ORIGINAL RESEARCH

Comparison of Molecular Characteristics Between Methicillin-Resistant and -Susceptible *Staphylococcus aureus* Clinical Isolates by Whole-Genome Sequencing

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Introduction: The transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *Staphylococcus aureus* (MSSA) are great public health concern worldwide. To better understand *S. aureus* evolution and dissemination, we compared the molecular features of MSSA and MRSA isolates.

Methods: In this study, 74 MSSA and 102 MRSA non-duplicate isolates were recovered from clinical samples between 2016 and 2020. Molecular epidemiology, antimicrobial resistance determinants, and virulence gene profiles were carried out by whole-genome sequencing (WGS).

Results: Twenty distinct sequence types were identified in MRSA isolates, with the most common being ST59, ST630, and ST338. The major genotypes of MSSA were ST188 and ST7. The toxin genes *clfA, sek*, and *seq* were significantly associated with MRSA, while *splA/B, clfB, map, sdrC/D*, and *sem-sen-seo-seu* were detected more frequently in MSSA isolates than MRSA (P < 0.05). The *tst* positive isolates were more commonly identified in CC1 and CC72, whereas *lukE/D* was mainly found in the CC7, CC15, CC88, and completely absent in CC59 clones.

Conclusion: Our results compared the genetic diversity between MRSA and MSSA strains, suggesting efforts to fight infections caused by MSSA need to be intensified due to MSSA isolates carrying wide range of virulence factors. Comparative epidemiological studies of large populations of MSSA and MRSA will be necessary in the future to understand how MSSA and MRSA populations may co-evolve and interact in the future.

Keywords: methicillin-susceptible *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, virulence, resistance, molecular characteristics, whole-genome sequencing

Introduction

Staphylococcus aureus (*S. aureus*) is one of the most infamous pathogens, causing an incalculable number of uncomplicated infections and probably tens of millions of more severe, rapidly progressive infections globally every year. A study from the University of Washington reported that *S. aureus* ranked in the two top priority pathogens for deaths associated with resistance. And during 2019, methicillin-resistant *S. aureus* (MRSA) accounting for more than 100, 000 deaths.[1] This invasive bacterial continues to evolve, and recognition of recent trends in molecular characteristics and future horizons are highly meaningful.

MRSA has been reported to evolve via two primary mechanisms: spread of existing resistant clones and acquisition of staphylococcal cassette chromosome *mec* (SCC*mec*) by a methicillin-susceptible *S. aureus* (MSSA) strain. To date, 13 SCC*mec*

types and three *mec* genes have been discovered in *S. aureus*.[2,3] The epidemiological influence of MRSA strains is considered to originate from combination of several hypervirulence loci and methicillin resistance genes, which enable these strains to colonize and invade healthy individuals and transmit rapidly through the population.[4] Molecular epidemiological studies have shown that a few epidemic MRSA lineages are given rise to the majority of infections worldwide.[5,6] Hence, it is crucial to understand the molecular characteristics of MRSA isolates to achieve infection control more effectively. Noteworthy, significant progress has been achieved in the research of antimicrobial activity and its mechanism of action.[7–13]

Most studies focus on MRSA, although infections with MSSA might be even more clinically important, given their higher incidence of invasive infections when compared with MRSA.[14] However, genetic diversity between MRSA and MSSA isolates may exist but remain unelucidated. Karauzum et al reported that MSSA ST8 is more virulent than MRSA ST8.[15] Antimicrobial resistance genes and virulence factors located in *staphylococci* are often carried on large mobile DNA elements that can be transferred horizontally. Horizontal gene transfer of these mobile elements may ultimately enhance pathogenicity, epidemics, and resistance to antibiotics.[16] Some previous studies have found an increasing transfer of mobile elements, leads to an increase in *S. aureus* pathogenicity and resistance.[17,18]

Whole-genome sequencing (WGS) provides a cost-effective approach to investigating and confirming or refuting outbreaks of *S. aureus*.[19,20] Such sequencing appears to be well suited for comprehensive analysis of the genetic relatedness among isolates, detect uncommon antibiotic resistance genes, and reveal complex patterns of virulence genes. Therefore, this study aimed to unravel the molecular characteristics between MRSA and MSSA isolated from patients admitted to a tertiary hospital in southern China over a 5-year period utilizing WGS.

Materials and Methods

Collection of Clinical Isolates and *S. aureus* Confirmation

From January 2016 to December 2020, 102 non-duplicate MRSA isolates were recovered from a university hospital in central China. In addition, 74 MSSA isolates were randomly collected during this period. *S. aureus* isolates were cultured on blood chocolate agar plates at 37 °C (±1 °C) for 16–18 h, and confirmed by colony morphology, Gram-staining, cell morphology, catalase, and coagulase tests according to routine laboratory procedures. *S. aureus* isolates were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF). Briefly, single colony was suspended in distilled water, and mixture was spotted onto a MALDI target plate. Then, the bacterial pellet was resuspended by adding 1 μL of an α-cyano-4- hydroxycinnamic acid matrix solution and allowed to dry. Before identification, quality control and calibration were carried out using a standard calibration mixture of an *Escherichia coli* (ATCC 8739) extract. Spectra were recorded using a MALDI-TOF mass spectrometer in positive linear mode. The pattern-matching algorithm in the software performed comparative analysis with reference spectra and processed spectra of bacteria. Further antimicrobial susceptibility tests of all isolates were implemented using the standard methods provided by VITEK 2 Compact system (bioMérieux, Marcy-l'Étoile, France).

Whole Genome Sequencing and Analysis of Isolates

DNA was extracted using a Bacteria GenDNA Kit (TIANGEN, China) following the manufacturer's protocols, and added 20 mg/mL lysostaphin solution. The NEBNext R UltraTM II DNA Library PrepKit for Illumina R was utilized to construct sequencing libraries and loaded onto NovaSeq S4 flow cell, in accordance with standard Illumina instructions (Illumina, Inc., United States). The raw sequenced reads quality was assessed using FastQC v.0.11.5. The WGS information were used for typing characterization, clonality, resistome, and virulence determinant analysis. Multilocus sequence typing (MLST) was performed using the MLST database website (https://cge.cbs.dtu.dk/services/MLST/). *Spa* typing was performed by submitting sequences to the *spa*Typer database website (https://cge.cbs.dtu.dk/services/*spa*typer/). BioNumerics software was applied to cluster related sequence types (STs), defined as clonal complexes (CCs). The SCC*mec* of the 102 MRSA isolates was determined using the database website SCC*mec*Finder. Resistome analysis was performed using the ResFinder database website (https://cge.cbs.dtu.dk/services/ResFinder/).

Statistical Analysis

All data were analyzed using the SPSS Data Editor, version 25 (IBM Corp, Armonk, NY, USA). Categorical variables were presented as counts and percentages (%) and compared using the chi-square test. $P > 0.05$ was considered statistically insignificant.

Results

Antimicrobial Susceptibility Testing and Concordance Between Phenotypic and Genotypic

All the strains were susceptible to linezolid and vancomycin (Table 1). Among the 102 MRSA isolates, the majority were resistant to penicillin (100.0%), erythromycin (72.5%), and clindamycin (57.8%); The resistance rates to other antibiotics tested were 34.3% to tetracycline, 16.7% to ciprofloxacin, 11.8% to gentamicin, and 5.9% to rifampicin. The correlation between detected resistance genes and penicillin, erythromycin and trimethoprim-sulfamethoxazole resistance were 92.9%, 96.0% and 93.6%, respectively. The correlation between other drugs and sequences data was 100%. The most prevalent *erm* gene at erythromycin-resistant *S. aureus* isolates were *ermB* (54.5%, 54/99), followed by *ermC* (45.5%, 45/99), *ermA* (7.1%, 7/99) and *mph* (1.0%, 1/99).

Virulence Gene Profiling of MRSA and MSSA

We compared the toxin gene frequency among MSSA and MRSA (Table 2). Almost all *S. aureus* (>95%) isolates carried certain toxin genes (*icaA, hly/hla, hysA*, and *sspA*/B), with a low prevalence (<10%) of other genes (*tst, sed, seh, ser*).

The *tst* gene was found in 7.8% of MRSA isolates, whereas 8.1% of MSSA strains contained the *tst* gene. In addition, *clfB, map, sdrC*/D, *sem-sen-seo-seu*, and *splE* genes were more common in MSSA than in MRSA. In our study, *pvl* gene was found in 24 isolates, with a prevalence of 17.6% (18/102) in MRSA and 8.1% (6/74) in MSSA. No *etb* genes were found among the 176 isolates, and *eta* gene was only found in one MSSA isolate.

Table 1 Correlation of Resistance Phenotype and Predicted Genotype for 176 *Staphylococcus aureus* Isolates

Tested Drugs	Resistance Phenotype (%)			Detected Resistance Genes and Mutations (n)	Correlation Between Phenotype and Genotype (%)
	Total (n =176)	MRSA (n =102)	MSSA (n=74)		
Oxacillin	102(58.0)	102(100)	0(0.00)	*mecA*(102)	100
Penicillin	170(96.6)	102(100)	68(91.9)	*blaZ*(158)	92.9
Erythromycin	99(56.3)	74(72.5)	25(33.8)	*ermA*(7), *ermB*(54), *ermC*(45), *msrA*(1)	96.0
Clindamycin	65(36.9)	59(57.8)	6(8.1)	*ermA*(7), *ermB*(54), *ermC*(45), *mph*(1)	100
Gentamicin	14(8.0)	12(11.8)	2(2.7)	*aac(6')-Iaph(2")*(17)	100
Vancomycin	0	0	0	*vanA(0)*	100
Ciprofloxacin	21(11.9)	17(16.7)	4(5.4)	*gyrA* E88G(1), *gyrA* G106D(5), *gyrA* S84A(1), *gyrA* S84L(17)	100
Linezolid	0	0	0	*cfr(0)*	100
Tetracycline	44(25.0)	35(34.3)	9(12.2)	*tetK*(37), *tetM*(1), *tetL*(8)	100
Rifampicin	7(4.0)	6(5.9)	1(1.4)	*rpoB* D471Y(1), *rpoB* H481N(10), *rpoB* L466S(5)	100
Trimethoprim-sulfamethoxazole	16(9.1)	12(11.8)	4(5.4)	*dfrC*(5), *dfrG*(6), *dfrK*(5), *linA*(1)	93.6

Table 2 Virulence Genes in *Staphylococcus aureus* Isolates as Determined by Whole-Genome Sequencing – Comparison Between MSSA and MRSA

Virulence Gene(s)	Total (n=176)	MSSA (n=74)	MRSA (n=102)	*P*
Adhesin				
icaA	171(97.2)	71(95.9)	100(98.0)	0.651
vwbp	29(16.5)	8(10.8)	21(20.6)	0.084
map	85(48.3)	55(74.3)	30(29.4)	0.000
clfA	83(47.2)	18(24.3)	65(63.7)	0.000
clfB	85(48.3)	48(64.9)	37(36.3)	0.000
sdrC	132(75.0)	64(86.5)	68(66.7)	0.003
sdrD	74(42.0)	47(63.5)	27(26.5)	0.000
sdrE	129(73.3)	51(68.9)	78(76.5)	0.264
Superantigen				
tst	14(8.0)	6(8.1)	8(7.8)	0.949
sea	25(14.2)	6(8.1)	19(18.6)	0.048
seb	66(37.5)	8(10.8)	58(56.9)	0.000
sec	24(13.6)	12(16.2)	12(11.8)	0.396
sed	4(2.3)	4(5.4)	0	0.030
seh	10(5.7)	4(5.4)	6(5.9)	0.893
sek	67(38.1)	1(1.4)	66(64.7)	0.000
sel	24(13.6)	12(16.2)	12(11.8)	0.396
sem	41(23.3)	24(32.4)	17(16.7)	0.015
sen	40(22.7)	24(32.4)	16(15.7)	0.009
seo	39(22.2)	23(31.1)	16(15.7)	0.015
sep	19(10.8)	11(14.9)	8(7.8)	0.138
seq	67(38.1)	1(1.4)	66(64.7)	0.000
ser	4(2.3)	4(5.4)	0(0.0)	0.030
seu	40(22.7)	24(32.4)	16(15.7)	0.009
Other Toxin				
eta	1(0.6)	1(1.4)	0(0.0)	0.420
etb	0	0	0	/
hly/hla	171(97.2)	73(98.6)	98(96.1)	0.400
hlb	73(41.5)	6(8.1)	67(65.7)	0.000
lukE/D	78(44.3)	51(68.9)	27(26.5)	0.000
pvl	24(13.6)	6(8.1)	18(17.6)	0.069
Immune-evasion gene				
chp	122(69.3)	43(58.1)	79(77.5)	0.006
sak	135(76.7)	59(79.7)	76(74.5)	0.419
scn	154(87.5)	66(89.2)	88(86.3)	0.564
Enzyme				
hysA	171(97.2)	72(97.3)	99(97.1)	1.000
splA/B	85(48.3)	59(79.7)	26(25.5)	0.000
splE	56(31.8)	49(66.2)	7(6.9)	0.000
sspA	172(97.7)	73(98.6)	99(97.1)	0.640
sspB	169(96.0)	71(95.9)	98(96.1)	1.000

Typing Characteristics Between MRSA and MSSA

The genetic differences of *S. aureus* isolates tested were assessed by MLST and *spa* typing, and 102 MRSA isolates were additionally tested for SCC*mec* typing. Among the 176 *S. aureus* isolates, 31 unique STs belonging to 16 CCs were observed, except for the untypable isolates (Supplementary Figure). Among the MRSA isolates, ST59 was most prevalent (41.2%, 42/102), followed by ST630 (9.8%, 10/102), ST338 (5.9%, 6/102), and ST88 (5.9%, 6/102) (Table 3). In the MSSA group, the major STs were ST188 (20.3%, 15/74) and ST7 (10.8%, 8/74), followed by ST6 (6.8%, 5/74) and ST15 (6.8%, 5/74) (Table 4).

Table 3 Molecular Characteristics and Antibiotic Resistance Genes Profiles of 102 MRSA Isolates

Clone Complex (n)	Major ST (n)	Predominant *spa*-Type (n)	SCC*mec* Type (n)	Resistance Gene (n)
CC59(53)	ST59(42), ST338(6), ST4513(2)	t437(31), t3523(5), t441(5), t172(4), t163(3)	IV(42), V(11)	*blaZ(49), ermB(42), ermC(4), tetK(27)*
CC239(15)	ST630(10), ST239(5)	t4549(9), t30(2)	III(5),10V(10)	*aacA-aphD(5), blaZ(10), fosB(15), fusB(10), ermA(3), ermC(5), tetM(5), dfrB(5)*
CC5(6)	ST5(3), ST764(1), ST965(2)	t002(1), t010(1), t062(2), t311(1), t5349(1)	II(2), IV(4)	*aacA-aphD(6), aadD(1), sat(2), blaZ(5), fosB(6), fusB(2), mupA(1), ermA(2), ermB(3), lnuA(1), qacA(1), qacR(1), tetK(3), tetM(1)*
CC88(6)	ST88(6)	t10450(1), t10738(1), t16257(1), t5351(1), t9790(1)	IV(4)	*aadD(3), blaZ(5), ermC(4), lnuA(1), dfrB(1), fusA(1), fosB(2), dfrG(2)*
CC1(5)	ST1(5)	t114(4), t127(1)	IV(5)	*blaZ(5), ermC(4)*
CC398(4)	ST398(4)	t1250(2), t1456(1), 1t011(1)	IX(1),V(3)	*aacA-aphD(1), aadD(2), blaZ(4), fosB(2), fusB(1), ermB(2), ermC(3), lnuA(1), tetK(1), tetL(1), tetM(1), dfrG(1)*
CC72(2)	ST72(2)	t148(2)	V(2)	*blaZ(2), ermC(2), tetK(1), fosB(2), ermB(1)*

Table 4 Molecular Characteristics and Antibiotic Resistance Genes Profiles of 74 MSSA Isolates

Clone Complex(n)	MLST (n)	*Spa* Type (n)	Resistance Gene
CC1(21)	ST188(15), ST1(4), ST3206(1), ST573(1),	t189(15), t127(3), t1839(1), t227(1), t345(1)	*aac(6')/aph(1), blaZ(21), erm(B)(1), ermC(4), dfrG(1), fosB(2)*
CC5 (14)	ST5(3), ST6(5), ST965(4), ST5662(1)	t548(3), t701(4), t062(4), t304(1)	*aac(6')/aph(2), aadD(1), blaZ(12), erm(B)(2), ermC(3), fosB(13), lnuA(1)*
CC7(8)	ST7(8)	t091(7)	*aadD(7), blaZ(8), lnuA(7), ermA(1), ermC(3), tetK(5)*
CC20(4)	ST20(2), ST2631(2)	t164(2), t2094(1)	*blaZ(3), ermC(1), lnuA(1), fosB(4)*
CC15(5)	ST15(5)	t084(1), t144(1), t1492(1), t436(1)	*blaZ(4), tetK(1), fosB(5), mph(1), msr(A)(1)*
CC398(4)	ST398(3), ST2772(1)	t1250(4)	*blaZ(4), ermC(1)*
CC239(3)	ST630(3)	t2196(2), t377(1)	*tetK(2), fosB(3)*
CC25(3)	ST25(3)	t078(3)	*blaZ(1), fosB(3), ermC(2), lnuA(1)*
CC22(2)	ST22(2)	t5335(1), t15234(1)	*aadD(2), blaZ(2), ermC(1), lnuA(2), tetK(1)*
CC88(2)	ST88(2)	t10450(1), t12444(1)	*blaZ(2), fosB(1), ermC(1)*
CC121(1)	ST121(1)	t8660(1)	*blaZ(1), ermC(1), lnuA(1), fosB(1)*

Using *spa* typing, 57 distinct types were found. The most prevalent *spa* types were t0437 (19.3%, 34/176), t0189 (9.1%, 16/176), t4549 (5.1%, 9/176), and t1250 (4.0%, 7/176). The MRSA isolates yielded 33 *spa* types, of which t437 was the most prevalent (Table 3). The MSSA isolates were assigned to 31 *spa* types, among which t189 was dominant (Table 4). Seven *spa* types—t002, t062, t10450, t1250, t127, t148, and t78—were identified in both MRSA and MSSA isolates.

When combined with MLST and *spa* typing, the most prevalent type was ST59-t437 (13.6%, 24/176), followed by ST188-t189 (8.5%, 15/176), ST630-t4539 (5.1%, 9/176), and ST7-t091 (4.0%, 7/176). It was interesting to note a significant association between certain STs and *spa* types: ST59 was associated primarily with t437 (57.1%, 24/42), ST188 with t189 (100.0%, 15/15), ST338 with t437 (83.3%, 5/6), and ST630 with t4549 (69.2%, 9/13). Remarkably, all ST59-t437 belonged to MRSA isolates, whereas all ST188-t189 belonged to MSSA isolates, indicated that some of the certain molecular types were closely correlated to methicillin resistance.

Sixteen clonal complexes (CCs) were obtained in five years (Supplementary Figure). The most abundant CCs were CC59, CC1, and CC5, representing 55.7% of the isolates. Specifically, the top three CCs in the MSSA group were CC1 (28.4%), CC5 (18.9%), and CC7 (10.8%), while the most dominant CCs in the MRSA group were CC59 (52.0%), CC239 (14.7%), and CC5 (5.9%).

Through SCC*mec* typing, five SCC*mec* types were identified in all MRSA strains, and two additional isolates were non-typeable. SCC*mec* IV, the most epidemic cassette type identified in this study (n = 62, 60. 8%) followed SCC*mec* V (n = 29, 28.4%). Traditional hospital-associated SCC*mec* types I–III were found in 7.8% of isolates, SCC*mec* III (n = 5, 4.9%), and

SCC*mec* II (n = 3, 2.9%), but SCC*mec* type I was not observed. Almost all SCC*mec* V were associated with CC59, C239, CC398, and CC72. In contrast, all SCC*mec* III isolates were associated with CC239. Notably, one isolate carried the SCC*mec* type IX and belonged to ST398.

Association of Virulence Genes Among the Clonal Complexes

Toxin factors varied among the major 13 CCs (Table 5). The *pvl* gene was only found in CC22 (100%), CC88 (25%), CC398 (25%), CC59 (24.5%). The *tst* was considerably more common in CC72 and CC1 isolates. The prevalence of *sep* gene in CC7 and CC88 was significantly higher compared with other CCs . *seb* was mainly present in CC59 strains (96.2%), followed by CC25 (75.0%) and CC45 (60.0%). *seb, sek*, and *seq* were significantly higher in CC59 than in other CCs, *clfA* was considerably higher than in CC15. The *lukE/D* gene was more frequently possessed by isolates from the CC239 strains than by those from the CC59 (44.4% vs 0%), mainly existed in CC7, CC15, CC72, and CC88 isolates. Neither of the CC239 and CC7 isolates carried *chp*, and no CC1 found *pvl*-positive isolate. What's more, we found an MSSA isolate that carried *sek* and *seq* gene.

Discussion

S. aureus is considered one of the most representative pathogen that causes endemic and epidemic infections, and the wide distribution of *S. aureus* has been a severe problem worldwide. These epidemic *staphylococcal* strains have evolved, developing new genetic elements via horizontal gene transfer, and accumulated mutations that have altered gene expression and function.

The majority of the clinical MRSA cases were divided to the CC59 clone (ST59-IV/V-t439), a clone contributing to the most outbreaks of community-associated MRSA in China even in Asian.[21,22] However, while previous studies found ST239 to be a major clone,[23,24] only five of the MRSA isolates in this study were ST239. A recent study indicated that the ongoing replacement of ST239 by ST59 in China, is probably due to the carriage of the *chp* gene that enhances ST59 virulence activity.[25] Up now, the transmission success of ST59 clones in hospital settings remains unclear. Jin et al suggested that clonal transmission was putatively associated with the intrinsic physiological properties of ST59, rather than with its resistance to antibiotics.[26] ST630, the second most prevalent MRSA clone strain in this report, was also an emerging lineage in Nordic countries,[27] and has spread to China[28] and Japan.[29] In addition, six types of SCC*mec* were identified, of which type IV accounted for 60.8% of the MRSA isolates, suggesting that cassette is frequently transferred. This is probably because the smaller size and simpler genetic makeup of SCC*mec* type IV are advantageous.[30]

The present study showed that the most predominant STs in MSSA were ST188 (20.3%), ST7 (10.8%), and ST6 (6.8%). ST188, with its significantly higher bacterial adhesion and biofilm formation ability than other STs, remained the most frequent MSSA clone in *S. aureus* infections among children and adults in China.[31,32] Although most documented infections caused by ST188 have been linked to MSSA, ST188-MRSA has been reported.[33–35] It has been reported that simultaneous acquisition of SCC*mec* may contribute to the emergence of ST188-MRSA.[36] The diverse epidemiology between MRSA and MSSA might be associated with evolutionary processes that affect staphylococcal genetic.[37] Furthermore, we found that up to seven STs included both MSSA and MRSA. A study performed in a Chinese hospital observed that roughly 14.2% of the MSSA isolates shared a common genetic background with MRSA lineages.[38] These data support the notion that some MSSA lineages may provide a stable genomic environment for integrating the SCC*mec* element. Probably, compared to other lineages, these MSSA lineages possess fitness characteristics that promote their persistence in the host and additional potential transfer between hosts. Hence, this indicated that these MSSA isolates probably integrated the SCCmec element to promote MRSA infection and dissemination in communities and healthcare settings.

In vitro antimicrobial susceptibility testing of 176 isolates showed all strains were susceptible to linezolid and vancomycin, while the resistance rates observed for penicillin, erythromycin were over 56%. In the current study, the susceptibility rate of rifampicin in *S. aureus* was low (4.0%, 7/176). However, the resistance rate of *S. aureus* to rifampicin has been reported with increasing frequency in many regions.[39,40] 96.0% isolates of resistant to erythromycin carried one or more genes, which called *ermA, ermB, ermC*, and *mph*. According to previous studies, *erm* are major erythromycin resistance genes.[41] In our study, we found that the *ermB* gene was detected in 54.5% of erythromycin-resistant strains, while the *ermA* gene was present in 7.1% of resistant strains. The efflux proteins, encoded by *tetK* gene, was detected from 84.1%

Table 5 Frequencies of Virulence Genes Among Different CC Clones (%)

Molecular Type (n)	pvl	lukE/D	icaA	clfA	sdrC	sdrD	scn	chp	hly	hlb	hld	tst	sea	seb	sec	sed	seq	sek	sep
CC59(53)	24.5	0	100	88.7	77.4	0	100	100	96.2	100	100	1.9	17.0	96.2	1.9	0	96.2	96.2	0
CC1(26)	0	84.6	100	30.8	92.3	42.3	88.5	69.2	100.	7.7	100	30.8	7.7	23.1	46.2	0	23.1	23.1	0
CC5(19)	0	73.7	100	0	89.5	89.5	94.7	47.4	100	5.3	100	10.5	42.1	10.5	5.3	21.1	0	0	15.8
CC7(6)	0	100	100	66.7	100	100	100	0	100	0	100	0	0	0	0	0	0	0	100
CC15(4)	0	100	100	100	100	75.0	100	100	100	0	100	0	0	0	0	0	0	0	0
CC20(4)	0	0	100	0	100	100	100	100	100	0	100	0	0	0	0	0	0	0	0
CC22(3)	100	0	100	0	100	100	100	100	100	0	100	0	0	0	0	0	0	0	0
CC25(4)	0	100	100	0	100	100	100	100	100	0	100	0	0	75	25	0	0	0	0
CC45(5)	0	80	100	20	80	80	100	100	100	0	100	0	0	60	40	0	0	0	0
CC72(3)	0	100	100	0	100	66.7	100	33.3	100	0	100	33.3	0	0	100	0	0	0	0
CC88(8)	25.0	100	100	0	87.5	100	87.5	87.5	100	12.5	100	0	0	0	0	0	0	0	87.5
CC239(18)	0	44.4	88.9	72.2	38.9	38.9	27.8	0	100	72.2	100	0	27.8	0	0	0	27.8	27.8	0
CC398(8)	25.0	0	100	12.5	0	0	87.5	75.0	100	12.5	100	0	0	0	0	0	0	0	0

of tetracycline-resistant strains.[42] In addition, *tetL* and *tetM* genes are also resulted to tetracycline resistance, but their frequency is lower compared to *tetK*. Numerous studies have proved that WGS enables the rapid identification of infecting isolates and investigation of antimicrobial resistance determinants. Concordance between predicted antimicrobial resistance genotypes from WGS data and phenotypic susceptibility in *S. aureus* has been reported. In current study, there was a high correlation between phenotypic resistance and the presence of predicted genotype. A correlation above 95% was seen for all antimicrobials except penicillin and trimethoprim-sulfamethoxazole with a correlation of 92.9%, 93.6%.

In our study, differences in the contribution of virulence genes were found between the MSSA and MRSA strains. For the MRSA isolates, 64.7% carried the *sek* gene, while only 25.5% were *splA/B*-positive. However, only one MSSA isolate carried the *sek* gene, whereas 79.7% harbored the *splA/B* genes. *sek* is frequently reported to be present in MRSA strains and has been postulated to play a key role in the evolution of community lifestyle.[43] In the present study, serine proteases A and B (*splA* and *splB*) were significantly more prevalent in MSSA than MRSA isolates. This observation similar to previous reports, as these genes are often isolated from countries with a low prevalence of MRSA.[44]

lukE/D has been reported as one of the most important pore-forming toxin genes in *S. aureus* isolates, which lyses host cells and promotes virulence of the bacteria.[45,46] However, the characteristics of *lukE/D*-positive strains have not yet been well investigated in our region. According to our study, the *lukE/D* gene was detected in 78 isolates (44.3%; 51 MSSA and 27 MRSA), substantially lower than reported in previous reports.[47,48] It is of interest to note that the distribution of *lukE/D* in CC clones was different from that in the previous reports. According to previous reports, the toxin locus of *lukE/D* was present in CC1, CC59, CC5, and CC7 but completely absent from CC22, CC30, and CC398.[49,50] In this study, we did not find any CC59 (ST59) isolates that carried *lukE/D*, while the detection rate of CC59 strain *lukE/D* isolated by Liu et al was 100%.[34] In a study by He et al,[47] the detection rate of *lukE/D* in ST59 isolates was 66.7%. In addition, the prevalence of *lukE/D* was not present in CC20 and CC22 isolates, but was found in CC1, CC5, CC7, CC15, and CC88 isolates, which is vastly different from previous studies. One possible explanation for this difference is that *lukE/D* is the main virulence factor for *S. aureus* causing infection in the blood, however, the total number of blood samples in present MRSA isolates is relatively small, or it may be due to potential regional differences, and the relationship between *lukE/D* gene and MSSA infection requires further investigation. The study also found the discrepancies between the *tst* gene distribution and epidemic CCs in *S. aureus*. A multicenter study showed that roughly 31% of *S. aureus* isolates were test-positive in China, and the most predominant CCs were CC398, CC15 and CC188.[51] However, the *tst* gene was present in 8.0% of isolates and was mostly identified in CC1 in our collection, significantly higher than the CC1-*tst* carrier rate in other areas. This is probably due to geographic heterogeneity or the dissemination of CC1-*tst* strains in our hospital even in our region.

In summary, our results showed that, although MRSA was still the major problem of *S. aureus* infections, efforts to fight infections caused by MSSA need to be intensified due to MSSA isolates carrying wide range of virulence factors. Comparative epidemiological studies of large populations of MSSA and MRSA will be necessary in the future to understand how MSSA and MRSA populations may co-evolve and interact in the future.

Abbreviations
MRSA, Methicillin-resistant S. aureus; SCCmec, Staphylococcal cassette chromosome mec; MSSA, methicillin-susceptible S. aureus; WGS, whole-genome sequencing; MLST, multilocus sequence typing; STs, sequence types; CCs, clonal complexes.

Ethics Statement
As the *Staphylococcus aureus* clinical isolation in this study was part of the routine hospital laboratory procedure, we have confirmed that the isolate has no identifiable patient data, the Second Affiliated Hospital of Nanchang University Medical Research Ethics Committee exempted this research for review.

Author Contributions
All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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