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Molecular Evidence for Spread of Two Major Methicillin-Resistant *Staphylococcus aureus* Clones with a Unique Geographic Distribution in Chinese Hospitals[▽]

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Methicillin (meticillin)-resistant *Staphylococcus aureus* (MRSA) is a serious problem worldwide. To investigate the molecular epidemiology of MRSA isolates in China, a total of 702 MRSA isolates collected from 18 teaching hospitals in 14 cities between 2005 and 2006 were characterized by antibiogram analysis, pulsed-field gel electrophoresis (PFGE), staphylococcal cassette chromosome *mec* (SCC*mec*) typing, and *spa* typing; and 102 isolates were selected for multilocus sequence typing (MLST). Overall, SCC*mec* type III was the most popular type and was found in 541 isolates (77.1%), followed by SCC*mec* type II (109/702; 15.5%). Twenty-four PFGE types were obtained among 395 isolates collected in 2005, and 18 *spa* types were obtained among 702 isolates. *spa* type t030, which corresponded to PFEG types A to E, constituted 52.0% (365/702) of all isolates, and isolates of this type were present in all 14 cities; *spa* type t037, which corresponded to PFGE types F and G, accounted for 25.5% (179/702) of all isolates, and isolates of this type were identified in 12 cities. The two *spa* genotypes belonged to sequence type 239 (ST239) and carried SCC*mec* type III. *spa* type t002, which included isolates of PFGE types L to T, made up 16.0% (112/702) of the isolates that belonged to ST5 and SCC*mec* type II, and isolates of this type were distributed in 12 cities. The distribution of *spa* types varied among the regions. *spa* type t002 was the most common in Dalian (53.4%) and Shenyang (44.4%); *spa* type t037 was predominant in Shanghai (74.8%), whereas *spa* type t030 was the most common in the other cities. Two isolates from Guangzhou that harbored SCC*mec* type IVa with ST59 and ST88 were identified as community-associated MRSA. The prevalence of the Panton-Valentine leukocidin gene was 2.3%. The data documented two major epidemic MRSA clones, ST239-MRSA-SCC*mec* type III and ST5-MRSA-SCC*mec* type II, with unique geographic distributions across China.

The emergence and spread of multidrug-resistant clones of methicillin (meticillin)-resistant *Staphylococcus aureus* (MRSA) are worldwide problems (7, 17). The recent emergence of highly virulent community-associated MRSA (CA-MRSA) strains and vancomycin-resistant, intermediate-resistant, or heteroresistant *S. aureus* strains further heightens public health concerns (3, 16, 20, 26, 29). According to a study conducted in 2005, the mean incidence of MRSA across China was over 50%, and in Shanghai, the prevalence was over 80% (30). Therefore, understanding and controlling the spread of MRSA in both hospital and community settings are now of paramount importance.

One of the efficient ways of controlling the spread of MRSA is through determination of the genotypic characteristics of MRSA clones as well as the genetic relatedness of the strains in different geographic regions (7, 10). Pulsed-field gel electrophoresis (PFGE) has been regarded as the "gold standard" method for MRSA typing (4, 27), and recently, the application of analysis of the staphylococcal cassette chromosome *mec* (SCC*mec*) element (13, 21, 23), multilocus sequence typing (MLST) (8), and *spa* typing have brought further progress to epidemiological studies of MRSA (12, 19, 25).

Previous studies demonstrated that two major MRSA clones are prevalent in Asian countries. On the basis of MLST and SCC*mec* typing, sequence type 5 (ST5)-MRSA-SCC*mec* type II (ST5-MRSA-II) was found to be prevalent in South Korea and Japan, while ST239-MRSA-III was prevalent in other Asian countries (1, 2, 5, 17). However, since the number of MRSA isolates from three hospitals in China analyzed in the previous studies was very limited, the data do not represent the nationwide distribution of MRSA genotypes in China (1, 5, 17). Therefore, further study of more isolates and isolates from more centers is needed. In this study, a combination of molecular typing methods, including PFGE, *spa* typing, MLST, and SCC*mec* typing, was used to characterize a collection of 702 MRSA isolates from 18

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TABLE 1. Distribution of SCC*mec* types of MRSA in the 14 cities during 2005 and 2006[a]

City	No. (%) of isolates of the following SCC*mec* types recovered:																				
	2005					2006							Between 2005 and 2006								
	Total	I	II	III	IV	NT	Total	I	II	III	IV	NT	Total	I	II	III	IV	NT			
Beijing	197	12 (6.1)	29 (14.7)	147 (74.6)		9 (4.6)	39		1 (2.6)	38 (97.4)			236	12 (5.1)	30 (12.7)	185 (78.4)	0	9 (3.8)			
Changchun[a]	3		1	1	1		12			11		1	15		1	12	1	1			
Dalian	14	1	5	8			16		12	4			30	1 (3.3)	17 (56.7)	12 (40.0)					
Guangzhou	14	1		13			36		3 (8.3)	30 (83.3)	2 (5.6)		50	1 (2.8)	1 (2.0)	43 (86.0)	2 (4.0)	1 (2.0)			
Hangzhou	35	4 (11.4)	3 (8.6)	25 (71.4)		3 (8.6)	23		6 (26.1)	16 (69.6)		1 (4.3)	58	4 (6.9)	3 (6.0)	41 (70.7)		4 (6.9)			
Nanjing	2			2			12		1	11			14		9 (15.5)	13					
Naming	4			3		1	4			4			8		1	7					
Qingdao	7	4		3			23			23 (100)			30	4 (13.3)		26 (86.7)		1			
Shanghai	57		6 (10.5)	50 (87.7)		1 (1.8)	46		11 (23.9)	32 (69.6)		3 (6.5)	103		17 (16.5)	82 (79.6)		4 (3.9)			
Shenyang	28		18 (64.3)	10 (35.7)			26	1 (3.8)	9 (34.7)	15 (57.7)		1 (3.8)	54	1 (1.9)	27 (50.0)	25 (46.2)		1 (1.9)			
Shenzhen	7			5		2	10			10			17			15		2			
Urumchi	7			7			7			6	1		14			13	1				
Wuhan	19		1	17		1	21		1 (4.8)	20 (95.2)			40		2 (5.0)	37 (92.5)		1 (2.5)			
Xi'an	1			1			32		2 (6.3)	29 (90.6)		1 (3.1)	33		2 (6.1)	30 (90.9)		1 (3.0)			

[a] The prevalence of SCC*mec* types was not calculated in cities from which the total number of isolates recovered was less than 20.

teaching hospitals in 14 cities recovered between 2005 and 2006 to show the genetic background of MRSA strains in China.

MATERIALS AND METHODS

Bacterial isolates. A total of 702 nonduplicate MRSA isolates were collected from 18 teaching hospitals in 14 cities from January 2005 to October 2006. The number of isolates from each city is summarized in Table 1, and the study centers are listed in Acknowledgments. The isolates were recovered from several clinical sources, including the respiratory tract (n = 371), blood (n = 93), secretions (n = 44), drainage (n = 40), pus (n = 40), wounds (n = 34), abdominal fluid (n = 15), and other sources (n = 65). Thirty-five isolates were obtained from outpatients, 10 isolates were obtained from emergency departments, 50 isolates were obtained from intensive care units, and the others were obtained from inpatients. The isolates were collected from nationwide surveillance networks organized by the Peking Union Medical College Hospital. Each center was required to send 50 nonrepetitive consecutive isolates of gram-positive cocci to the central laboratory; thus, the number of *S. aureus* isolates may vary among the centers. In vitro susceptibility tests were performed with all *S. aureus* isolates at the central laboratory. All isolates were confirmed to be MRSA by multiplex PCR for the detection of the *mecA* and *femB* genes (18). CA-MRSA was defined as described by Klevens et al. (16).

Antimicrobial susceptibility testing. Antimicrobial susceptibility profiles were determined by the agar dilution method on Mueller-Hinton agar, according to the guidelines of Clinical and Laboratory Standards Institute (formerly the NCCLS) (22). The antimicrobial agents tested included oxacillin (Sigma Chemical Co., St Louis, Mo.), clindamycin (Sigma), ciprofloxacin (Bayer AG, Leverkusen, Germany), chloramphenicol (Sigma), erythromycin (Sigma), gentamicin (Sigma), rifampin (rifampicin; Sigma), tetracycline (Sigma), trimethoprim-sulfamethoxazole (Sigma), and vancomycin (Sigma). Clinical and Laboratory Standards Institute breakpoints were used for MIC interpretation (6). *S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as quality controls in each set of tests.

DNA extraction. DNA was extracted as described by Unal et al. (28). The DNA was used as the template in all PCRs described below.

Molecular typing methods. All 702 isolates were analyzed by SCC*mec* typing and Panton-Valentine leukocidin gene (*pvl*) gene detection. PFGE and *spa* typing were used to characterize 395 isolates in 2005, and in 2006, only *spa* typing was used for 307 isolates. One hundred two isolates were selected as representatives of each PFGE and *spa* type for MLST typing. To be specific, isolates that had the same PFGE pattern or *spa* type but different antibiotic resistance profiles or SCC*mec* types were chosen for MLST typing.

(i) SCC*mec* typing. The SCC*mec* types were determined by a multiplex PCR strategy, as described previously (23). Nontypeable (NT) types were defined as isolates showing unexpected fragments or lacking some fragments by multiplex PCR. International clones of SCC*mec* types I to V were used as quality controls.

(ii) Detection of *pvl*. *pvl* was detected by PCR, as described previously (20). The identity of the PCR products was confirmed by sequencing with an ABI 3700 sequencer.

(iii) PFGE. DNA extraction and SmaI restriction were performed as described previously (14). A bacteriophage lambda DNA PFGE molecular size standard was included in each gel. The PFGE patterns were examined visually and were interpreted according to the criteria of Tenover et al. (27).

(iv) *spa* typing. *spa* typing was performed as described previously (12, 19). Purified *spa* PCR products were sequenced, and short sequence repeats were assigned by using the *spa* database website (http://www.ridom.de/spaserver). The *spa* complex was defined by visual analysis, whereby *spa* types with similar short sequence repeats were clustered into the complexes previously described by Ruppitsch et al. (25).

(v) MLST and data analysis. MLST was performed as described previously (8). PCR fragments were purified and sequenced with an ABI 3700 sequencer. The sequences of the PCR products were compared with the existing sequences available on the MLST website (http://*saureus*.mlst.net) for *S. aureus*, and the allelic number was determined for each sequence. Clustering of related STs, which were defined as clonal complexes (CCs), was determined by using the program eBURST (based upon related sequence types) (9).

(vi) Collection of clinical data. The medical records of all patients from Peking Union Medical College Hospital (n = 198) and those of patients with *pvl*-positive isolates (n = 16) and SCC*mec* type IVa isolates (n = 4) were reviewed by physicians. The following variables were collected: patient demographics (gender and age); ward transfer; underlying diseases; the length of time after admission

TABLE 2. PFGE types of MRSA isolates recovered in China in 2005 and their respective antibiotic resistance profiles

PFGE type (no. of isolates)	Predominant antibiotic resistance profile (no. of isolates)[a]	No. of isolates of the following SCC*mec* type:					*spa* type	MLST allele no. (*arc-aro-glp-gmk-pta-tpi-yqi*)	ST	CC	Distribution by city (no. of cases)[b]
		I	II	III	IV	NT					
A (195)	TEDGRCi (116)	15	6	166		8	t030	2-3-1-1-4-4-3	239	8	BJ (141), CC (1), GZ (2), HZ (16), NJ (2), NN (1), SH (4), SY (7), SZ (1), Ur (5), WH (14), XA (1)
B (3)	TEDGRCi (2)			2		1	t030	2-3-1-1-4-4-3	239	8	BJ (3)
C (2)	EDGHSCi (2)			2			t030	2-3-1-1-4-4-3	239	8	BJ (2)
D (2)	TEDGRCi (2)			1		1	t030	2-3-1-1-4-4-3	239	8	BJ (2)
E (2)	EDGRSCi (1)			2			t030	2-3-1-1-4-4-3	239	8	BJ (2)
F (93)	TEDGH(S)Ci (70)	1	1	90		1	t037	2-3-1-1-4-4-3	239	8	DL (10), GZ (11), HZ (15), NN (2), QD (1), SH (48), SZ (4), WH (2)
G (3)	TEDGSHCi (2)			2		1	t037	2-3-1-1-4-4-3	239	8	BJ (3)
H (7)	TEDGCi (6)	1		6			t1152	2-3-1-1-4-4-3	239	8	BJ (7)
I (1)	TEDGCi (1)					1	t1152	2-3-1-1-4-4-3	239	8	SZ (1)
J (2)	TEDGRSCi (1)			2			t1152	2-3-1-1-4-4-3	239	8	SY (2)
K (2)	E (2)			2			t803	2-3-1-1-4-4-3	239	8	SY (2)
L (39)	TEDGCi (33)	1	27	10		1	t002	1-4-1-4-12-1-10	5	5	BJ (30), GZ (1), HZ (3), SH (3), Ur (1), WH (1)
M (23)	TEDGCi (21)		20	2		1	t002	1-4-1-4-12-1-10	5	5	DL (4), NN (1), SY (17), WH (1)
N (3)	TEDGHCi (3)		2	1			t002	1-4-1-4-12-1-10	5	5	BJ (2), Ur (1)
O (2)	TEDCi (2)		2				t002	1-4-1-4-12-1-10	5	5	SH(2)
P (1)	TEDGCi (1)		1				t002	1-4-1-4-12-1-10	5	5	BJ (1)
Q (1)	EDCi (1)					1	t002	1-4-1-4-12-1-10	5	5	SZ (1)
R (1)	TEDCi (1)		1				t002	1-4-1-4-12-1-10	5	5	WH (1)
S (1)	Ci (1)		1				t002	1-4-1-4-12-1-10	5	5	CC (1)
T (1)	Ci (1)		1				t002	1-4-1-4-12-1-10	5	5	BJ (1)
U (6)	TEDCi (4)	4		2			t189	3-1-1-8-1-1-1	188	1	HZ (1), QD (5)
V (3)	TEDGRCi (3)			2		1	t163	19-23-15-2-19-20-15	59	59	BJ (2), QD (1)
W (1)	TEDCi (1)				1		t3155	22-1-14-23-12-4-31	88	88	CC (1)
X (1)	ECi (1)		1				t318	2-2-2-2-6-3-2	30	30	BJ (1)

[a] T, tetracycline; E, erythromycin; D, clindamycin; G, gentamicin; H, chloramphenicol; R, rifampin; S, trimethoprim-sulfamethoxazole; Ci, ciprofloxacin; V, vancomycin. Use of parentheses around an abbreviation indicates that not all isolates were resistant to a particular antibiotic.

[b] BJ, Beijing; CC, Changchun; DL, Dalian; GZ, Guangzhou; HZ, Hangzhou; NJ, Nanjing; NN, Nanning; QD, Qingdao; SH, Shanghai; SY, Shenyang; SZ, Shenzhen; Ur, Urumchi; WH, Wuhan; XA, Xi'an.

that a sample for culture was obtained; the presence of an invasive device (e.g., a vascular catheter or a gastric feeding tube) at the time of admission; and a history of MRSA infection or colonization, surgery, hospitalization, dialysis, or residence in a long-term care facility in the 12 months preceding the culture.

RESULTS

SCC*mec* types. All of the 702 MRSA isolates were analyzed by SCC*mec* typing. Overall, four SCC*mec* types, namely, types I, II, III, and IVa, were found. The most common SCC*mec* type was type III, which found in 541 isolates (541/702; 77.1%) and which existed in all cities, especially Wuhan (92.5%) and Xi'an (90.9%). SCC*mec* type II was found to be the second most predominant type, which accounted for 109 (109/702; 15.5%) of all isolates and which existed in 10 cities. SCC*mec* type II was identified to be prevalent in Dalian (56.7%) and Shenyang (50.0%). Twenty-three of 702 (3.3%) isolates belonged to SCC*mec* type I distributed in six cities; they belonged to three STs: ST239, ST5, or ST188. SCC*mec* type IVa was found in four isolates, and isolates of SCC*mec* type IVa belonged to two STs: ST59 or ST88. A total of 25 isolates (25/702; 3.6%) were NT by the multiplex SCC*mec* typing method.

The change in the distribution of SCC*mec* types between 2005 and 2006 is summarized in Table 1. The prevalence of SCC*mec* type II increased in Dalian, Shanghai, and Hangzhou and decreased in Shenyang and Beijing. On the contrary, the prevalence of SCC*mec* type III correspondingly decreased or increased in these cities during the two study periods.

Antibiotic resistance profiles and PFGE types. The PFGE types of the MRSA isolates recovered in 2005 and their respective antibiotic resistance profiles are listed in Table 2. Overall, 24 PFGE types and 42 subtypes were obtained. Four PFGE types (types A, F, L, and M) were found to be the predominant types, constituting 49.4% (195/395), 23.5% (93/395), 9.9% (39/395), and 5.8% (23/395) of all isolates, respectively. PFGE type A existed in 12 cities, type F existed in 8 cities, and type L existed in 6 cities, while type M existed in 4 cities.

The PFGE types were generally associated with unique antibiotic resistance profiles, and these are illustrated in Table 2. PFGE type H, I, L, M, and P isolates had the same antibiotic resistance profile of resistance to tetracycline, erythromycin, clindamycin, gentamicin, and ciprofloxacin (the TEDGCi resistance profile). PFGE type A, B, D, and V isolates were additionally resistant to rifampin, while PFGE type F and G isolates were additionally resistant to chloramphenicol and trimethoprim-sulfamethoxazole but were susceptible to rifampin. PFGE type O, R, U, and W isolates had the same resistance profile as type C isolates but were susceptible to gentamicin. PFGE type K, S, T, and X isolates were resistant only to erythromycin or ciprofloxacin, or both. All isolates

TABLE 3. Molecular characteristics of representative *spa* types of MRSA isolates recovered in China in 2006 and their respective antibiotic resistance profiles

spa type (no. of isolates)	Predominant antibiotic resistance profile (no. of isolates)[a]	Distribution by city (no. of cases)[b]	No. of isolates with the following SCC*mec* type:					MLST allele no. (*arc-aro-glp-gmk-pta-tpi-yqi*)	MLST-CC
			I	II	III	IV	NT		
t002 (40)	TEDGCi (29)	BJ (1), DL (12), GZ (3), HZ (3), SH (11), SY (7), WH (1), XA (2)		40				1-4-1-4-12-1-10	ST5-CC5
t030 (161)	TEDGRCi (125)	BJ (31), CC (11), DL (3), GZ (17), HZ (11), NJ (6), NN (1), QD (19), SH (6), SY (14), SZ (1), WH (19), XA (22)	1		158		2	2-3-1-1-4-4-3	ST239-CC8
t037 (84)	TEDGHSCi (68)	BJ (3), GZ (11), HZ (6), NJ (5), NN (3), QD (3), SH (29), SZ (9), Ur (6), WH (1), XA (8)			80		4	2-3-1-1-4-4-3	ST239-CC8
t377 (1)	T (1)	NJ (1)		1				2-3-1-1-4-4-3	ST239-CC8
t437 (4)	TEDCi (2)	GZ (3), Ur (1)			2	2		19-23-15-2-19-20-15	ST59-CC59
t459 (1)	TEDGRSCi (1)	QD (1)			1			2-3-1-1-4-4-3	ST239-CC8
t570 (2)	TEDGCi (2)	SY (2)		2				1-4-1-4-12-1-10	ST5-CC5
t601 (3)	TEDGCi (3)	HZ (3)		3				1-4-1-4-12-1-10	ST5-CC5
t632 (7)	TEDGRCi (5)	BJ (4), SY (3)			7			2-3-1-1-4-4-3	ST239-CC8
t796 (1)	TEDG (1)	CC (1)					1	5-4-1-4-4-6-3	ST7-CC7
t899 (1)	TEDGRSCi (1)	GZ (1)					1	3-3-1-1-1-1-10	ST9-CC9
t1152 (1)	TEDGRCi (1)	DL (1)			1			2-3-1-1-4-4-3	ST239-CC8
t2649 (1)	E (1)	GZ (1)				1		22-1-14-23-12-4-31	ST88-CC88

[a] T, tetracycline; E, erythromycin; D, clindamycin; G, gentamicin; H, chloramphenicol; R, rifampin, S, trimethoprim-sulfamethoxazole; Ci, ciprofloxacin; V, vancomycin.

[b] BJ, Beijing; CC, Changchun; DL, Dalian; GZ, Guangzhou; HZ, Hangzhou; NJ, Nanjing; NN, Nanning; QD, Qingdao; SH, Shanghai; SY, Shenyang; SZ, Shenzhen; Ur, Urumchi; WH, Wuhan; XA, Xi'an.

tested were susceptible to vancomycin, but a shift in the MIC was identified. In 2005, the vancomycin MIC_{50} was 0.5 mg/liter, while in 2006, the vancomycin MIC_{50} increased to 1 mg/liter.

***spa* types.** Typing of all isolates yielded 18 *spa* types (Table 3). The most predominant *spa* type was t030, which contained PFEG types A to E, constituted 52.0% (365/702) of all isolates, and was present in all of the cities. The second common *spa* type was t037, which contained PFGE types F and G. *spa* type t037 accounted for 25.5% (179/702) of all isolates and was identified in 12 cities. *spa* type t002 was the third most prevalent *spa* type and included PFGE types L to T, made up 16.0% (112/702) of all isolates, and was detected in 12 cities. Similar to the antibiotic resistance profiles of the PFGE types, *spa* type t002, t570, and t601 isolates showed the same TEDGCi antibiotic resistance profile; and *spa* type t030, t632, and t1152 isolates presented additional resistance to rifampin. *spa* type t037 isolates had a profile of resistance to tetracycline, erythromycin, clindamycin, gentamicin, chloramphenicol, trimethoprim-sulfamethoxazole, and ciprofloxacin, while *spa* type t437 isolates were susceptible to gentamicin and had a profile of resistance to tetracycline, erythromycin, clindamycin, and ciprofloxacin.

The distribution of *spa* types varied among the cities (Table 4). *spa* type t002 was the most common *spa* type in Dalian (53.4%) and Shenyang (44.4%); *spa* type t037 was predominant in Shanghai (74.8%), Shenzhen, and Nanning; in the other cities, *spa* type t030 was the most common type. The second predominant *spa* type also showed different patterns of prevalence among the cities. The second most common *spa* type in Shanghai and Beijing was t002; that in Dalian, Hangzhou, and Xi'an was t037; and that in Shenyang and Guangzhou was t030.

MLST analysis. One hundred two isolates (53 isolates recovered in 2005, 49 isolates recovered in 2006) were selected for MLST analysis. The STs of representatives of the PFGE types or the *spa* types are presented in Table 2 and Table 3. Overall, eight STs were found among the 102 isolates. Clustering analysis by use of the eBURST program showed that these STs belonged to eight CCs. The most dominant ST was ST239, which belonged to CC239, constituted 80.8% (567/702) of all isolates, and existed in all cities. CC239 is a distinct branch within CC8. ST239 included PFGE types A to K and *spa* types t030, t037, t377, t459, t632, t803, and t1152. ST5, which belonged to CC5, was found to be the second common ST (117/702; 16.7%) and included PFGE types L to T and *spa* types t002, t570, and t601. The distribution of STs also exhibited different patterns among the cities. ST5 was the most

TABLE 4. Distribution of *spa* types by city in China[a]

City	Total no. of isolates	% (no.) of isolates of the following *spa* type:			
		t030	t037	t002	Other
Beijing	236	76.7 (181)	2.5 (6)	14.9 (35)	5.9 (14)
Changchun	15	− (12)		− (1)	− (2)
Dalian	30	10.0 (3)	33.3 (10)	53.4 (16)	3.3 (1)
Guangzhou	50	38.0 (19)	44.0 (22)	8.0 (4)	10 (5)
Hangzhou	58	46.6 (27)	34.5 (20)	10.3 (6)	8.6 (5)
Nanjing	14	− (8)	− (5)		− (1)
Nanning	8	− (2)	− (5)	− (1)	
Qingdao	30	63.4 (19)	13.3 (4)		23.3 (7)
Shanghai	103	9.7 (10)	74.8 (77)	15.5 (16)	
Shenyang	54	38.9 (21)		44.4 (24)	16.7 (9)
Shenzhen	17	− (2)	− (13)	− (1)	− (1)
Urumchi	14	− (5)	− (6)	− (2)	− (1)
Wuhan	40	82.5 (33)	7.5 (3)	10.0 (4)	
Xi'an	33	69.7 (23)	24.2 (8)	6.1 (2)	

[a] The prevalence of *spa* types was not calculated in cities where the total number of isolates was less than 20.

TABLE 5. Characteristics of Panton-Valentine leukocidin-positive MRSA isolates

Isolate no.[a]	Patient age (yr)	Specimen type	Antibiogram[b]									SCC*mec* type	*spa* type	MLST type	CC
			CIP	ERY	CLI	GEN	TET	RIF	CHL	SXT	VAN				
BJ043	69	Abdominal drainage	R	R	R	R	R	R	S	S	S	II	t030	ST239	8
BJ068	33	Sputum	R	R	R	S	R	R	S	R	S	III	t030	ST239	8
BJ078	80	Sputum	R	R	S	R	R	R	S	S	S	III	t030	ST239	8
BJ082	86	Bile	R	R	R	R	R	R	S	S	S	III	t030	ST239	8
BJ085	89	Sputum	R	R	R	R	R	R	S	S	S	III	t030	ST239	8
BJ088	97	Sputum	R	R	R	R	R	R	S	S	S	III	t030	ST239	8
BJ210	58	Sputum	R	R	R	S	R	R	S	S	S	II	t002	ST5	5
BJ249	94	Sputum	R	R	R	R	R	R	S	S	S	III	t030	ST239	8
BJ250	40	Sputum	R	R	R	R	R	R	S	S	S	III	t030	ST239	8
BJ274	80	Sputum	R	R	R	R	R	R	S	S	S	III	t030	ST239	8
BJ363	74	Secretion	R	R	R	R	R	R	S	S	S	III	t030	ST239	8
BJ372	40	Sputum	R	R	R	R	R	R	S	S	S	III	t030	ST239	8
BJ390	75	Secretion	R	R	R	R	R	R	S	S	S	III	t030	ST239	8
CC023	18	Sputum	R	R	R	R	R	S	S	S	S	IV	t3155	ST88	88
DL010	76	Sputum	R	R	R	R	R	S	S	S	S	II	t002	ST5	5
SZ009	45	Pus	R	R	R	R	R	S	S	R	S	III	t037	ST239	8

[a] BJ, Beijing; CC, Changchun; DL, Dalian; SZ, Shenzhen. All isolates were HA-MRSA.
[b] CIP, ciprofloxacin; ERY, erythromycin; CLI, clindamycin; GEN, gentamicin; TET, tetracycline; RIF, rifampin, CHL, chloramphenicol; SXT, trimethoprim-sulfamethoxazole; VAN, vancomycin; R, resistant; S, susceptible.

commonly identified ST in Dalian (16/30; 53.3%) and Shenyang (26/54; 48.1%), whereas ST239 was the most common ST identified in the other cities.

Prevalence of *pvl* gene. All the isolates were analyzed for the detection of the *pvl* gene; only 16 isolates were *pvl* positive (16/702, 2.3%). All 16 of these isolates were considered to be hospital-acquired MRSA (HA-MRSA), according to the information in the patients' medical records (Table 5). In total, the most common specimen type was sputum (11/17; 68.8%). Four *spa* types and three STs were found. The most prevalent clone was ST239 and SCC*mec* type III; it was *spa* type t030 and had the TEDGRCi antibiotic resistance profile.

Detection of CA-MRSA. Two isolates from Guangzhou without the *pvl* gene were confirmed to be CA-MRSA from the detailed clinical information. One isolate, which was resistant only to erythromycin, was characterized as ST59-MRSA-IVa and *spa* type t437, while the other isolate, which had a profile of resistance to erythromycin, clindamycin, and ciprofloxacin, belonged to ST88, harbored SCC*mec* type IVa, and had *spa* type t2649. However, one isolate from Changchun that had the genotype of ST88-MRSA-IVa and the *pvl* gene was confirmed from the clinical information to be a HA-MRSA isolate. Another isolate from Urumchi could not be identified as CA-MRSA because of a lack of adequate clinical data.

DISCUSSION

Elucidation of the mechanism of the geographic spread of MRSA has been greatly facilitated by the techniques of molecular epidemiology. Both *spa* typing and PFGE were performed in 2005; however, the previous study revealed that *spa* typing possessed significant advantages over PFGE in terms of speed, ease of interpretation, and exportability and was a reliable method for long-term, nationwide epidemiological surveillance studies (11), so we conducted *spa* typing instead of PFGE in 2006.

Previous studies have demonstrated that most HA-MRSA infections are due to a relatively small number of epidemic MRSA clones (7, 10). In Asia, the most popular clones are Brazil/Hungary (ST239-III) and New York/Japan (ST5-II) (1, 2, 5, 15, 17). The data from the present study confirm that these two clones with different *spa* types are spreading across China and have unique geographic distributions.

The most common clone found was ST239, which existed in all cities covering most areas in China, from Urumchi in the northwest to Shenzhen in the southeast. This clone included isolates with seven *spa* types, belonging to SCC*mec* type III with ST239, and had a profile of resistance to multiple drugs: tetracycline, erythromycin, clindamycin, gentamicin, and ciprofloxacin, with some isolates also being resistant to chloramphenicol, rifampin, and trimethoprim-sulfamethoxazole. In previous studies, data revealed that some isolates from mainland China belonged to this clone and were multidrug resistant (resistant to tetracycline, erythromycin, clindamycin, gentamicin, chloramphenicol, and ciprofloxacin) (1, 5, 17). The ST239-III group, which belongs to CC239 and which represents a distinct branch within CC8 in the evolutionary model of the emergence of MRSA (24), has been reported to be widespread in most areas in Asia except Japan and South Korea and in many countries worldwide (1, 2, 5, 17). This clone was also the most dominant one in Hong Kong (15), whereas ST241 (a single-locus variant of ST239) with SCC*mec* type III was prevalent in Taiwan (1).

The second most predominant clone was ST5, which belonged to CC5. This clone existed in 12 cities and was also recovered from most areas across China, from Jilin in the north to Shenzhen in the south. This clone had three *spa* types, possessed SCC*mec* type II, and showed a TEDGCi multidrug resistance profile. This clone was common in Dalian, Shenyang, Shanghai, Beijing, and Hangzhou (it made up >10% of the isolates in each city). It was suggested that these isolates fell in the ST5-II group, which was derived from the same ancestor as the New York/Japan clone within CC5 (24). MRSA isolates of ST5 have spread widely in many countries worldwide and are the predominant clone in South Korea and

Japan (5, 17). Interestingly, this clone was also found to be the second predominant one in Hong Kong (15).

CA-MRSA was most commonly implicated in skin and soft tissue infections and carried SCC*mec* type IV or V, as well as the *pvl* gene, in the majority of cases (20, 26, 29, 31). In this study, from the review of the patients' medical records, we identified two isolates that were CA-MRSA. Both of them carried SCC*mec* type IVa and were from patients with skin infections, but they did not possess the *pvl* gene. It has been reported that CA-MRSA strains may spread in the hospital and cause nosocomial infections (26), but in this study, based on 18 hospitals across China, we did not identify this phenomenon.

In this study, 16 isolates harbored the *pvl* gene and belonged to three STs: ST239, ST5, and ST88. The clinical data demonstrated that all these isolates were obtained from patients with hospital-associated infections. Among these isolates, ST239 was the most predominant clone. These data may indicate that the sporadic Panton-Valentine leukocidin-positive isolates classified as HA-MRSA exist in Chinese hospitals but in a low proportion. Nevertheless, the risk of the emergence of Panton-Valentine leukocidin-positive HA-MRSA clones is an issue of concern and could result in the emergence of multidrug-resistant HA-MRSA isolates with increased virulence. Further investigation of the prevalence of carriage of *pvl* among CA-MRSA isolates is necessary.

There may be some limitations to this study. As it was a retrospective study, the medical records from emergency department patients and outpatients and from other hospitals was hard to obtain, and some of the data obtained may not have been able to provide enough information to entitle the isolates to be classified as CA-MRSA, so this study is just the tip of the iceberg on the prevalence of CA-MRSA. Because there are few studies on the prevalence of CA-MRSA in mainland China, a prospective study that includes accurate clinical data and more isolates and that uses the techniques of molecular epidemiology is needed.

In summary, our study documented that two major pandemic MRSA clones (ST239 and ST5) have spread across China and have unique geographic distributions. Further study of CA-MRSA isolates should be conducted to elucidate the current status of CA-MRSA in China.

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REFERENCES

1. **Aires de Sousa, M., M. I. Crisóstomo, I. Santos Sanches, J. S. Wu, J. Fuzhong, A. Tomasz, and H. de Lencastre.** 2003. Frequent recovery of a single clonal type of multidrug-resistant *Staphylococcus aureus* from patients in two hospitals in Taiwan and China. J. Clin. Microbiol. **41:**159–163.
2. **Arakere, G., S. Nadig, G. Swedberg, R. Macaden, S. K. Amarnath, and D. Raghunath.** 2005. Genotyping of methicillin-resistant *Staphylococcus aureus* strains from two hospitals in Bangalore, South India. J. Clin. Microbiol. **43:**3198–3202.
3. **Bert, F., J. Clarissou, F. Durand, D. Delefosse, C. Chauvet, P. Lefebvre, N. Lambert, and C. Branger.** 2003. Prevalence, molecular epidemiology, and clinical significance of heterogeneous glycopeptide-intermediate *Staphylococcus aureus* in liver transplant recipients. J. Clin. Microbiol. **41:**5147–5152.
4. **Blanc, D. S., M. J. Struelens, A. Deplano, R. de Ryck, P. M. Hauser, C. Petignat, and P. Francioli.** 2001. Epidemiological validation of pulsed-field gel electrophoresis patterns for methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **39:**3442–3445.
5. **Chongtrakool, P., T. Ito, X. X. Ma, Y. Kondo, S. Trakulsomboon, C. Tiensasitorn, M. Jamklang, T. Chavalit, J. H. Song, and K. Hiramatsu.** 2006. Staphylococcal cassette chromosome *mec* (SCC*mec*) typing of methicillin-resistant *Staphylococcus aureus* strains isolated in 11 Asian countries: a proposal for a new nomenclature for SCC*mec* elements. Antimicrob. Agents Chemother. **50:**1001–1012.
6. **Clinical and Laboratory Standards Institute.** Performance standards for antimicrobial susceptibility testing: 16th informational supplement. M100-S16. Clinical and Laboratory Standards Institute, Wayne, PA.
7. **Enright, M. C., D. A. Robinson, G. Randle, E. J. Feil, H. Grundmann, and B. G. Spratt.** 2002. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc. Natl. Acad. Sci. USA **99:**7687–7692.
8. **Enright, M. C., N. P. Day, C. E. Davies, S. J. Peacock, and B. Spratt.** 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J. Clin. Microbiol. **38:**1008–1015.
9. **Feil, E. J., B. C. Li, D. M. Aanensen, W. P. Hanage, and B. G. Spratt.** 2004. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. J. Bacteriol. **186:**1518–1530.
10. **Gomes, A. R., H. Westh, and H. de Lencastre.** 2006. Origins and evolution of methicillin-resistant *Staphylococcus aureus* clonal lineages. Antimicrob. Agents Chemother. **50:**3237–3244.
11. **Hallin, M., A. Deplano, O. Denis, R. De Mendonca, R. De Ryck, and M. J. Struelens.** 2007. Validation of pulsed-field gel electrophoresis and *spa* typing for long-term, nationwide epidemiological surveillance studies of *Staphylococcus aureus* infections. J. Clin. Microbiol. **45:**127–133.
12. **Harmsen, D., H. Claus, W. Witte, J. Rothgänger, H. Claus, D. Turnwald, and U. Vogel.** 2003. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. J. Clin. Microbiol. **41:**5442–5448.
13. **Ito, T., Y. Katayama, and K. Hiramatsu.** 1999. Cloning and nucleotide sequence determination of the entire *mec* DNA of pre-methicillin-resistant *Staphylococcus aureus* N315. Antimicrob. Agents Chemother. **43:**1449–1458.
14. **Ip, M., D. J. Lyon, F. Chio, M. C. Enright, and A. F. Cheng.** 2003. Characterization of isolates of methicillin-resistant *Staphylococcus aureus* from Hong Kong by phage typing, pulsed-field gel electrophoresis, and fluorescent amplified-fragment length polymorphism analysis. J. Clin. Microbiol. **41:**4980–4985.
15. **Ip, M., R. W. H. Yung, T. K. Ng, W. K. Luk, C. Tse, P. Hung, M. Enright, and D. J. Lyon.** 2005. Contemporary methicillin-resistant *Staphylococcus aureus* clones in Hong Kong. J. Clin. Microbiol. **43:**5069–5073.
16. **Klevens, R. M., M. A. Morrison, J. Nadle, S. Petit., K. Gershman, S. Ray, L. H. Harrison, R. Lynfield, G. Dumyati, J. M. Townes, A. S. Craig, E. R. Zell, G. E. Fosheim, L. K. McDougal, R. B. Carey, S. K. Fridkin, and Active Bacterial Core Surveillance (ABCs) MRSA Investigators.** 2007. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA **298:**1763–1771.
17. **Ko, K. S., J. Y. Lee, J. Y. Suh, W. S. Oh, K. R. Peck, N. Y. Lee, and J. H. Song.** 2005. Distribution of major genotypes among methicillin-resistant *Staphylococcus aureus* clones in Asian countries. J. Clin. Microbiol. **43:**421–426.
18. **Kobayashi, N., H. Wu, K. Kojima, K. Taniguchi, S. Urasawa, N. Uehara, Y. Omizu, Y. Kishi, A. Yagihashi, and I. Kurokawa.** 1994. Detection of *mecA*, *femA*, and *femB* genes in clinical strains of staphylococci using polymerase chain reaction. Epidemiol. Infect. **113:**259–266.
19. **Koreen, L., S. V. Ramaswamy, E. A. Graviss, S. Naidich, J. M. Musser, and B. N. Kreiswirth.** 2004. *spa* typing method for discriminating among *Staphylococcus aureus* isolates: implications for use of a single marker to detect genetic micro- and macrovariation. J. Clin. Microbiol. **42:**792–799.
20. **Lina, G., Y. Piemont, F. Godail-Gamot, M. Bes, M.-O. Peter, V. Gauduchon, F. Vandenesch, and J. Etienne.** 1999. Involvement of Panton-Valentine leu-

kocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin. Infect. Dis. **29:**1128–1132.
21. **Milheirico, C., D. C. Oliveira, and H. de Lencastre.** 2007. Update to the multiplex PCR strategy for assignment of *mec* element types in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **51:**3374–3377.
22. **National Committee for Clinical Laboratory Standards.** 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 6th ed. Approved standard. NCCLS publication no. M7-A6. National Committee for Clinical Laboratory Standards, Wayne, PA.
23. **Oliveira, D. C., C. Milheirico, and H. De Lencastre.** 2006. Redefining a structural variant of staphylococcal cassette chromosome *mec*, SCC*mec* type VI. Antimicrob. Agents Chemother. **50:**3457–3459.
24. **Robinson, D. A., and M. C. Enright.** 2003. Evolutionary models of the emergence of methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **47:**3926–3934.
25. **Ruppitsch, W., A. Indra, A. Stöger, B. Mayer, S. Stadlbauer, G. Wewalka, and F. Allerberger.** 2006. Classifying *spa* types in complexes improves interpretation of typing results for methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **44:**2442–2448.
26. **Seybold, U., E. V. Kourbatova, J. G. Johnson, S. J. Halvosa, Y. F. Wang, M. D. King, S. M. Ray, and H. M. Blumberg.** 2006. Emergence of community-associated methicillin-resistant *Staphylococcus aureus* USA300 genotype as a major cause of health care-associated blood stream infections. Clin. Infect. Dis. **42:**647–656.
27. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
28. **Unal, S., J. Hoskins, J. E. Flokowitsch, C. Y. Wu, D. A. Preston, and P. L. Skatrud.** 1992. Detection of methicillin-resistant staphylococci by using the polymerase chain reaction. J. Clin. Microbiol. **30:**1685–1691.
29. **Vandenesch, F., T. Naimi, M. C. Enright, G. Lina, G. R. Nimmo, H. Heffernan, N. Liassine, M. Bes, T. Greenland, M. E. Reverdy, and J. Etienne.** 2003. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg. Infect. Dis. **9:**978–984.
30. **Wang, H., Y. Liu, H. Sun, Y. Xu, X. Xie, and M. Chen.** 2008. In vitro activity of ceftobiprole, linezolid, tigecycline, and 23 other antimicrobial agents against *Staphylococcus aureus* isolates in China. Diagn. Microbiol. Infect. Dis. **62:**226–229.
31. **Yu, F., Z. Chen, C. Liu, X. Zhang, X. Lin, S. Chi, T. Zhou, Z. Chen, and X. Chen.** 2008. Prevalence of *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes among isolates from hospitalized patients in China. Clin. Microbiol. Infect. **14:**381–384.