCIs were unlikely. Twenty-two of the 25 NORSA FQ strains were nontypeable for the *SCCmec* cassette, and two strains were *SCCmec* type IV. This indicated that the horizontally transferable (24, 29) mobile genetic element (*SCCmec*) carrying the *mecA* gene in these strains may be relatively small. Concurrently with the increase in the incidence of NORSA FQ BSI, we observed an increase in the incidence of *tst* gene-positive *S. aureus* BSI, mostly due to genetically diverse *tst*-positive MSSA strains (Fig. 2). Also, the three *tst* gene-positive MRSA strains belonged to distinct PFGE divisions. There also, as the *tst*-positive strains originated from different HCIs, the occurrence of outbreaks at an institution level was excluded. If the diffusion at a regional level of these potentially virulent clones of strains could be, at least partly, the consequence of frequent patient transfers between HCIs, their ability to emerge and spread rapidly needs to be studied further (fitness, epidemicity. . .).

The observed increase in the incidence of NORSA FQ strains and *tst*-positive MSSA strains raises questions about the role of FQs (4, 8, 18), particularly because the prescription of FQs has increased recently in France (http://associations.societegenerale.fr/EIA-Visiteurs_medicaux_invites_a_reduire_la_promotion_de_certaines_medicaments-sv-asso-rq-afp-actu-4452.html). Indeed, in vitro, FQs are able (i) to promote mutations affecting genes encoding topoisomerase IV (14), (ii) to induce an SOS response in *S. aureus* promoting horizontal transfer of antibiotic resistance and virulence genes including the *tst* gene (2, 7, 11, 17, 26), and possibly (iii) to promote natural transformation of small genetic elements (in particular, the small genetic *SCCmec* element), as recently shown in *S. pneumoniae* strains (19, 22). These genetic events could occur independently in different populations of strains. It would be useful to conduct further studies to clarify whether the clinical use of FQs contributes to the acquisition of antibiotic resistance and virulence genes by *S. aureus* strains. c

ACKNOWLEDGMENTS

This work was supported by the Centre de Coordination de la Lutte contre les Infections Nosocomiales of West France (CCLIN Ouest),

the Agence Régionale de l'Hospitalization du Centre, and the Centre Hospitalier Universitaire de Tours.

We are grateful to P.-Y. Donnio, H. de Lencastre, and C. Olivier for helpful discussions concerning the interpretation of the SCCmec typing results.

The members of the Bloodstream Infection Study Group of the Relais d'Hygiène du Centre are M.-N. Adam, J. Akli, P. Amirault, J. P. Arnould, M.-N. Bachelier, M. Beigneux, H. Berjon, G. Bisi, D. Bloc, P. Bourdillat, M. Boyer, Z. Benseddik, M. Cahiez, B. Cattier, M. Chabaud-Meyer, C. Chandesris, F. Cotty, F. Coulomb, G. Courouble, M.-C. Courtin, L. Danse, P. de Mauregard, M. C. Farcy, B. Fauqueur, C. Fièvre, P. Foloppe, F. Fongauquier, R. Fournier-Hoock, A.-M. Gingras-Roux, I. Goard, V. Gorin, J.-L. Graveron, Y. Gretha, F. Grosbost, O. Guignard, M. F. Guillon, F. Guinard, P. Harriau, C. Hombrouck-Alet, D. Imbault, F. Jacqmin, G. Jamault, J.-F. Jamet, P. Laudat, O. Lehiani, J. Loulergue, N. van der Mee-Marquet, V. Morange, E. Morel-Desjardins, L. Mereghetti, S. Monin, E. Morin, C. Naudion, M. Odaert, L. Ollivier, K. Opsomer, F. Perigois, F. Petit, G. Petit, S. Petit, S. Picault, D. Poisson, J.-P. Pourrat, R. Quentin, D. Ratovohery, S. Rossard, A. Roussin, Y. Salaun, A. Secher, C. Suard, J.-F. Theron le Gargasson, and R. Vergez-Pascal.

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