

Molecular Characteristics, Antimicrobial Resistance and Virulence Gene Profiles of *Staphylococcus aureus* Isolates from Wuhan, Central China

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Purpose: This study aimed to investigate the molecular characteristics, antimicrobial resistance and virulence genes profiles of *S. aureus* isolates from Wuhan, central China.

Materials and Methods: A total of 302 non-duplicate *S. aureus* isolates were collected successively during January–December 2018 and subjected to multi-locus sequence typing (MLST), *staphylococcal protein A (spa)* typing and *Panton–Valentine leucocidin (PVL)* and *staphylococcal enterotoxin A, B, C, D, E, G, H and I (sea, seb, sec, sed, see, seg, seh and sei)* detection. All methicillin-resistant *S. aureus* (MRSA) isolates were additionally subjected to staphylococcal chromosomal cassette *mec*(SCC*mec*) typing.

Results: Of the 302 *S. aureus* isolates, 131 were categorised as MRSA, yielding a rate 1.4 times the average rate in China during 2018 (43.4% vs 30.9%). Thirty-one sequence types (STs) and 82 *spa* types were identified. The most prevalent clones were ST5-t2460 (10.9%), ST239-t030 (9.3%), ST188-t189 (7.9%) and ST59-t437 (6.3%). Notably, the continued prevalence of ST239-t030 in Wuhan differs from other areas in China. SCC*mec* types and subtypes I, II, III, IVa and V were present in 0.8%, 36.6%, 26.0%, 20.6% and 8.4% of MRSA isolates. A comprehensive analysis identified ST5-t2460-SCC*mec* II (25.2%), ST239-t030-SCC*mec* III (19.8%) and ST59-t437-SCC*mec* IVa (7.6%) as the major clones among MRSA isolates. The genes *pvl*, *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh* and *sei* were detected at respective frequencies of 11.9%, 42.1%, 49.7%, 45.0%, 20.9%, 33.8%, 60.5%, 25.8% and 66.9%.

Conclusion: ST239-t030 remains one of the most prevalent clones in *S. aureus* isolates from Wuhan, leading us to conclude that *S. aureus* isolates from Wuhan possess unique molecular characteristics. The *S. aureus* isolates also exhibit unique antimicrobial resistance profiles and harbour relatively high numbers of enterotoxin virulence genes, compared with other reports from China.

Keywords: *Staphylococcus aureus*, molecular characteristics, antimicrobial resistance, virulence

Introduction

Staphylococcus aureus (*S. aureus*), a common conditionally pathogenetic bacterium that colonises the surface skin and mucosa of humans, is a leading cause of bacteraemia and infective endocarditis, as well as osteoarticular, dermal, soft tissue, pleuropulmonary and device-related infections¹ and aerobic vaginitis which is associated with a significantly increased risk of preterm labour and low birth weight

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and so on.² *S. aureus* harbours a diverse range of genotypes and virulence factors, which may influence the severity of infections and the occurrence of associated complications and mortality. For example, infection with *S. aureus* sequence type 121 (ST121), a strain with a high virulence potential, is often associated with longer durations of hospitalisation and antimicrobial therapy.³ The isolates clonal complex 30 (CC30) and CC5 frequently and easily induce hematogenous complications,⁴ while bloodstream infection with CC398, a methicillin-susceptible *S. aureus* (MSSA) strain, is associated with high mortality.⁵ Therefore, analyses of the molecular characteristics and virulence gene profiles of *S. aureus* are important for predicting the prognosis of a patient with *S. aureus* infection.

The molecular characteristics of *S. aureus* differ among countries. ST239 has been the most common clone in China and other Asian countries since the 1970s, where it accounts for approximately 75% of identified cases of hospital-acquired methicillin-resistant *S. aureus* (HA-MRSA).⁶ In contrast, ST8 (USA300) is the most frequently identified clone in the United States.⁷ What's more, the distinction between the CA-MRSA and HA-MRSA has eroded slowly over time⁸ and it showed that the staphylococcus aureus strains have changed. Even within China, the molecular characteristics of *S. aureus* isolates vary among cities. For example, the predominant types are ST188 in Wenzhou,⁹ ST398 in Hainan,¹⁰ ST5 in Dalian and Shenyang¹¹ and ST59 in Chengdu.¹² Moreover, the most prevalent *staphylococcal protein A (spa)* types are t030 in Asia,^{13,14} t032 in Europe,¹⁵ t008 in America, t037 in Africa and t020 in Australia.¹⁶ The molecular characteristics of *S. aureus* also change over time. For example, ST239-t030 has rapidly replaced ST239-t037 since 2000 and has become the major clone among *S. aureus* isolates,¹⁷ but reports from some areas of China indicate that this clone was substituted with ST59-t437 in 2013 and with ST5-t2460 in 2017.¹⁸ Therefore, it is preferable to focus on a specific region of interest at a particular time when monitoring the molecular characteristics of *S. aureus* isolates.

The definition for community-associated methicillin-resistant *Staphylococcus aureus*(CA-MRSA) is the development of infection by individuals in an outpatient setting or by inpatients discharged within 48 hours of admission without a history of MRSA infection, surgery, dialysis, the presence of an indwelling catheter or devices that penetrate through the skin, or admission to an extended care facility.¹⁹ A previous epidemiological study conducted in Wuhan

aimed simply to investigate the molecular characteristics and virulence gene profiles of community-acquired MRSA (CA-MRSA) isolates that caused skin and soft tissue infections. That study identified the ST59-SCCmecIVa-PVL-positive clone as predominant among the isolates.²⁰ Another multi-centre study that included Wuhan, Changsha and Zhengzhou identified ST239-t030-SCCmecIII-agr-I and ST188-t189-agr-I as the most common MRSA and MSSA clones, respectively.²¹ Wuhan is the capital of Hubei province and the largest city in central China, with a population that has increased to 15 million. However, no previous study focused on the molecular characteristics and virulence gene profiles of *S. aureus* isolates, including MRSA and MSSA. Moreover, the molecular characteristics, antimicrobial resistance and virulence gene profiles of *S. aureus* isolates vary dramatically over time in the same region. Therefore, successive surveillance is an extremely critical component of *S. aureus* control and prevention. This study aimed to investigate the molecular characteristics, antimicrobial resistance and virulence gene profiles of *S. aureus* isolates sampled in Wuhan during 2018.

Materials and Methods

Clinical Isolates and Primers

From January to December 2018, 302 non-duplicate clinical isolates of *S. aureus* were collected successively from Zhongnan Hospital, Wuhan University. These isolates were derived from wound secretions (n = 111), sputum (n = 95), blood (n = 27) and other samples (n = 69), including tissues, cerebrospinal fluid, pleural effusion and ascites, catheter tips and urine. All isolates were first identified using traditional microbiological approaches, including Gram staining and coagulase and catalase tests, and were further identified using a VITEK 2 Compact system and the VITEK 2 GP Test Kit (bioMérieux, Inc., Durham, NC, USA). All samples were then stored at -80°C for further molecular experiments. All primers used in the present study were synthesised by Tianyihuiyuan Co. Ltd. (Wuhan, China). This study was approved by the Ethics Committee of Zhongnan Hospital, Wuhan University, which waived the requirement for informed consent because the details of patients with *S. aureus* infection were anonymised.

S. aureus Chromosomal DNA Extraction

All *S. aureus* isolates were cultured overnight on tryptic soy broth (TSB) agar plates at 35°C. Subsequently, single

colonies were transferred into 5 mL of TSB and grown overnight at a rotation speed of 200 rpm. Chromosomal DNA was extracted from *S. aureus* pellets using TIANamp Bacteria DNA kit (Tiangen, China), as described previously.^{10,22} The extracted DNAs were stored at -20°C for further studies.

Staphylococcal Protein A (Spa) Typing

The X region, which comprises a variable repeat region of the *spa*, was amplified using a PCR assay as described previously.^{10,22} The resultant amplicons were then purified and subjected to Sanger dideoxy DNA sequencing (Tianyihuiyuan, China). All sequences were analysed using the Ridom web server (<http://www.ridom.de/spaserver>), and each clinical isolate was assigned to a *spa* type.

Multi-Locus Sequence Typing (MLST)

Seven house-keeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi* and *ymjI*) were amplified from *S. aureus* chromosomal DNA for MLST as described previously.^{10,22-24} The amplified products were also purified and subjected to Sanger dideoxy DNA sequencing (Tianyihuiyuan, China). The sequences of these genes were then compared with known alleles at each locus, which were stored in the MLST database (<http://www.mlst.net>). Each clinical isolate was then explained by the sequence type.

Staphylococcus Chromosomal Cassette *mec*(SCC*mec*) Typing

All MRSA isolates were subjected to SCC*mec* typing as described previously.^{10,25} In brief, a multiple PCR assay containing nine pairs of primers specific for S CC*mec* types and subtypes, including I, II, III, IVa, IVb, IVc, IVd and V and *mecA* was used for SCC*mec* typing. Subsequently, the classification of SCC*mec* types and subtypes I, II, III, IVa, IVb, IVc, IVd and V was performed based on the molecular weights of the amplified products. MRSA isolates without any specific band were classified as non-typable (NT).

Detection of *pvl* and Enterotoxin Genes

The *S. aureus* isolates were screened for the presence of the *Panton-Valentine leucocidin* (PVL) and enterotoxin genes, including *staphylococcal enterotoxins A, B, C, D, E, G, H* and *I* (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh* and *sei*, respectively), using nine independent PCR assays with specific primers and reaction conditions as described

previously.²⁶ In brief, all PCR reaction mixtures contained 1 μL of DNA template, 2 μL of a primer pair (10 μM), 12.5 μL of 2 \times Taq Master Mix and 9.5 μL of double-distilled water. All PCR products were detected by electrophoresis on 1% agarose gels.

Antimicrobial Susceptibility Test (AST)

AST was performed using a VITEK 2 Compact system and the VITEK 2 AST-GP67 Test Kit (bioMérieux, Inc., Durham, NC, USA). The VITEK 2 AST-GP67 Test Kit contains 16 antibiotics: levofloxacin (LVX), gentamicin (GEN), trimethoprim-sulfamethoxazole (TST), penicillin (PEN), ciprofloxacin (CIP), vancomycin (VAN), erythromycin (ERY), clindamycin (CLI), linezolid (LZD), tetracycline (TET), rifampin (RIF), oxacillin (OXA), ceftiofloxacin (FOX), nitrofurantoin (NIT), quinupristin/dalfopristin (QUD) and moxifloxacin (MOF). *S. aureus* strains ATCC 25923 and 29213 were used as the quality control strains for AST. The results were analysed according to the Clinical and Laboratory Standards Institute (CLSI) M100-S27 standard.

Statistical Analysis

The statistical analysis was performed using SPSS Statistics 24.0 for Windows (IBM Corp., Armonk, NY, USA). The chi-square test was used to compare the resistance rates, prevalence of MDR and distributions of PVL and enterotoxin genes between the MSSA and MRSA isolates. A *p* value <0.05 was considered to indicate a statistically significant difference.

Results

The results of AST

The detailed results from the AST of 302 *S. aureus* isolates are presented in Table 1. Notably, all tested *S. aureus* isolates were susceptible to VAN, LZD and QUD, and most were also susceptible to NIT (99.3%, 300/302), TST (92.1%, 278/302) and RIF (87.1%, 263/302). However, most isolates were resistant to PEN (93.0%, 281/302). The rates of susceptibility to the remaining antibiotics ranged from 37.4% to 76.5%. Of the 302 *S. aureus* isolates, 131 (43.4%) were categorised as MRSA based on the OXA susceptibility test and FOX screening test, including 116 HA-MRSA and 15 CA-MRSA isolates as determined from patient medical records. The frequency of MRSA isolates was 1.4 times

Table 1 The Distribution of Antimicrobial Resistance of MRSA, MSSA and *S. aureus* Isolates and Its Statistical Significance

Isolates (n)	Resistant Isolates n(%)															
	CLI	ERY	FOX	OXA	PEN	RIF	LVX	TET	CIP	GEN	MOF	TST	LZD	QUD	NIT	VAN
MRSA(131)	103(78.6)	105(80.2)	131(100)	131(100)	131(100)	37(28.2)	79(60.3)	76(58.0)	79(60.3)	55(42.0)	77(58.8)	0	0	0	2(1.5)	0
MSSA(171)	79(46.2)	84(49.1)	0	0	150(87.7)	2(1.2)	12(7.0)	20(11.7)	13(7.6)	16(9.4)	10(5.8)	24(14.0)	0	0	0	0
Total(302)	182(60.3)	189(62.6)	131(43.4)	131(43.4)	281(93.0)	39(12.9)	91(30.1)	96(31.8)	92(30.5)	71(23.5)	87(28.8)	24(7.9)	0	0	2(0.7)	0
χ^2	32.572	30.5	278.065	286.093	15.444	45.971	100.047	73.394	92.261	43.912	101.328	-	-	-	-	-
p value*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-	-	-	-	-

Note: *The resistance rate to antibiotics in MRSA isolates were compared with those in MSSA isolates.

Abbreviations: PEN, penicillin; ERY, erythromycin; CLI, clindamycin; OXA, oxacillin, TEI, tetracycline; CIP, ciprofloxacin; LVX, levofloxacin; MOF, moxifloxacin; GEN, gentamycin; RIF, rifampin; VAN, vancomycin; LZD, linezolid; NIT, nitrofurantoin; QUD, quinupristin/dalfopristin; TST, trimethoprim-sulfamethoxazole; FOX, ceftaxime.

the average level of 30.9% reported in China during 2018 (www.carss.cn).

The resistance rates of the MRSA isolates to CLI, ERY, PEN, RIF, LVX, TET, CIP, GEN and MOF were 78.6% (103/131), 80.2% (105/131), 100.0% (131/131), 28.2% (37/131), 60.3% (79/131), 58.0% (76/131), 60.3% (79/131), 42.0% (55/131) and 58.8% (77/131). These rates were significantly higher than those observed for MSSA (Table 1). The multidrug resistance (MDR) rates among all *S. aureus*, MRSA and MSSA isolates were 66.6%, 89.3% and 49.1%, respectively. The prevalence of MDR was significantly higher among MRSA isolates than among MSSA isolates ($\chi^2 = 53.827$, $P < 0.001$; chi-square test).

MLST, *Spa* and SCCmec Typing

All 302 *S. aureus* isolates were successfully typed for MLST and assigned to 31 sequence types. The detailed results of MLST typing are listed in Table 2. The most prevalent ST type was ST5 (20.5%, 62/302), followed by ST239 (11.6%, 35/302), ST59 (10.3%, 31/302) and ST188 (8.9%, 27/302). Notably, 67.7% (42/62) of ST5, 77.4% (24/31) of ST59 and 97.1% (34/35) of ST239 isolates were MRSA, whereas 100% of ST188 isolates were MSSA. Among the 131 MRSA isolates, ST5 (32.1%, 42/131) was the most prevalent, followed by ST239 (26.0%, 34/131) and ST59 (18.3%, 24/131). Among MSSA isolates, ST188 (15.8%, 27/171) was the most common clone, followed by ST7 (14.0%, 24/171) and ST5 (11.7%, 20/171). In addition to ST1281, 30 other STs were identified as belonging to 16 clonal complexes (CCs) by eBURST. As shown in Fig. 1 and Table 2, CC5 (22.5%, 68/302) was the most prevalent clone complex, followed by CC8 (15.9%, 48/302), CC59 (10.6%, 32/302) and CC188 (8.9%, 27/302).

Of the 302 isolates, 294 were successfully typed and assigned to 82 *spa* types, whereas eight isolates were NT (Table 2). The most prevalent *spa* types were t2460 (11.2%, 33/302), t030 (9.5%, 28/302), t189 (8.2%, 24/302) and t437 (6.8%, 20/302). The MRSA isolates were assigned to 33 *spa* types, of which t2460 was the most prevalent. The MSSA isolates yielded 63 *spa* types, among which t189 was dominant (Table 2). Fourteen *spa* types—t002, t030, t062, t084, t127, t701, t309, t3527, t437, t441, t2091, t13828, t034 and t4549—were identified in both MRSA and MSSA isolates. In a combined analysis of the ST and *spa* types, strong associations were observed between the main ST and *spa* types. Specifically, ST188 and ST239 were primarily associated with t189 (88.9%, 24/27) and t030 (80.0%, 28/35), respectively, whereas ST5 and ST59 were mainly associated with t2460 (53.2%, 33/62) and t437 (61.3%, 19/31), respectively.

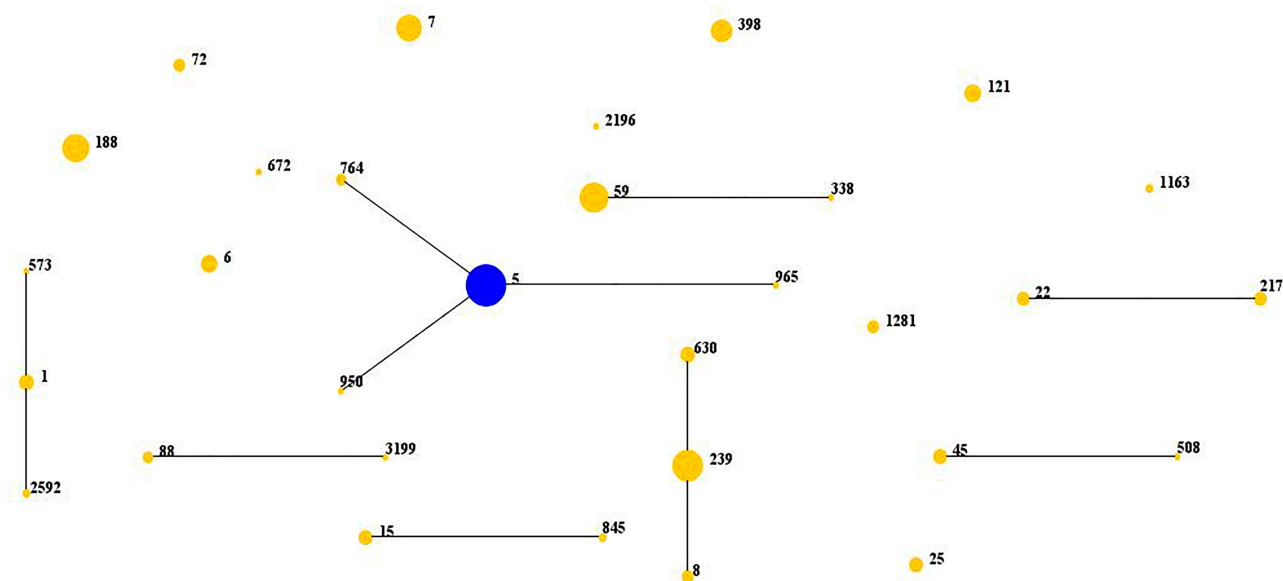


Figure 1 Distribution of sequence types (STs) in the clone complexes.

Notes: The diagram generated by eBURST based on the MLST data of this study, representing the relationships of the 302 *S. aureus* isolates. Each number is on behalf of an ST, STs that are linked by lines belong to the same clone complexes. The diameter of the circle stands for the number of the same ST isolates.

Therefore, the major epidemic clones were ST5-t2460 (10.9%, 33/302), ST239-t030 (9.3%, 28/302), ST188-t189 (7.9%, 24/302) and ST59-t437 (6.3%, 19/302).

SCCmec typing was performed successfully for 121 out of the 131 MRSA isolates (92.4%). The SCCmec types and subtypes I, II, III, IVa and V were identified in 1 (0.8%), 48 (36.6%), 34 (26.0%), 27 (20.6%) and 11 (8.4%) MRSA isolates, respectively (Table 2). SCCmec type II was the most prevalent followed by III and IVa. The majority (85.4%, 41/48) of the SCCmec type I MRSA isolates were ST5, while most type III MRSA isolates (94.1%, 32/34) were ST239 and IVa was primarily associated with ST59 (74.1%, 20/27). A comprehensive analysis of the results from MLST, spa and SCCmec typing identified ST5-t2460-SCCmec II (25.2%, 33/131), ST239-t030-SCCmec III (19.8%, 26/131) and ST59-t437-SCCmec IVa (7.6%, 10/131) as the major clones of MRSA isolates.

PVL and Enterotoxin Gene Profiles

The frequencies of the PVL and enterotoxin genes in the 302 *S. aureus* isolates are listed in Table 3. Thirty-six of the 302 isolates (11.9%) were positive for PVL, and 14 of these were identified as MRSA (including nine CA-MRSA isolates). The frequency of PVL positivity differed significantly between the 15 CA-MRSA and 116 HA-MRSA isolates (64.3% vs 4.3%, $\chi^2 = 37.520$, $p < 0.001$). The most frequently identified enterotoxin gene was *sei* (66.9%), followed by *seg* (60.5%). The frequencies of the other enterotoxin genes ranged from 20.9%

to 49.7%. Compared with the MSSA isolates, the MRSA isolates had higher frequencies of *sec* and *see* positivity and a lower rate of *seb* and *seh* positivity (Table 3). On average, each isolate harboured 3.5 enterotoxin genes, although 156 isolates harboured four or more enterotoxin virulence genes. Specifically, 5, 13, 55 and 83 isolates harboured seven, six, five or four enterotoxin virulence genes, respectively. The frequency of clones harbouring four or more enterotoxin genes did not differ significantly between the MRSA and MSSA isolates ($\chi^2 = 2.438$, $p > 0.05$). The most common enterotoxin virulence genotype was *seb-sec-seg-sei* (8.9%, 27/302), followed by *seb-seg-sei* (3.0%, 9/302).

Discussion

In this study, 302 *S. aureus* isolates were subjected to AST, and 131 (43.4%) were classified as MRSA according to OXA and FOX susceptibility. All 131 isolates were *mecA*-positive, which further supported methicillin resistance. Notably, the prevalence of MRSA in this study was 1.4 times the average level of 30.9% in China during 2018 (maximum, 46.8%; minimum, 15.5%) (www.carss.cn). In 2017, the respective frequencies of MRSA in Russia, Japan and Australia were 16.0%, 41.0% and 19.0% (resistancemap.cddep.org/AntibioticResistance.php). In other words, the prevalence of MRSA varies among countries and cities. Most MRSA isolates evaluated in this study were classified as SCCmec II, although III and IVa were also abundant. In contrast, types I and V were less

Table 2 The Results of Different Typing of All the 302 Non-Duplicate *S. aureus* Isolates

Clone Complex(n)	MLST(n)	SPA Type (n)	SCCmec Type (n)
CC5(68)	ST5(62) ST764(4) ST950(1) ST965(1)	t002(1), t311(7) t2460(33) t5353(1) t002(7), t088(1), t548(4), t640(1), t796(1), t954(2), t1793(1), t3794(1), t8660(1), t9476(1) t002(2) t601(1), t1084(1) t895(1) t062(1)	II (8) II (33) IVa(1) II (1), IVa(1) II (2) NT(1)
CC8(48)	ST239(35) ST8(5) ST630(8)	t030(28) t459(6) t1510(1) t024(1) t7087(2) NT(2) t2196(1) t4549(4) t377(2), t4047(1)	III(26), NT(1) III(5), NT(1) III(1) NT(1) V (1) V (3)
CC59(32)	ST59(31) ST338(1)	t172(5) t437(19) t1751(1), t3523(1), NT(1) t441 (2) t3527(2) t437(1)	IVa(5) II (2), III(1), IVa (10), V (1) IVa(3) IVa(1) IVa(1) V (1)
CC45(8)	ST45(7) ST508(1)	t116(3) t1510(1) NT(3) t9058(1)	IVa(2), NT(1) IVa(1) NT(1) IVa(1)
CC398(17)	ST398(17)	t034(7) t5462(1), t5635(1) t1451(1), t1580(1), t2765(1), t3625(1), t548(1), t571(3)	NT(1) V (2)
CC22(12)	ST22(6) ST217(6)	t309(6) t309(4) t13828(2)	V (1) V (1) V (1)
CC88(5)	ST88(4) ST3199(1)	t3622(2) t3155(1), t18556(1) t1376(1)	II (1), NT(1)
CC1(11)	ST1(8) ST573(1) ST2592(2)	t127(7) t1784(1) t3876(1) t114(1) t416(1)	II (1) NT(1)
CC6(10)	ST6(10)	t701(9) t1131(1)	NT(1)

(Continued)

Table 2 (Continued).

Clone Complex(n)	MLST(n)	SPA Type (n)	SCCmec Type (n)
CC72(5)	ST72(5)	t148(3) t664(1) t3092(1)	IVa(1)
CC121(10)	ST121(10)	t2091(2) t021(1), t954(1), t1425(1), t2019(2), t2086(2) NT(1)	I (1)
CC15(9)	ST15(7) ST845(2)	t084(4), t120(1), t12513(1), t14014(1) t084(1) t346(1)	III(1)
CC188(27)	ST188(27)	t062(1), t189(24), t2765(1), t2769(1)	
CC7(26)	ST7(24) ST1163(2)	t091(14), t548(1), t605(1), t796(6), t3884(1), t6835(1) t021(2)	
CC672(2)	ST672(1) ST2196(1)	t014(1) NT(1)	
CC25(7) NT(5)	ST25(7) ST1281(5)	t078(2), t081(1), t280(1), t528(1), t3033(1), t7642(1) t164(5)	

represented. Our finding differ from the recent studies that the most common SCCmec types were III in Guangdong and Xinjiang^{27,28} and IV in Shanghai²⁹ and Trento.³⁰ In this study, AST also revealed that all or most of the isolates were susceptible to VAN, LZD, QUD, TST and NIT but were resistant to PEN, consistent with the results of most previous studies conducted in China and worldwide.^{31–33} Our findings support previous evidence suggesting that QUD and LZD are valuable substitutes for VAN in the treatment of MRSA infections.³⁴ In addition, the rates of resistance to five antibiotics including

CIP, CLI, GEN, LVF and RIF among the MRSA isolates in this study differed from the average levels reported in China in 2018 (www.chinets.com). Therefore, we conclude that *S. aureus* isolates in Wuhan has an unique antibiotic susceptibility, which might be associated with differences in health care programmes and drug usage patterns.

Thirty-one different sequence types were identified among the 302 *S. aureus* isolates in this study. The most prevalent sequence types in both the total *S. aureus* and MRSA isolates were ST5 and ST239, and this finding differs from other reports.²⁷ Specifically, the predominant MRSA isolates were ST5 and ST398 in Shanghai in 2017,¹⁸ ST239 and ST338 in Guangdong during 2010–2016,²⁸ ST239 and ST15 in Iran during 2015–16³⁵ and ST5 and ST8 in the United States during 2010–2011.³⁶ The predominant MSSA genotypes were ST188 and ST7, which was in accordance with the majority of previous studies.³⁷ Therefore, the common STs may change over time and are frequently associated with specific geographic areas.

In this study, 294 *S. aureus* isolates were assigned to 82 *spa* types, indicating a high level of diversity and complexity with respect to the genetic backgrounds. We identified t2460, t030 and t189 as the predominant *spa* types among the *S. aureus* isolates. Of these three types, t2460 was

Table 3 The Frequencies of PVL and Enterotoxin Genes of the 302 Non-Duplicate *S. aureus* Strains Both in MRSA and MSSA

Virulence Genes	<i>S. aureus</i> N (%)	MRSA N (%)	MSSA N (%)	P value
sea	127(42.1)	63(48.1)	64(37.4)	0.063
seb	150(49.7)	53(40.4)	97(56.7)	0.005
sec	136(45.0)	71(54.2)	65(38.0)	0.005
sed	63(20.9)	25(19.1)	38(22.2)	0.506
see	102(33.8)	54(41.2)	48(28.1)	0.017
seg	183(60.5)	86(65.6)	97(56.7)	16
seh	78(25.8)	24(18.3)	54(31.6)	0.009
sei	202(66.9)	92(70.2)	110(64.3)	0.280
pvl	36(11.9)	14(10.7)	22(12.9)	0.563

predominant among MRSA isolates, whereas t189 was most common among MSSA isolates. We reviewed 253 articles that describe studies in Europe, Asia, America, Africa and Australia and were published online before 17 December 2017. According to these reports, the most prevalent *spa* types were t030, t037 and t002 in Asia; t032, t008 and t002 in Europe; t008, t002 and t242 in America; t037, t84 and t064 in Africa; and t202, t037 and t437 in Australia.¹⁶ Our study and other studies conducted in China during 2018–2019 identified t2460, rather than t030, as the most prevalent *spa* type among *S. aureus* and MRSA isolates.^{18,22,38,39} Therefore, we conclude that both the common *spa* type and common sequence type also change over time and are frequently associated with specific geographic areas. These observations suggest that the polymorphic X region may be associated with the ability of the organism to adapt to conditions such as diverse geographic areas, temperatures and host populations.

In this study, ST188, ST239, ST5 and ST59 were associated primarily with t189, t030, t2460 and t437, respectively, consistent with the findings of most previous studies.^{10,18,40} We then combined the sequence and *spa* types and identified ST5-t2460 as the most prevalent pandemic clone, followed by ST239-t030, ST188-t189 and ST59-t437. A multi-centre study of *S. aureus* bacteraemia at eight hospitals from four cities in China during 2017 also identified ST5-t2460 as the most common genotype, followed by ST188-t189 and ST59-t437.⁴⁰ Another study of *S. aureus* in Shanghai during 2017 also identified ST5-t2460 as the predominant genotype, followed by ST5-t002, ST398-t034 and ST7-t091.¹⁸ Our results further demonstrate that ST239-t030, a truly pandemic MRSA isolate that has circulated in China and worldwide since the 1970s, remains one of the most prevalent clones in Wuhan, despite its exclusion from the top rank. This observation differs from those reported in other areas of China,^{18,40} leading us to conclude that *S. aureus* isolates from Wuhan possess unique molecular characteristics.

S. aureus isolates that harbour *pvl* can potentially secrete the cytotoxin PVL, which causes tissue necrosis and leukocyte destruction. This gene is generally considered a stable marker of CA-MRSA in some European countries and the United States.^{41–43} In this study, *pvl* was detected in 36 (11.9%) *S. aureus* isolates, of which 14 were MRSA and 5 were HA-MRSA. Our statistical analysis revealed a higher rate of *pvl* positivity among CA-MRSA relative to HA-MRSA isolates, suggesting that this gene could be used to identify CA-MRSA. Previous studies revealed low rates of *pvl* positivity among MRSA

isolates in Korea (1.7%)⁴⁴ and various European countries, including Germany and Spain (0–4.4%).⁴⁵ In contrast, MRSA was isolated from 48% of patients with skin and skin structural infections at an emergency department in the United States, and 92.8% of these MRSA isolates harboured *pvl*.⁴⁶ Therefore, the prevalence of *pvl* positivity differs among countries. In this study, we tested eight enterotoxin virulence genes and identified *sei* as the most prevalent, followed by *seg*. Of the tested enterotoxin virulence genes, six were present in >25.8% of *S. aureus* isolates, whereas in a previous study conducted in Shanghai, 13 enterotoxin genes (including those tested in our study) were present in 5.1–23.1% of *S. aureus* isolates.²⁹ Moreover, we observed an average rate of 3.6 enterotoxin genes per isolate, which was about two times the rate reported previously in Shanghai.²⁹ Another study performed in Guangzhou reported that the prevalence of *sea*, *seb*, *sec* and *sed* in MRSA isolates ranged from 0.97% to 21.4%,²⁹ whereas the corresponding rates in this study were 42.1%, 49.7%, 45.0% and 20.9%, respectively. Therefore, we conclude that *S. aureus* isolates from Wuhan more frequently harbour enterotoxin genes than isolates from Shanghai and Guangdong.

In conclusion, this study is the first to simultaneously reveal the antibiotic resistance, molecular characteristics and virulence genes profiles of *S. aureus* isolates from Wuhan, China. ST239-t030 remains one of the most prevalent clones in *S. aureus* isolates from Wuhan. The *S. aureus* isolates from this city exhibit unique antimicrobial resistance profiles and harbour relatively high numbers of enterotoxin virulence genes, compared with other reports from China.

Disclosure

The authors report no conflicts of interest in this work.

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