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ORIGINAL ARTICLE

Molecular characteristics and virulence factors in methicillin-susceptible, resistant, and heterogeneous vancomycin-intermediate *Staphylococcus aureus* from central-southern China

Cailin Liu [a], Zhong-ju Chen [a], Ziyong Sun [a,*], Xianju Feng [b], Mingxiang Zou [c], Wei Cao [d], Shanmei Wang [e], Ji Zeng [f], Yue Wang [a], Mingyue Sun [a]

[a] *Department of Laboratory, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China*
[b] *Department of Laboratory, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China*
[c] *Department of Laboratory, Xiangya Hospital of Central South University, Changsha, China*
[d] *Department of Laboratory, The Second Xiangya Hospital of Central South University, Changsha, China*
[e] *Department of Laboratory, Henan Province People's Hospital, Zhengzhou, China*
[f] *Department of Laboratory, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China*

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Heterogeneous vancomycin-intermediate *Staphylococcus aureus*;
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Molecular;

Background: *Staphylococcus aureus* is a leading cause of nosocomial infections. The purpose of this study was to evaluate the prevalence of methicillin-resistant *S. aureus* (MRSA) and heterogeneous vancomycin-intermediate *S. aureus* (hVISA), and compare the antimicrobial susceptibility, molecular characteristic, and virulence factors in methicillin-susceptible *S. aureus* (MSSA), MRSA, and hVISA from central-southern China.
Methods: A total of 184 *S. aureus* were isolated from sterile body fluids. All isolates were subjected to population analysis profiling for the identification of hVISA phenotype and polymerase chain reaction analysis for genotyping and 31 virulence genes.
Results: The prevalence of MRSA isolates was 41.8% in central-southern China. Of 77 MRSA isolates, 17 (22.1%) were identified as hVISA. The most common MRSA and MSSA clones were

* Corresponding author. Department of Laboratory, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 430030 Wuhan, China.
E-mail address: tjszyong@163.com (ZY. Sun).

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Staphylococcus aureus;
Virulence gene

ST239-MRSA-*SCCmec*III-t030-*agr*-I (55.8%) and ST188-MSSA-t189-*agr*-I (20.6%), respectively. The frequency of carriage of *pvl*, hemolysins, *tst*, and staphylococcal enterotoxin genes among MSSA isolates was significantly higher than that for MRSA isolates ($p < 0.05$); 98 MSSA isolates (53.3%) carried ≥10 tested virulence genes simultaneously, which was significantly higher than that of MRSA isolates (33.8%; $p = 0.004$). The 17 hVISA isolates carried a significantly small number of virulence genes; only two hVISA isolates carried ≥10 tested virulence genes simultaneously, and two hVISA isolates harbored only four virulence genes. Compared with other clonal complexes (CCs), CC1 and CC398 isolates harbored a higher frequency of exfoliatin genes, CC1 and CC59 harbored a higher frequency of *pvl* gene, and only CC1 isolates harbored *lukED*.

Conclusion: The prevalence of hVISA was considerably high in central-southern China. Simultaneous carriage of multiple virulence genes was common in *S. aureus* isolates; the virulence genes were more diverse and frequent among MSSA isolates than among MRSA isolates. Furthermore, the distribution of some virulence genes was correlated with the different *S. aureus* CCs.

Introduction

Since Alexander Ogston first isolated *Staphylococcus aureus* from a surgical abscess in 1880 and described its role in localized infection and septicemia,[1,2] *S. aureus* has been recognized as an important cause of human disease for nearly 130 years. The pathogenesis of *S. aureus* infections is related to the expression of a wide variety of virulence factors, including coagulase, hemolysins (which damage cell membrane of various cells), staphylococcal enterotoxins (SEs; which cause food poisoning), toxic shock syndrome toxin-1 (TSST-1; which increases sensitivity to endotoxin), exfoliative toxins (which are implicated in staphylococcal scalded-skin syndrome), staphylococcal protein A (spa), and Panton—Valentine leukocidin (pvl, which has been preferentially linked to furuncles, cutaneous abscesses, and severe necrotic skin infections).[3—5] Generally, serious *S. aureus* infections are caused by the combined actions of several virulence factors; *S. aureus* strains causing infections have variable combinations of virulence genes.[6,7]

Since its emergence in the 1980s, methicillin-resistant *S. aureus* (MRSA) has become a major cause of nosocomial infections worldwide. Vancomycin, a glycopeptide antibiotic introduced 50 years ago, is regarded as the mainstay of treatment for MRSA infections. Since first reported in Japan in 1997,[8] heterogeneous vancomycin-intermediate *S. aureus* (hVISA) have been detected throughout the world, which has threatened the rank of vancomycin as the first-line antibiotic for MRSA infections. The clinical significance of hVISA has been difficult to assess. It is unknown whether these isolates are fully virulent or just more virulent than vancomycin-susceptible *S. aureus* isolates and whether their levels of resistance are responsible for treatment failure.[9] The prevalence of hVISA varied significantly, which can be attributed to many factors, including differences in laboratory definitions, testing strategies, and regional variability.[10,11]

The prevalence of hVISA among MRSA isolates from China in 2007 was 15.7%[12]; however, the hVISA phenotype was determined by a combination of the following factors: (1) measurement by the modified population analysis profile/area under the curve method (PAP/AUC) and (2) estimation based on the measured sensitivity and specificity of a screening method, which was somewhat limited. The aim of this study was to investigate the prevalence of hVISA and compare the antimicrobial susceptibility, molecular characteristics, and virulence-associated gene carriage in methicillin-susceptible, resistant, and heterogeneous vancomycin-intermediate *S. aureus* from six tertiary teaching hospitals in Hubei, Hunan, and Henan provinces, central-southern China.

Materials and methods

Bacterial isolates

A total of 184 consecutive and nonduplicated *S. aureus* isolates were collected from six tertiary teaching hospitals in central-southern China, from June 2011 to May 2012. The hospitals included Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (57 isolates), the First Affiliated Hospital of Zhengzhou University (45 isolates), Xiangya Hospital of Central South University (39 isolates), the Second Xiangya Hospital of Central South University (24 isolates), Henan Province People's Hospital (12 isolates), and Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (7 isolates). The strains were isolated from blood (141 isolates), cerebrospinal fluid (15 isolates), ascites (11 isolates), pleural effusion (10 isolates), and synovial fluid (7 isolates). All isolates were stored at −80°C until testing.

Antimicrobial susceptibility testing

Identification of *S. aureus* isolates was performed using standard methods and the Vitek 2 compact automated system (bioMérieux, Marcy-l'Étoile, France). Minimum inhibitory concentrations of antibiotics were determined using the agar dilution method, according to the Clinical

and Laboratory Standards Institute recommendations. The reference strain ATCC 29213 was used as a control.

Modified PAP/AUC

PAP/AUC was performed as described by Wootton et al.[13] Following 24 hours of incubation in Tryptone Soya Broth (Oxoid, Basingstoke, England), neat culture and dilutions of 10^{-3} (10^5 CFU/mL) and 10^{-6} (10^2 CFU/mL) were plated onto Brain Heart Infusion agar (Oxoid) plates containing 0.0 mg/L, 0.5 mg/L, 1.0 mg/L, 2.0 mg/L, 2.5 mg/L, 4.0 mg/L, and 8.0 mg/L vancomycin. Colonies were counted after 48 hours. Control strains [ATCC 29213 (methicillin-susceptible *S. aureus*, MSSA), Mu50, and Mu3] were included in each run. Calculated CFU/mL values were plotted against vancomycin concentration using Prism version 5.0 (GraphPad Software, San Diego, CA, USA). The ratio of the AUC of the test isolate to the AUC of Mu3 was calculated and interpreted as follows: for VSSA, a ratio of <0.9; for hVISA, a ratio of 0.9—1.3; and for VISA, a ratio of ≥ 1.3.

DNA isolation

All isolates were cultured on blood agar and incubated overnight at 37°C. Genomic DNA was extracted using the Puregene Yeast/Bact. Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol for Gram-positive bacteria. DNA samples were stored at −20°C until used.

Detection of virulence genes

The *pvl* gene was detected by amplification of the *lukS-PV* and *lukF-PV* genes, as described by Lina et al.[14] *S. aureus* ATCC 49775, harboring *pvl* gene, was used as a control strain. The presence of the virulence gene-encoded hemolysins (*hla*, *hlb*, *hld*, *hlg*, and *hlg* variant), leukotoxins (*lukED* and *lukM*), exfoliatins (*eta*, *etb*, and *etd*), *edin*, toxic shock syndrome toxin (*tst*), and SEs (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, *seo*, *sep*, *seq*, *ser*, and *seu*) were investigated by multiplex polymerase chain reaction (PCR).[6,15]

Molecular typing

Multiplex PCR was used to amplify the *mecA* gene and determine the *SCCmec* type (I—V) of all MRSA isolates, according to the method published by Milheirico et al.[16] Multilocus sequence typing was examined following a previously described method.[17] The accessory gene regulator (*agr*) polymorphism was determined by multiplex PCR using a previously described method.[18] According to the method described by Harmsen et al,[19] *spa* typing was performed, and *spa* types with identical or similar repeat profiles were grouped into clusters (http://www.ridom.de/spaserver).

Statistical analysis

Chi-square test or Fisher's exact test for categorical variables were used to calculate *p* values. A *p* value <0.05 was considered statistically significant. All susceptibility data and molecular test results were analyzed using WHONET 5.6 software (http://www.whonet.org.cn/).

Results

Antimicrobial susceptibility

The prevalence of MRSA isolates was 41.8% (77/184) in central-southern China. A total of 17 hVISA strains were identified by modified PAP/AUC; the percentage of hVISA among MRSA isolates was 22.1% (17/77). The antimicrobial resistance profiles for 184 *S. aureus* isolates are listed in Table 1. All isolates were susceptible to vancomycin, teicoplanin, linezolid, daptomycin, and tigecycline. Significant differences were observed in antimicrobial resistance

Table 1 Antimicrobial resistance rates of 184 *Staphylococcus aureus* isolates

Antimicrobial agents	MSSA (n = 107)	MRSA (n = 77)	p[a]	Non-hVISA (n = 60)	hVISA (n = 17)	p[a]
	R (%)	*R* (%)		*R* (%)	*R* (%)	
Gentamicin	23.4	80.5	<0.001	75.0	100	0.032
Levofloxacin	15.0	80.5	<0.001	75.0	100	0.032
Tobramycin	15.0	89.6	<0.001	86.7	100	0.018
Erythromycin	55.1	89.6	<0.001	86.7	100	0.018
Clindamycin	26.2	74.0	<0.001	70.0	88.2	0.210
Chloramphenicol	7.5	14.3	0.148	10.0	29.4	0.058
Tetracycline	27.1	67.5	<0.001	58.3	100	0.001
Rifampin	12.1	84.4	<0.001	81.7	94.1	0.282
Trimethoprim/sulfamethoxazole	4.7	9.1	0.244	3.3	29.4	0.005
Vancomycin	0	0	—	0	0	—
Teicoplanin	0	0	—	0	0	—
Linezolid	0	0	—	0	0	—
Daptomycin	0	0	—	0	0	—
Tigecycline	0	0	—	0	0	—

[a] Chi-square test or Fisher's exact test.
hVISA = heterogeneous vancomycin-intermediate *S. aureus*; MRSA = methicillin-resistant *S. aureus*; MSSA = methicillin-susceptible *S. aureus*.

to gentamicin, levofloxacin, tobramycin, erythromycin, clindamycin, tetracycline, and rifampin between MRSA and MSSA isolates ($p < 0.05$). Among MRSA isolates, hVISA isolates had significantly higher resistance to gentamicin, levofloxacin, tobramycin, erythromycin, tetracycline, and trimethoprim/sulfamethoxazole than non-hVISA isolates ($p < 0.05$).

Prevalence of virulence genes among S. aureus

Of 184 *S. aureus* isolates, the most frequent toxin genes were hemolysin genes (except *hlg*); all isolates exhibited carriage of at least two hemolysin genes, followed by *lukED* (91.3%), *seg* (42.4%), *sei* (32.6%), and *seq* (31.5%) (Table 2).

The frequency of carriage of *pvl*, *tst*, *hlb*, *hlg*-v, *seb*, *sed*, and *sei* among MSSA isolates was significantly higher than that of MRSA isolates ($p < 0.05$). A total of 98 MSSA isolates (53.3%) carried ≥10 tested virulence genes simultaneously, which was significantly higher than that of MRSA isolates (33.8%, 26/77; $p = 0.004$).

Among MRSA isolates, the frequency of carriage of *hlb*, *hld*, and *seg* in non-hVISA isolates was significantly higher than that of hVISA isolates ($p < 0.05$). Comparing with MSSA isolates and non-hVISA isolates, hVISA isolates carried a significantly small number of virulence genes; only two hVISA isolates carried ≥10 tested virulence genes simultaneously, and two hVISA isolates harbored only four virulence genes.

Molecular characteristics of *S. aureus*

A total of 21 STs and 39 spa types were identified in 184 *S. aureus* isolates. Clustering analysis by eBURST showed that these STs belonged to 13 clonal complexes (CCs; Table 3). The predominant types among MRSA isolates were CC8 (71.4%, 55/77), t030 (55.8%, 43/77), and *SCCmec* type III (85.7%, 64/77). However, the predominant types in the MSSA group were CC1 (33.6%, 36/107) and t189 (20.6%, 22/107).

Overall, ST239-MRSA-*SCCmec*III-t030-*agr*-I was the most common clone (55.8%, 43/77) among MRSA isolates. Among

Table 2 Prevalence of 31 virulence genes among *Staphylococcus aureus* strains isolated from sterile body fluids

Virulence genes	*S. aureus* ($n = 184$) (%)	MSSA ($n = 107$) (%)	MRSA ($n = 77$) (%)	p[a]	Non-hVISA ($n = 60$) (%)	hVISA ($n = 17$) (%)	p[b]
hla	96.7	99.1	93.5	0.084	96.7	82.4	0.068
hlb	88.0	91.6	77.9	0.010	85.0	52.9	0.009
hld	96.7	99.1	93.5	0.084	98.3	76.5	0.008
hlg	11.4	13.1	9.1	0.485	8.3	11.8	1.000
hlg-v	88.6	95.3	79.2	0.001	75.0	88.2	0.332
lukED	91.3	93.5	88.3	0.290	91.7	76.5	0.102
lukM	2.2	3.7	0.0	0.141	0.0	0.0	—
eta	6.5	5.6	7.8	0.540	6.7	11.8	0.608
etb	14.1	16.8	10.4	0.284	11.7	5.9	0.676
etd	4.9	3.7	6.5	0.494	6.7	5.9	1.000
sea	26.1	28.0	23.4	0.501	23.3	23.5	1.000
seb	25.0	33.6	13.0	0.002	15.0	5.9	0.443
sec	6.0	4.7	7.8	0.530	5.0	17.6	0.118
sed	6.0	9.3	1.3	0.027	1.7	0	1.000
see	4.9	6.5	2.6	0.308	3.3	0.0	1.000
seg	42.4	44.9	39.0	0.648	46.7	11.8	0.011
seh	7.1	7.5	6.5	1.000	5.0	11.8	0.580
sei	32.6	39.3	23.4	0.026	21.7	29.4	0.526
sej	8.7	9.3	7.8	0.795	5.0	17.6	0.118
sek	27.2	16.8	41.6	<0.001	40.0	47.1	0.781
sel	8.7	11.2	5.2	0.190	5.0	5.9	1.000
sem	7.6	5.6	10.4	0.266	11.7	5.9	0.676
sen	6.5	4.7	9.1	0.365	6.7	17.6	0.337
seo	5.4	4.7	6.5	0.744	6.7	5.9	1.000
sep	1.1	1.9	0.0	0.511	0.0	0.0	—
seq	31.5	19.6	48.1	<0.001	40.0	76.5	0.012
ser	6.5	8.4	3.9	0.247	5.0	0.0	0.589
seu	14.1	18.7	7.8	0.052	10.0	0	0.329
edin	6.0	7.5	3.9	0.364	3.3	0.0	1.000
pvl	10.3	16.8	1.3	0.001	1.7	0.0	1.000
tst	7.6	11.2	2.6	0.045	3.3	0.0	1.000

[a] The prevalence of virulence genes among MRSA were compared with those among MSSA isolates.
[b] The prevalence of virulence genes among non-hVISA MRSA were compared with those among hVISA MRSA isolates.
hVISA = heterogeneous vancomycin-intermediate *S. aureus*; MRSA = methicillin-resistant *S. aureus*; MSSA = methicillin-susceptible *S. aureus*.

Table 3 Molecular characteristics of 184 *Staphylococcus aureus* isolates

CC (no.)	MLST (no.)	MRSA (no.)	MSSA (no.)	*spa* type (no.)	*SCCmec* type (no.)	*agr* type (no.)	Virulence genes (%)
8 (55)	239 (50)	50	0	t030 (43), t037 (4), t437 (2), t2207 (1),	III (49), NT (1)	I (50)	*hla* (94.5), *hlb* (81.8), *hld* (94.5), *hlg* (9.1), *hlg-v* (80.0), *lukED* (90.9), *eta* (7.3), *etb* (9.1), *etd* (5.5), *sea* (18.2), *seb* (5.5), *sec* (9.1), *seg* (38.2),
	432 (3)	3	0	t2207 (3)	III (3)	I (3)	*seh* (5.5), *sei* (27.3), *sej* (10.9), *sek* (47.3), *sem* (10.9), *sen* (9.1),
	250 (1)	1	0	t2526 (1)	NT (1)	I (1)	*seq* (52.6), *seu* (7.3), *pvl* (1.8), *tst* (3.6)
	630 (1)	1	0	t441 (1)	III (1)	I (1)	
1 (37)	188 (31)	1	30	t189 (23), t127 (6), t030 (2)	IV (1)	I (25), III (3), II (3)	*hla* (100), *hlb* (97.3), *hld* (100), *hlg* (27.0), *hlg-v* (100), *lukED* (100), *lukM* (10.8), *eta* (8.1), *etb* (24.3), *etd* (5.4), *sea* (48.6), *seb* (48.6),
	1 (6)	0	6	t127 (4), t114 (1), t7117 (1)	NA	III (3), II (3)	*sec* (10.8), *seg* (48.6), *seh* (21.6), *sei* (48.6), *sej* (21.6), *sek* (27.0), *seq* (40.5), *ser* (16.2), *seu* (27.0), *edin* (21.6), *pvl* (32.4), *tst* (27.0)
398 (16)	398 (16)	1	15	t571 (6), t011 (6), t034 (3), t1451 (1)	II (1)	I (12), II (3), NT (1)	*hla* (100), *hlb* (100), *hld* (100), *hlg* (18.8), *hlg-v* (100), *lukED* (100), *eta* (12.5), *etb* (31.2), *etd* (12.5), *sea* (37.5), *seb* (50.0), *see* (18.8), *seg* (50.0), *sei* (50.0), *sej* (12.5), *sek* (25.0), *sel* (50.0), *sem* (18.8), *seq* (18.8), *pvl* (12.5)
59 (13)	59 (13)	3	10	t437 (10), t5350 (2), t163 (1)	IV (3)	I (8), II (5)	*hla* (92.3), *hlb* (100), *hld* (100), *hlg* (7.7), *hlg-v* (100), *lukED* (100), *sea* (15.4), *seb* (30.8), *sec* (38.5), *sed* (30.8), *see* (15.4), *seg* (46.2), *sei* (46.2), *sek* (30.8), *sen* (38.5), *seo* (38.5), *seq* (7.7), *ser* (23.1), *seu* (30.8), *pvl* (30.8), *tst* (15.4)
7 (12)	7 (12)	0	12	t796 (5), t091 (5), t189 (2)	NA	I (12)	*hla* (100), *hlb* (83.3), *hld* (100), *hlg-v* (100), *lukED* (100), *eta* (8.3), *sea* (33.3), *seb* (33.3), *sed* (16.7), *seg* (66.7), *sei* (66.7), *sel* (33.3), *sem* (16.7), *sep* (16.7), *seq* (16.7), *seu* (41.7)
30 (11)	36 (9)	9	0	t233 (9)	III (7), II (2)	I (9)	*hla* (90.9), *hlb* (81.8), *hld* (100), *hlg* (9.1), *hlg-v* (81.8), *lukED* (81.8),
	910 (1)	0	1	t318 (1)	NA	IV (1)	*eta* (9.1), *sea* (27.3), *seb* (27.3), *seg* (54.5), *sei* (18.2), *sek* (45.5),
	30 (1)	0	1	t338 (1)	NA	III (1)	*sel* (9.1), *seo* (18.2), *seq* (45.5), *edin* (27.3)
5 (9)	5 (7)	5	2	t002 (4), t1376 (1), t045 (1), t548 (1)	III (4), NT (1)	I (6), IV (1)	NC
	1920 (1)	1	0	t286 (1)	III (1)	I (1)	
	965 (1)	0	1	t062 (1)	NA	NT I (1)	
25 (9)	25 (9)	1	8	t078 (8), t081 (1)	III (1)	I (8), IV (1)	NC
121 (8)	121 (6)	0	6	t2091 (6)	NA	II (4), IV (2)	NC
	1301 (2)	1	1	t2019 (1), t6662 (1)	II (1)	IV (2)	NC
6 (5)	6 (5)	0	5	t701 (5)	NA	I (5)	NC
88 (4)	88 (4)	0	4	t1950 (1), t6979 (1), t5269 (1), t377 (1)	NA	II (3), III (1)	NC
20 (3)	20 (3)	0	3	t164 (2), t1089 (1)	NA	I (3)	NC
15 (2)	15 (2)	0	2	t084 (2)	NA	I (2)	NC

CC = clonal complex; hVISA = heterogeneous vancomycin-intermediate *S. aureus*; MLST = multilocus sequence typing; MRSA = methicillin-resistant *S. aureus*; MSSA = methicillin-susceptible *S. aureus*; NA = not applicable; NC = not counted; NT = not typeable.

MSSA isolates, the predominant clone was ST188-MSSA-t189-*agr*-I (20.6%, 22/107). The 17 hVISA isolates were isolated from blood (12 isolates), cerebrospinal fluid (2 isolates), ascites (2 isolates), and pleural effusion (1 isolate). ST239-*SCCmec*III-t030-*agr*-I was the most common clone (15 isolates); other clones identified included ST5-*SCCmec*III-t002-*agr*-II and ST1301-*SCCmec*II-t2019-*agr*-IV (1 isolate each).

The distribution of virulence genes in major CCs is shown in Table 3. Among 55 CC8 isolates, the most prevalent SE genes were *seq* (52.6%), *sek* (47.3%), *seg* (38.2%), and *sei* (27.3%). Among CC1 isolates were *sea* (48.6%), *seb* (48.6%), *seg* (48.6%), and *sei* (48.6%), and among CC398 isolates, *seb* (50.0%), *sej* (50.0%), *sei* (50.0%), and *sea* (37.5%). Compared with other CC isolates, CC1 and CC398 isolates harbored a higher frequency of exfoliatin genes, CC1 and CC59 harbored a higher frequency of *pvl* gene, and only CC1 isolates harbored *lukED* gene.

Discussion

In the present study, 184 *S. aureus* isolates obtained from six tertiary teaching hospitals in central-southern China were surveyed. The prevalence of MRSA isolates was 41.8%. The strains were isolated from sterile body fluids, mainly from blood (141 isolates); the prevalence of MRSA among *S. aureus* blood stream infection (BSI) was 40.4% (57/141). *S. aureus* is the leading cause of BSI in many countries.[20–22] A previous study reported that 41.6% of *S. aureus* BSI isolates from Zhejiang province were MRSA.[23] Our study further indicates that MRSA is an important etiologic agent of severe, life-threatening infections in China and suggests the need for active surveillance to identify patients at risk. A total of 17 hVISA isolates were identified by modified PAP/AUC; the prevalence of hVISA among MRSA isolates was 22.1%. The highest hVISA prevalence rate among MRSA isolates in Asia was 15.7% in China,[12] followed by 12.5% in Singapore;[24] our results showed a higher prevalence. To our knowledge, it was the highest rate of hVISA in China.

Our results showed that MRSA isolates exhibited low resistance to chloramphenicol (14.3%) and trimethoprim/sulfamethoxazole (9.1%). This supports the potential utility of chloramphenicol and trimethoprim/sulfamethoxazole as empiric treatment agents for *S. aureus* in China. Among MRSA isolates, hVISA had significantly higher resistance to gentamicin, levofloxacin, tobramycin, erythromycin, tetracycline, and trimethoprim/sulfamethoxazole than non-hVISA isolates, which indicated that antimicrobial options for hVISA are very limited. This finding is similar to that of a previous study, which showed that hVISA isolates had higher resistance to ciprofloxacin, gentamicin, trimethoprim/sulfamethoxazole, and tetracycline.[25]

The clinical outcome of *S. aureus* infections is influenced by antimicrobial resistance and virulence factors. This is believed to be the first study to report the presence of 31 virulence genes in MSSA, MRSA, and hVISA from China. The results showed that MSSA harbored a higher frequency of virulence genes than MRSA; 98 MSSA isolates (53.3%) carried $\geq$10 tested virulence genes simultaneously, which was significantly higher than that of MRSA isolates (33.8%; $p = 0.004$). A total of 24 non-hVISA isolates (40.0%) carried $\geq$10 tested virulence genes simultaneously, which was significantly higher than that of hVISA isolates (11.8%; $p = 0.041$); two hVISA isolates harbored only four virulence genes. This result was different from a previous report, which showed that MRSA isolates harbored more superantigen and *pvl* genes than MSSA isolates.[23] It has been reported that the acquisition of antibiotic resistance in *S. aureus* involves changes in virulence factor secretion due to the fitness cost associated with the expression of resistance, and it is reflected in decreased toxin expression.[26–28] Therefore, it is reasonable that the results of our study confirmed this by showing that virulence factor gene carriage was more diverse and abundant in MSSA than in MRSA strains.

In this study,184 *S. aureus* isolates belonged to 13 CCs: CC8 (29.9%), CC1 (20.1%), CC398 (8.7%), CC59 (7.1%), CC7 (6.5%), CC30 (6.0%), CC5 (4.9%), CC25 (4.9%), CC121 (4.3%), CC6 (2.7%), CC88 (4 isolates), CC20 (3 isolates), and CC15 (2 isolates). Distribution of some virulence genes was correlated with the different *S. aureus* lineages.[29] Compared with other CC isolates, CC1 and CC398 isolates harbored a higher frequency of exfoliatin genes, CC1 and CC59 harbored a higher frequency of *pvl* gene, and only CC1 isolates harbored *lukED*, indicating that different CCs had different virulence profiles.

SCCmec, multilocus sequence, *agr*, and *spa* typing were performed in this study; our results revealed that molecular characteristics were significantly different between MRSA and MSSA. ST239-MRSA-*SCCmec*III and ST5-MRSA-*SCCmec*II were identified as major epidemic clones among MRSA isolates in China.[30,31] In this study, ST239-MRSA-*SCCmec*III-t030-*agr*-I was the most common MRSA clone (55.8%). Among 17 hVISA isolates, ST239-MRSA-*SCCmec*III-t030-*agr*-I was the predominant clone (15 isolates); a similar finding was noted previously.[12] Among MSSA isolates, the dominant clone was ST188-MSSA-t189-*agr*-I (20.6%).

In conclusion, this study showed the prevalence of MRSA isolates collected from sterile body fluids was 41.8% in central-southern China; the prevalence of hVISA in MRSA isolates was considerably high, up to 22.1%. The predominant MRSA, hVISA, and MSSA clones were ST239-*SCCmec*III-t030-*agr*-I and ST188-MSSA-t189-*agr*-I, respectively. Furthermore, we found that the frequency of carriage of *pvl*, hemolysins, *tst*, and SE genes among MSSA isolates was significantly higher than that among MRSA isolates; virulence genes were more diverse and frequent in MSSA isolates than those in MRSA isolates, and the distribution of some virulence genes was correlated with the different *S. aureus* CCs.

Conflicts of interest

The authors declare no conflicts of interest.

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