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ORIGINAL RESEARCH

Genetic Diversity, Antibiotic Resistance, and Virulence Gene Features of Methicillin-Resistant *Staphylococcus aureus* Epidemics in Guiyang, Southwest China

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Purpose: Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common pathogens of community- and hospital-acquired infections, and its prevalence is increasing globally. Guiyang is the capital city of Guizhou Province, Southwest China; as the transport and tourism centre of Southwest China, Guizhou Province is bordered by Yunnan, Sichuan, Chongqing, and Guangxi Provinces. Although MRSA prevalence is increasing, little is known about its aspects in the area. The purpose of this study was to analyse MRSA molecular characteristics, antimicrobial resistance, and virulence genes in Guiyang.

Methods: In total, 209 MRSA isolates from four hospitals (2019–2020) were collected and analysed by antimicrobial susceptibility testing and molecular classification by the MLST, spa, and SCC*mec* typing methods. Isolate antibiotic resistance rates were detected by a drug susceptibility assays. PCR amplification was used to detect the virulence gene-carrying status.

Results: Twenty-four STs, including 4 new STs (ST7346, ST7347, ST7348, and ST7247) and 3 new allelic mutations, were identified based on MLST. The major prevalent ST type and clone complex were ST59 (49.8%) and CC59 (62.7%), respectively. *Spa* type t437 (42.1%) and SCC*mec* IV (55.5%) were identified by spa and SCC*mec* typing methods as the most important types. Drug sensitivity data showed that the multidrug resistance rate was 79.0%. There were significant differences in multidrug resistance rates and virulence gene-carrying rates for *seb, hla, hlb, cna* and *bap* between ST59 and non-ST59 types.

Conclusion: ST59-SCC*mec*IV-t437 is a major epidemic clone in Guiyang that should be monitored by local medical and health institutions. The situation differs from other adjacent or middle provinces of China, which may be due to the special geographical location of the region and the trend in antibiotic use or lifestyle. This study provides empirical evidence for local medical and health departments to prevent and control the spread of MRSA.

Keywords: methicillin-resistant *Staphylococcus aureus*, MRSA, multilocus sequence type, MLST, *Staphylococcal* protein A type, SPA, *Staphylococcal* chromosomal cassette *mec* type, SCC*mec*, multidrug-resistant organism, virulence gene

Introduction

Antimicrobial resistance (AMR) of clinical bacteria threatens human health. According to the latest data by the China Antimicrobial Surveillance Network (CHINET), antibiotic-resistant *Staphylococcus aureus* ranked third in the country's

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clinical detection rate in 2021, with methicillin-resistant *Staphylococcus aureus* (MRSA) comprising 30% of all antibiotic-resistant *S. aureus*.[1] MRSA was once strictly associated with healthcare settings, termed hospital-acquired MRSA (HA-MRSA), including hospitals, healthcare centres and hospital staff. However, since community-acquired MRSA (CA-MRSA) infection was first reported in the 1980s, CA-MRSA infection has gradually become the major type of MRSA infection.[2] In recent years, the AMR of HA-MRSA and CA-MRSA has changed.[3] Overall, MRSA has a higher mortality rate than methicillin-sensitive *S. aureus* (MSSA), leading to longer hospitalizations and higher associated treatment costs. Therefore, analysis of the molecular characteristics, antimicrobial resistance, and virulence gene profiles of MRSA isolates are very important for controlling outbreaks of high-antimicrobial resistance and high-virulence strains.[4] Moreover, such information will lay the foundation for developing new anti-MRSA agents.

Biofilm is an important factor of MRSA pathogenicity,[5] involving 4 stages: initial attachment, irreversible attachment, bacterial growth and ECM generation, and biofilm maturation.[6] eDNA (extracellular DNA), PIA (Polysaccharide Intercellular Adhesion), and CWA (Cell Wall Associated) proteins, nucleases and proteases participate in construction of the biofilm material matrix and are regulated by different signalling pathways.[7] Biofilm formation is a dynamic process. Once it matures, the bacteria encased are released and spread to a new site to form another biofilm. Thus, these bacteria are the main targets of many new anti-MRSA agents.[8–13] Additionally, biofilms are an obstacle for antibiotic resistance treatment of MRSA bacteria inside the biofilm, which is why MRSA strains are difficult clear from the infection site. Based on this, many novel anti-MRSA agents have been investigated. Most importantly, several detection methods[14,15] and vaccines for biofilms via polysaccharide intercellular adhesion (PIA) antigen or PIA-rSesC have been developed.[16–19]

A variety of molecular classification methods are helpful for determining the type of MRSA isolate. Multilocus sequence typing (MLST) consists of seven housekeeping genes (a*rcC, aroE, glpF, gmk, pta, tpi*, and *yqil*).[20] Among them, types with one or two different alleles can be classified as the same clonal complexes (CCs).[21–23] *Staphylococcal* protein A typing (*spa*) is based on amplification and sequencing of the surface protein A gene. *Staphylococcal* chromosomal cassette *mec* (SCC*mec*) is a mobile genetic element used for typing. To date, 14 different types of SCC*mec* elements have been found.[24,25]

During *S. aureus* infection, it produces a quantity of virulence factors, often leading to toxin-mediated diseases, including toxic shock syndrome, staphylococcal food-borne diseases, and scalded skin syndrome.[26,27] Exotoxins are classified into three categories based on known functions: cytotoxins, superantigens, and cytotoxic enzymes.[21,28] These include haemolysin (Hl), Panton-Valentine leukocidin (PVL), staphylococcal enterotoxin (Se), toxic shock syndrome toxin-1 (TSST-1), fibronectin-binding protein (Fnb), intracellular adhesin (Ica), collagen adhesin (Cna), and biofilm-associated protein (Bap).[28,29] These exotoxins jointly regulate the host immune system and cell adhesion and play a vital role in *S. aureus* infection.[28]

In recent years, the global prevalence of MRSA has been changing, resulting in different epidemic clones and antimicrobial resistance profiles in different regions and at different times, with regional variability.[30] Guizhou Province is bordered by Yunnan, Sichuan, Chongqing, and Guangxi Provinces in southwestern China. However, no multicentre study on MRSA isolates has been carried out in this area. In this study, MLST, *spa*-type, and SCC*mec* methods were used for the first time to analyse MRSA isolates from provincial, city, and county hospitals to detect the main epidemic MRSA strains in Guiyang. The antimicrobial resistance and virulence genes of these isolates were also investigated. The findings provide a guiding reference for the clinical treatment of MRSA-related infections.

Materials and Methods

Clinical Bacterial Isolate Collection, Culture, and Genomic DNA Extraction

All MRSA strains were isolated and banked in 4 hospitals (including 3 tertiary teaching hospitals) in Guiyang from 2019 to 2020: The First Affiliated Hospital of Guizhou Medical University, The First People's Hospital of Guiyang, Guihang Guiyang Hospital, and People's Hospital of Kaiyang. All isolates were first identified using Gram staining and coagulase and catalase tests. Then, they were further identified using a Mérieux automated bacterial tester, VITEK 2 AST-GP67 Test Kit, and *mecA* gene testing with PCR amplification. All isolates were stored at −80°C for further experiments. After phenotype and genotype identification, 209 MRSA isolates were ultimately identified and included in the research. The sample sources of these isolates included cutaneous abscesses and wound secretions (n=76, 36.4%), sputum and pharynx

swabs (n=113, 45.1%), blood (n=9, 4.3%), and others (catheter tip, pleural fluid, drainage liquid, ascites, joint fluid, and urine) (n=11, 5.3%) (according to laboratory department statistics data). It should be clarified that all clinical MRSA strains were obtained from the laboratory department in these hospitals. The clinical samples were not specifically isolated for this research. Thus, all these strains were obtained as part of the routine hospital laboratory procedure. We did not collect samples from patients directly at all. Before we took the stock strains from the laboratory department of hospital, all personal information was removed for patient protection.

All primers used in the present study were synthesized by Sangon Biotech (Shanghai) Co., Ltd., as listed in Table 1. Any strain with PENG and CFX (cefoxitin) resistance and *mec*A positivity was considered an MRSA strain. All strains

Table 1 Primers Used in This Study

Primer	Nucleotide Sequence (5′-3′)	Amplicon Size (bp)	Reaction Condition	References
Arc-F	TTGATTCACCAGCGCGTATTGTC	570	[a]	[31]
Arc-R	AGGTATCTGCTTCAATCAGCG			
Aro-F	ATCGGAAATCCTATTTCACATTC	536	[a]	
Aro-R	GGTGTTGTATTAATAACGATATC			
Glp-F	CTAGGAACTGCAATCTTAATCC	576	[a]	
Glp-R	TGGTAAAATCGCATGTCCAATTC			
Gmk-F	ATCGTTTTATCGGGACCATC	488	[a]	
Gmk-R	TCATTAACTACAACGTAATCGTA			
Pta-F	GTTAAAATCGTATTACCTGAAGG	575	[a]	
Pta-R	GACCCTTTTGTTGAAAAGCTTAA			
Tpi-F	TCGTTCATTCTGAACGTCGTGAA	475	[a]	
Tpi-R	TTTGCACCTTCTAACAATTGTAC			
Yqi-F	CAGCATACAGGACACCTATTGGC	598	[a]	
Yqi-R	CGTTGAGGAATCGATACTGGAAC			
MecA-F	GTGAAGATATACCAAGTGATT	147	[b]	[57]
MecA-R	ATGCGCTATAGATTGAAAGGAT			
SCCmecI-F	GCTTTAAAGAGTGTCGTTACAGG	613	[c]	[34,58]
SCCmecI-R	GTTCTCTCATAGTATGACGTCC			
SCCmecII-F	CGTTGAAGATGATGAAGCG	398	[c]	
SCCmecII-R	CGAAATCAATGGTTAATGGACC			
SCCmecII-F2	TAGCTTATGGTGCTTATGCG	128	[c]	
SCCmec CII-R2	GTGCATGATTTCATTTGTGGC			
SCCmecIII-F	CCATATTGTGTACGATGCG	280	[c]	
SCCmecIII-R	CCTTAGTTGTCGTAACAGATCG			
SCCmec III-F5	GAAACTAGTTATTTCCAACGG	257	[c]	
SCCmec III-R6	GTGTAATTTCTTTTGAAAGATATGG			

(*Continued*)

Table 1 (Continued).

Primer	Nucleotide Sequence (5′-3′)	Amplicon Size (bp)	Reaction Condition	References
SCCmecIVa-F	GCCTTATTCGAAGAAACCG	776	[c]	
SCCmecIVa-R	CTACTCTTCTGAAAAGCGTCG			
SCCmecIVb-F	TCTGGAATTACTTCAGCTGC	493	[c]	
SCCmecIVb-R	AAACAATATTGCTCTCCCTC			
SCCmecIVc-F	ACAATATTTGTATTATCGGAGAGC	200	[c]	
SCCmecIVc-R	TTGGTATGAGGTATTGCTGG			
SCCmec IVc-F2	CCTGAATCTAAAGAGATACACCG	200	[c]	
SCCmec IVc-R2	GGTTATTTTCATAGTGAATCGC			
SCCmecIVd-F	CTCAAAATACGGACCCCAATACA	881	[c]	
SCCmecIVd-R	TGCTCCAGTAATTGCTAAAG			
SCCmec IVe-F3	CAGATTCATCATTTCAAAGGC	175	[c]	
SCCmec IVe-R4	AACAACTATTAGATAATTTCCG			
SCCmecV-F	GAACATTGTTACTTAAATGAGCG	325	[c]	
SCCmecV-R	TGAAAGTTGTACCCTTGACACC			
mecI-F	CGTTATAAGTGTACGAATGGTTTTTG	126	[d]	[24]
mecI-R	TCATCTGCAGAATGGGAAGTT			
ccrB4-F	CGAAGTATAGACACTGGAGCGATA	134	[d]	
ccrB4-R	CGACTCTCTTGGCGTTTA			
IS1272J-F	GAAGCTTTGGGCGATAAAGA	98	[d]	
IS1272J-R	GCACTGTCTCGTTTAGACCAATC			
SCCmec VII-F	GTGACGTTGATATTGCAGTGGT	473	[d]	
SCCmec VII-R	TGAAGAAGTTTGTTCCGCGT			
SCCmec VIII-F	AGCGACGATGAACAACACCGCTACTTACTCAA	138	[d]	
SCCmec VIII-R	TTGGTTGAGAATGAGAACAGTGGTAAGATC			
SCCmec IX-F	TGGCATGGTTGATAGAACAGTG	642	[e]	
SCCmec IX-R	TCACTAATTTTGCCTCACGTCT			
SCCmec X-F	ATTTACGCCGATGCGTTGAC	708	[e]	
SCCmec X-R	TATGCGATTGCGCAGGTGAT			
SCCmec XI-F	GGCGATACAACGACACATCC	255	[e]	
SCCmec XI-R	TGTTAGTGCTTGACCGCTCTT			
SCCmec XII-F	AGAAGACGGAGGACATCGACA	371	[e]	
SCCmec XII-R	TCGCTTCTTCAACGCCATCTT			

(*Continued*)

Table 1 (Continued).

Primer	Nucleotide Sequence (5′-3′)	Amplicon Size (bp)	Reaction Condition	References
spa-F	GCCAAAGCGCTAACCTTTTA	600	[f]	[32]
spa-R	CTCCAGCTAATAACGCTGCAC			
fnbA-F	GCGGAGATCAAAGACAA	1279	[f]	[59]
fnbA-R	CCATCTATAGCTGTGTGG			
fnbB-F	GTAACAGCTAATGGTCGAATTGATACT	524	[g]	[60]
fnbB-R	CAAGTTCGATAGGAGTACTATGTTC			
pvl(lukS-PV/lukF-PV)-F	TCATTAGGTAATAAAATGTCTGGACATGATCCA	433	[g]	[61]
pvl(lukS-PV/lukF-PV)-R	GCATCAAATGTATTGGATAGCAAAAGC			
sea-F	GAAAAAAGTCTGAATTGCAGGGAACA	560	[g]	[60]
sea-R	CAAATAAATCGTAATTAACCGAAGGTTC			
seb-F	ATTCTATTAAGGACACTAAGTTAGGGA	404	[g]	
seb-R	ATCCCGTTTCATAAGGCGAGT			
hla-F	CTGATTACTATCCAAGAAATTCGATTG	209	[g]	[62]
hla-R	CTTTCCAGCCTACTTTTTTATCAGT			
hlb-F	GTGCACTTACTGACAATAGTGC	309	[g]	
hlb-R	GTTGATGAGTAGCTACCTTCAGT			
tsst-F	AGCATCTACAAACGATAATATAAAGG	481	[g]	[60]
tsst-R	CATTGTTATTTTCCAATAACCACCCG			
icaA-F	ACACTTGCTGGCGCAGTCAA	188	[g]	[63]
icaA-R	TCTGGAACCAACATCCAACA			
icaD-F	ATGGTCAAGCCCAGACAGAG	198	[g]	
icaD-R	AGTATTTTCAATGTTTAAAGCAA			
cna-F	AGTGATGTTTCGGGATTTG	285	[g]	This study
cna-R	TAACTGCTGTCCACCTTGA			
bap-F	CCCTATATCGAAGGTGTAGAATTGCAC	971	[f]	
bap-R	GCTGTTGAAGTTAATACTGTACCTGC			

Notes: [a]Amplification comprised 4 min at 94°C, followed by 35 cycles of 30s at 94°C, 30s at 56°C and 30s at 72°C, with a final extension for 10 min at 72°C. [b]Amplification comprised 4 min at 94°C, followed by 35 cycles of 30s at 94°C, 30s at 50°C and 45s at 72°C, with a final extension for 10 min at 72°C. [c]Thermocycling conditions were set at 94°C for 5 min, followed by 10 cycles of 94°C 45s, 65°C 45s, 72°C 1.5 min. A further 25 cycles of 94°C 45s, 52°C 45s, 72°C 2 min were followed by a 10 min incubation at 72°C and a hold at 4°C. [d]Amplification comprised 2 min at 95°C, followed by 35 cycles of 30s at 95°C, 60s at 54°C and 80s at 72°C, with a final extension for 7 min at 72°C. [e]Amplification comprised 2 min at 95°C, followed by 32 cycles of 30s at 95°C, 60s at 47°C and 80s at 72°C, with a final extension for 7 min at 72°C. [f]Amplification comprised 5 min at 95°C, followed by 35 cycles of 45s at 95°C, 45s at 60°C and 90s at 72°C, with a final extension for 10 min at 72°C. [g]Amplification comprised 5 min at 94°C, followed by 35 cycles of 45s at 94°C, 55s at 58°C and 60s at 72°C, with a final extension for 10 min at 72°C.

were cultured by streaking onto sheep blood agar culture plates and growing for 16–18 h overnight. Then, single colonies were selected and inoculated into liquid medium. After 12 h of growth, MRSA DNA was extracted using a bacterial genomic DNA rapid extraction kit. The DNA obtained was dissolved in 100 μL of TE buffer and stored at −20°C. *S. aureus* ATCC25923, ATCC25913, and ATCC43300 were used as quality control strains.

MLST, spa, and *SCCmec* Typing

MLST Typing

According to the standard protocol of primer design and PCR amplification conditions,[31] 7 housekeeping gene fragments (*arcC, aroE, glpF, gmk, pta, tpi*, and *yqil*) of each MRSA isolate were amplified and sequenced (Sangon Biotech (Shanghai) Co., Ltd.). These sequences were submitted to the MLST database (https://pubmlst.org/) and analysed for allele numbers or ST type. Unique sequences of MRSA strains that could not be compared to any known ST types were submitted to the MLST database and assigned as new alleles or ST types. In this study, 4 isolates could not be assigned to any known ST; these novel alleles were submitted to the MLST database, and 3 new alleles were assigned, namely, arcC (845), glpF (900), and pta (857). (https://pubmlst.org/bigsdb?db=pubmlst_saureus_seqdefandpage=alleleInfoandlocus=arcCandallele_id=845, arcC:845; https://pubmlst.org/bigsdb?db=pubmlst_saureus_seqdefandpage=alleleInfoandlocus=glpFandallele_id=900, glpF:900; https://pubmlst.org/bigsdb?db=pubmlst_saureus_seqdefandpage=alleleInfoandlocus=ptaandallele_id=857, pta:857.). At the same time, 4 new STs were identified: ST7346, ST7347, ST7348 and ST7247 (Figure 1).

By clustering analysis, isolate strains with 6 identical allelic loci were defined as clonal complexes (CCs).

spa Typing

Similar as above, typing was obtained by amplifying[32] and sequencing the variable region (X) of the MRSA *spa* gene in different strains. Then, the sequence was submitted to the *spa* type database (http://spatyper.fortinbras.us/) for *spa* typing.

SCC*mec* Typing

MRSA isolate strains were classified by multiplex PCR[24,33,34] and agarose gel electrophoresis for SCC*mec* type. According to the literature, *S. aureus* can be identified as I–XIV types.[25] Some of the MRSA isolates that could not be classified as any known SCC*mec* type were defined as non-type (NT).

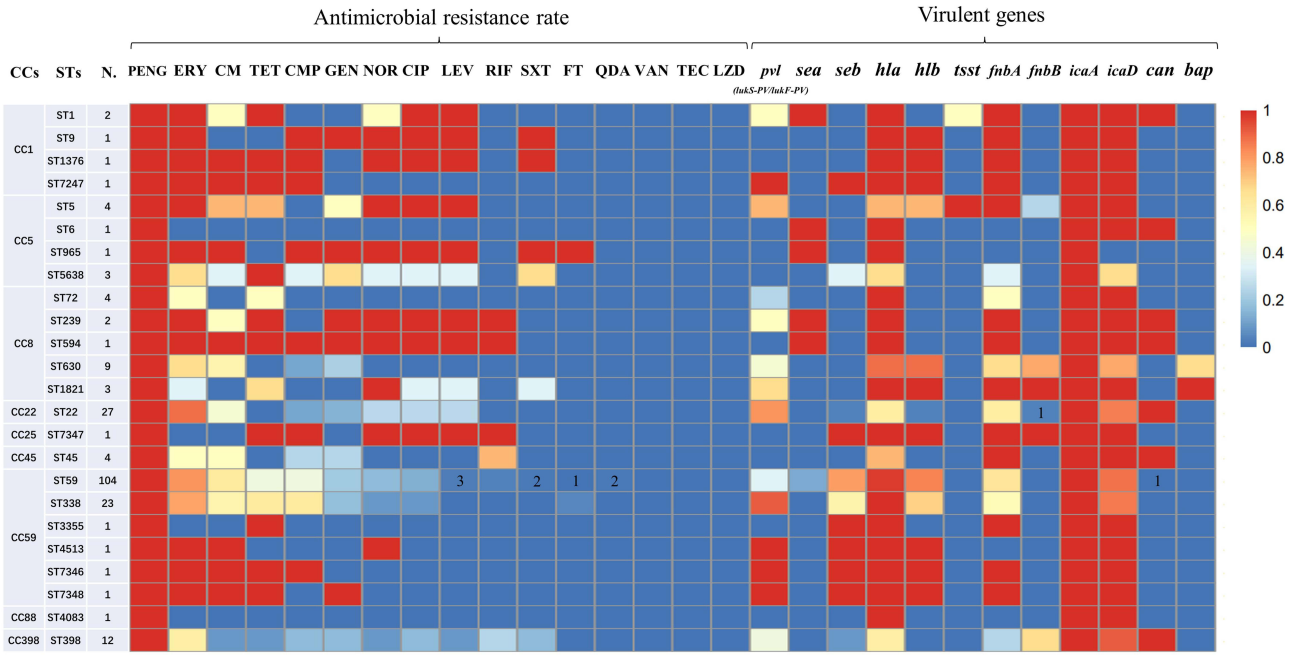

Figure 1 Virulence genes and antimicrobial resistance rates of MRSA clinical isolates linked to STs. Antibiotic and virulent genes were detected in less than 3% of isolates with a particular ST; the number of MRSA isolates is given.

Antibiotic Susceptibility Test

Antibiotic susceptibility testing was conducted for all *S. aureus* isolates using VITEK 2 AST-GP67 Test Kit (Compact system) and the Kirby-Bauer disc diffusion method (Oxoid) according to the guidelines of the Clinical and Laboratory Standard Institute (CLSI) M100-S29, 2020. The antibiotics tested were FT, SXT, CIP, CM, QDA, CMP, NOR, GEN, LEV, ERY, RIF, LZD, TET, TEC, VAN, PENG and CFX (all antibiotic abbreviations are shown in the abstract). Isolates resistant to three or more antimicrobial agents were considered multidrug-resistant strains.[35]

Detection of Virulence Genes

The 12 virulence factor gene fragments of MRSA isolates were screened using independent PCR assays, including *sea, seb, pvl* (lukS-PV/lukF-PV), *tsst, hla, hlb, fnbA, fnbB, icaA, icaD, cna* and *bap*. The PCR mixtures contained 1 μL DNA template, 2 μL primers (10 μM), 12.5 μL 2×Taq Master Mix (GenStar, China), and 9.5 μL double-distilled water. All PCR products were detected by 1.0% agarose gel electrophoresis. One of the PCR products was identified as a positive control by sequencing and sequence alignment analysis. All the primers used in this study are listed in Table 1.

Statistical Analysis

In this study, SPSS 26.0 software was used for statistical analysis of experimental data. A P value <0.05 was considered statistically significant. WHONET software was used to analyse antimicrobial sensitivity data. Minimum spanning tree diagram analysis was performed using goeBURST software.

Ethical Approval

This study was approved by the People's Hospital of Kaiyang, Guizhou Medical University Teaching Hospital management (20190203001).

Results

Molecular Typing of MRSA Strains in Guiyang

All 209 MRSA clinical isolates were successfully typed for MLST and assigned to 24 STs (sequence types). The specific results of the MLST typing are provided in Table 2. The most prevalent ST type was ST59 (49.76%, 104/209), followed by ST22 (12.9%, 27/209), ST338 (11.0%, 23/209), ST398 (5.7%, 12/209) and ST630 (4.3%, 9/209). All STs were identified as belonging to 9 clonal complexes (CCs) by goeBURST and the International MLST database. As shown in Figures 1–3 and Table 2, CC59 62.7% (131/209) was the most prevalent CC, followed by CC22 12.9% (27/209), CC8 9.1% (19/209), CC398 5.7% (12/209) and CC5 4.3% (9/209). In addition, 4 isolates could not be assigned to any known ST. These novel alleles were submitted to the MLST database, and 3 new alleles were assigned: *arc* (845), *glpF* (900), and *pta* (857). Four new STs were also identified: ST7346, ST7347, ST7348 and ST7247 (Figure 1).

In total, 36 *spa* types were found by *spa* typing. The most prevalent was t437 42.1% (88/209), followed by t309 9.1% (19/209), t441 6.7% (14/209), t4549 6.7% (14/209) and t172 5.3% (11/207). The t032 and t034 types were detected in 7 isolates, and t2460, t3485 and t3523 were identified in four isolates each (Table 2).

SCC*mec* typing was performed successfully for 181 of the 209 MRSA isolates (86.6%). Six SCC*mec* types, namely, types I, II, III, IV, V, and XII, were detected. The most common SCC*mec* type was IV, which was observed in 116 isolates 55.5% (116/209), and the second most common SCC*mec* type was II 20.1% (42/209). Only 1 isolate was SCC*mec* XII 0.5% (1/209), 4 isolates were SCC*mec* III 1.9% (4/209), 6 isolates were SCC*mec* I 2.9% (6/209) and 12 isolates were SCC*mec* V 5.7% (12/209). In addition, 28 isolates were classified as NT (non-type) by SCC*mec* typing (Table 2).

Antimicrobial Susceptibility of MRSA Strains in Guiyang

According to antimicrobial sensitivity testing, all 209 MRSA clinical isolates were susceptible to VAN, TEC, and LZD. However, no MRSA isolate was susceptible to PENG or CFX. The antimicrobial resistance profiles of the MRSA isolates are shown in Table 3. The multidrug resistance (MDR) rate among all MRSA isolates was 79.0%.

Table 2 The MLST, *spa* and SCC*mec* Typing of MRSA Isolate Strains

CCs (n=209)	CC (%)	MLST (n=209)	MLST (%)	*spa* (n=209)	*spa* (%)	SCC*mec* (n=209)	SCC*mec* (%)
CC59 (131)	62.7	ST59 (104)	49.8	t437(68),t172(11),t441(10), tt3523(4),tt3485(3),t3401(2), t163(1),tt347(1),t1212(1), t3385(1),t3590(1),t7501(1)	t437 42.1% (88), t309 9.1% (19), t441 6.7% (14), t4549 6.7% (14), t172 5.3% (11), t032 3.3% (7), t034 3.3% (7), t2460 1.9% (4), t3485 1.9% (4), t3523 1.9% (4), t030 1.4% (3), t116 1.4% (3), t899 1.4% (3), t011 1.0% (2), t347 1.0% (2), t664 1.0% (2), t3401 1.0% (2), t3590 1.0% (2), t015 0.5% (1), t062 0.5% (1), t111 0.5% (1), t114 0.5% (1), t148 0.5% (1), t163 0.5% (1), t311 0.5% (1), t324 0.5% (1), t349 0.5% (1), t588 0.5% (1), t688 0.5% (1), t1212 0.5% (1), t1255 0.5% (1), t1751 0.5% (1), t2310 0.5% (1), t3385 0.5% (1), t7501 0.5% (1), t8045 0.5% (1).	I(2),II(9),IV(89),NT(4)	SCC*mec* IV 55.5% (116), SCC*mec* II 20.1% (42), SCC*mec* V 5.7% (12), SCC*mec* I 2.9% (6), SCC*mec* III 1.9% (4), SCC*mec* XII 0.5% (1), SCC*mec* NT 13.4% (28).
		ST338 (23)	11.0	t437(18),t347(1),t441(2), t1751(1),t3590(1)		II(15),IV(2),V(3),NT(3)	
		ST3355 (1)	0.5	t437(1)		IV(1)	
		ST4513 (1)	0.5	t437(1)		IV(1)	
		ST7346 (1)	0.5	t441(1)		IV(1)	
		ST7348 (1)	0.5	t441(1)		IV(1)	
CC22 (27)	12.9	ST22 (27)	12.9	t309(19),t032(7),t8045(1)		II(7),IV(8),V(1),NT(11)	
CC8 (19)	9.1	ST630 (9)	4.3	t4549(9)		II(4),V(2),NT(3)	
		ST72 (4)	1.9	t664(2),t148(1),t324(1)		I(1),IV(2),V(1)	
		ST1821 (3)	1.4	t4549(3)		V(2),NT(1)	
		ST239 (2)	1.0	t030(2)		III(2)	
		ST594 (1)	0.5	t030(1)		III(1)	
CC398 (12)	5.7	ST398 (12)	5.7	t034(7),t011(2),t588(1),t1255(1),t4549(1)		I(2),II(3),III(1),V(1),NT(5)	
CC5 (9)	4.3	ST5 (4)	1.9	t2460(4)		II(4)	
		ST5638 (3)	1.4	t111(1),t311(1),t688(1)		IV(1),V(2)	
		ST6 (1)	0.5	t3485(1)		IV(1)	
		ST965 (1)	0.5	t062(1)		IV(1)	
CC1 (5)	2.4	ST1 (2)	1.0	t114(1),t4549(1)		IV(2)	
		ST9 (1)	0.5	t899(1)		XII(1)	
		ST1376 (1)	0.5	t899(1)		IV(1)	
		ST7247 (1)	0.5	t899(1)		IV(1)	
CC45 (4)	1.9	ST45 (4)	1.9	t116(3),t015(1)		IV(4)	
CC25 (1)	0.5	ST7347 (1)	0.5	t349(1)		NT(1)	
CC88 (1)	0.5	ST4083 (1)	0.5	t2310(1)		I(1)	

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; MLST, Multilocus sequence typing; *spa, Staphylococcal* protein A; SCC*mec, Staphylococcal* chromosome cassette *mec*; NT, non-type; ST; sequence type; CC, Clonal Complex.

Virulence Gene Profile of MRSA Strains in Guiyang

In total, 209 MRSA clinical isolates were amplified with primers for 12 virulence genes by PCR amplification. The detection frequency of each virulence gene is shown in Figure 1. Among them, the detection frequencies of the cytotoxic genes *hla, hlb*, and *pvl* (lukS-PV/lukF-PV) were 89.0%, 60.3%, and 47.8%, respectively. The detection rates of the staphylococcal superantigen genes *sea, seb*, and *tsst* were 9.6%, 49.8%, and 2.4%, respectively. Of the genes associated with biofilm formation, the intracellular adhesion molecule genes *icaA* and *icaD* were present in 100.0% and 88.0% of the isolates, respectively. Detection rates of the *fnbA, fnbB*, and *cna* microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) genes were 61.7%, 9.6%, and 23.9%, respectively. The *bap* gene was detected in 9 isolates (4.3%). Interestingly, 119 isolates (56.9%) carried 6 or more virulence genes (Figure 1 and Table 4).

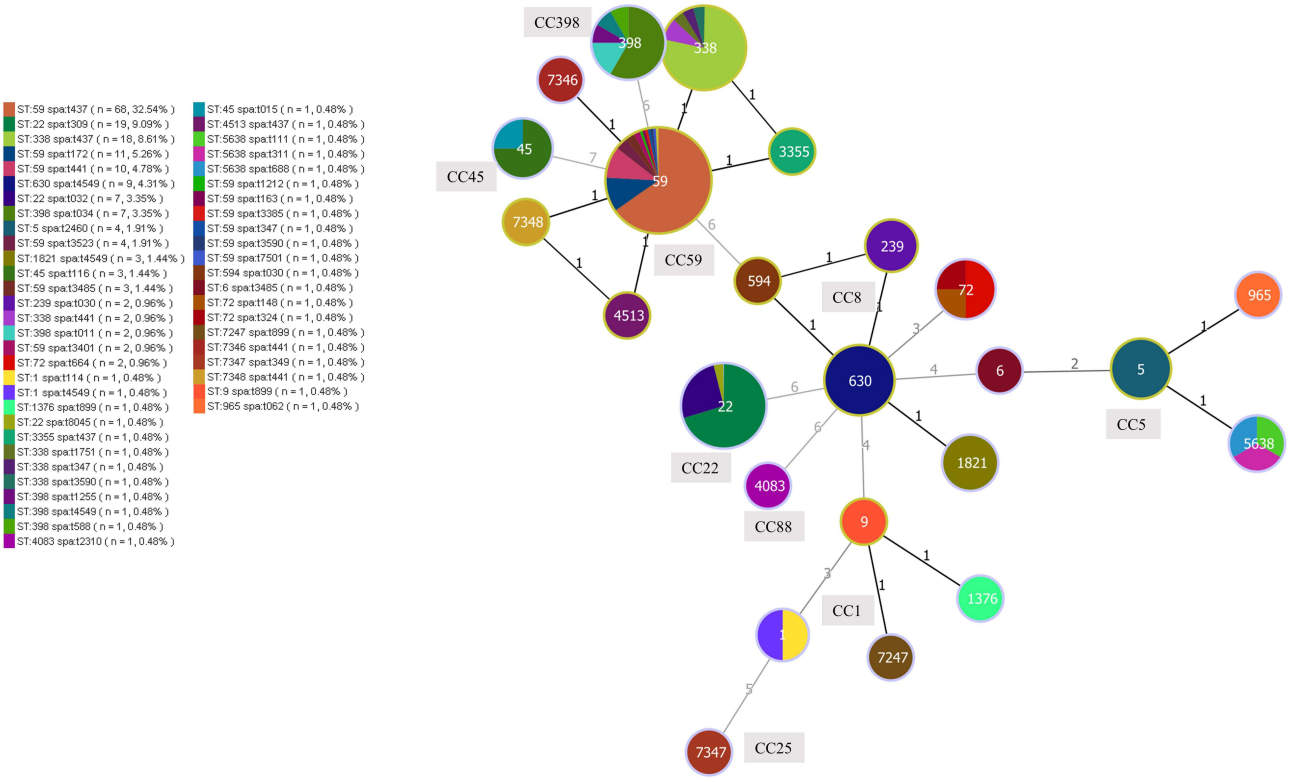

Figure 2 Minimum spanning tree constructed by goeBURST based on the MLST data of this study. The number between lines indicates locus differences. The size of each node corresponds to the number of strains. The colour partition of each disc corresponds to the proportion of the SPA types. Figures on the nodes are ST numbers.

Correlation and Difference Between *MLST*, spa *Type*, *SCCmec Type*, Antimicrobial Susceptibility, and Virulence Genes of MRSA in Guiyang

Intriguingly, there was a strong correlation between the main ST and *spa* types among the MRSA isolates (Figure 1 and Table 2). ST59-t437 65.4% (68/104), ST338-t437 78.3% (18/23), ST22-t309 70.4% (19/27), ST630-t4549 100.0% (9/9), ST398-t034 58.3% (7/12) and ST5-t2460 100.0% (4/4) were strongly associated types. The majority (76.2%, 89/116) of the SCC*mec* IV MRSA isolates were ST59, and SCC*mec* II was primarily associated with ST338 (31.0%, 13/42). Comprehensive analysis of MLST, *spa* type, and SCC*mec* typing the results identified ST59-SCC*mec* IV-t437 27.8% (58/209) as the major clone among the MRSA isolates.

The multidrug resistance of the 209 MRSA isolates mainly concentrated around classes 3–5 antimicrobials (69.9%, 146/209). CC8-t030 and CC1-t899 exhibited high-intensity multidrug resistance to 6–8 classes of antimicrobials. Among the 209 MRSA isolates, only 2 ST59 isolates were resistant to QDA. The ST59 isolates found among the 209 MRSA isolates were less resistant to LEV than CIP and NOR among fluoroquinolones. Moreover, ST45, ST72 and ST630 isolates were completely resistant to fluoroquinolones, whereas ST22, ST45, and ST630 isolates were completely sensitive to TET (Table 5).

According to the virulence gene detection test, all ST22, ST398, ST45, and ST239 isolates carried the *cna* gene. However, the *bap* gene was only detected in the 4 CC8-t4549 isolates. CC59 isolates did not carry the *fnbB* gene. The *pvl* (lukS-PV/lukF-PV) gene encompassed multiple STs, among which the ST22 (22/27) and ST338 (21/23) isolates were the two types with the highest rate of carriage (Table 6). The detection rate of the *seb* gene was higher than that of the *sea* gene, which was mainly carried by CC59 isolates. Although the detection rate of the *tsst* gene was low, ST5-t2460 isolates had a high rate of the *tsst* gene positivity.

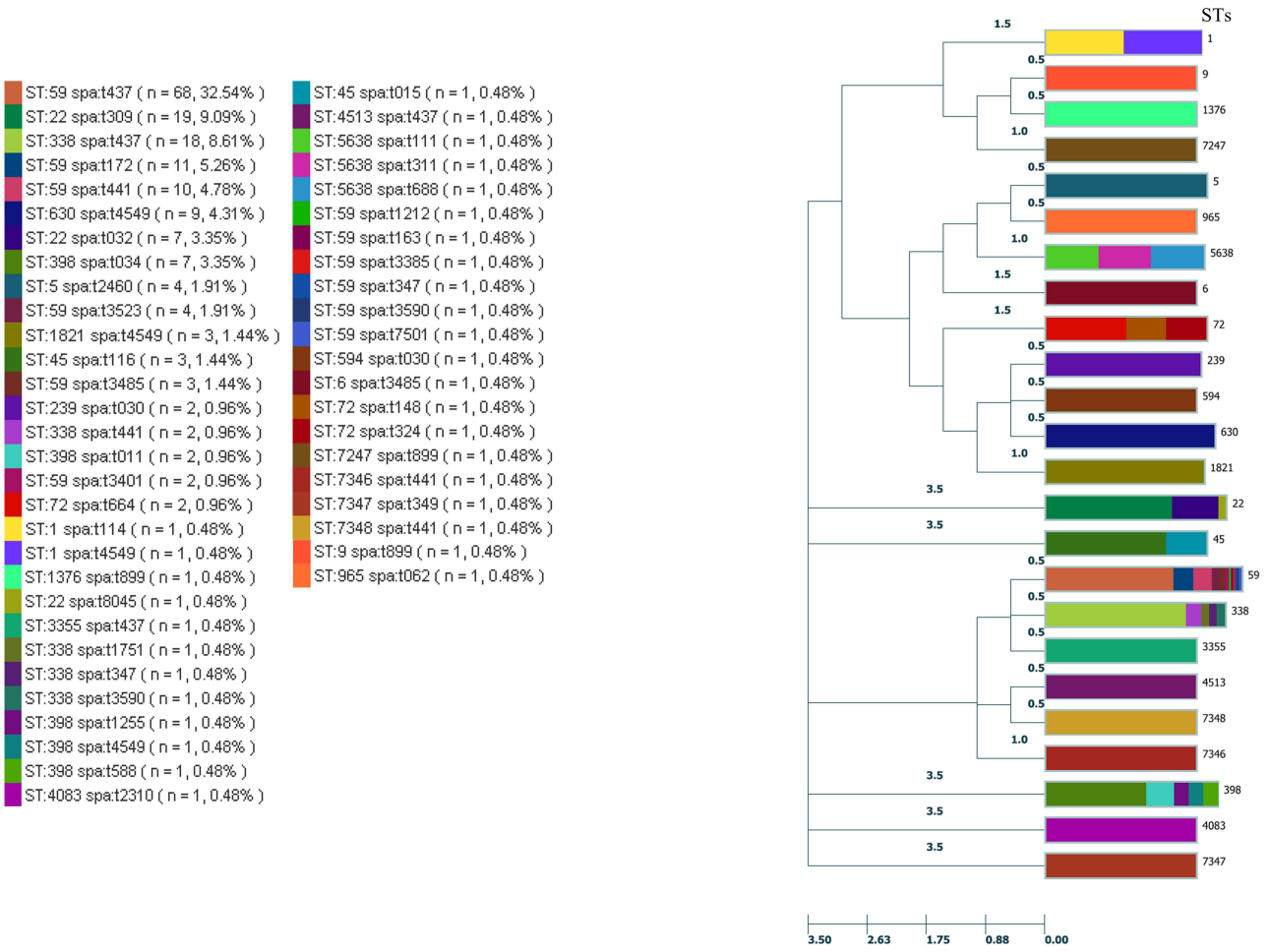

Figure 3 Dendrogram constructed based on the MLST data of this study.

Comparation of ST59 and Non-ST59 MRSA Strains in Guiyang

The antimicrobial resistance rate and virulence gene-carrying status of ST59 isolates and non-ST59 isolates were analysed. The MDR rate of the ST59 isolates was higher than that of non-ST59 isolates, and the ST59 isolates showed higher resistance rates to CM and CMP and lower resistance rates to CIP and LEV. Nonetheless, no significant difference in resistance rate to any other antibiotics was found between ST59 and non-ST59 isolates. Among the 12 tested virulence genes, *seb, hla*, and *hlb* were more frequent in ST59 isolates than in non-ST59 isolates. In contrast, the detection rates of *pvl* (lukS-PV/lukF-PV), *fnbB, cna*, and *bap* in ST59 isolates were lower. Detection rates of the other virulence genes were not significantly different between the two isolate types (Table 6).

Discussion

Increasing antibiotic resistance is a global public health problem. MRSA prevalence has increased in recent years.[4,23,27,36–38] In addition, antibiotic resistance and MRSA prevalence trends change dynamically according to different medication use habits and time periods. Thus, monitoring the prevalence and antibiotic resistance as well as timely updates are valuable and essential. This study focused on the molecular characteristics, antimicrobial resistance, and virulence gene profiles of 209 MRSA isolates in Guiyang, a multi-ethnic city in Southwest China, from 2019 to 2020.

The results showed that the most common MLST type was CC59-ST59, and ST59-SCC*mec* IV-t437 was identified as the main common clone type in Guiyang (Figure 2). According to the literature, 5 CCs are most reported among HA-MRSA strains around the world, namely, CC5, CC8, CC22, CC30, and CC45.[30] CC8-ST239, CC5-ST5, and CC22-ST22 are also the most common in Asian countries.[39] Regarding the CA-MRSA epidemic, CC5, CC8, CC22, CC30, CC59, and

Table 3 The Distribution of Antimicrobial Resistance of 209 MRSA Isolate Strains

Antibiotic[a]	Quantity[b] N (n=209)[c]	Antimicrobial Resistance Rate (%)
Penicillin G	209	100.0
Erythromycin	163	78.0
Clindamycin	109	52.2
Tetracycline	77	36.8
Chloramphenicol	72	34.5
Gentamicin	45	21.5
Norfloxacin	45	21.5
Ciprofloxacin	41	19.6
Levofloxacin	26	12.4
Rifampicin	14	6.7
Trimethoprim/sulfamethoxazole	10	4.8
Nitrofurantoin	3	1.4
Quinupristin-dalfopristin	2	1.0
Vancomycin	0	0.0
Teicoplanin	0	0.0
Linezolid	0cc	0.0

Notes: [a]Names of different antibiotics. [b]The number of 209 MRSA isolate strains resistant to the same antimicrobial. [c]Analysis of antimicrobial resistance of 209 MRSA isolate strains.

Table 4 The Frequencies of Virulence Genes of 209 MRSA Isolate Strains

Virulent Genes[a]	Quantity[b] (n=209)[c]	Virulence Gene Carrying Rate (%)
pvl(lukS-PV/lukF-PV)	100	47.9
sea	20	9.6
seb	104	49.8
hla	186	89.0
hlb	126	60.3
tsst	5	2.4
fnbA	129	61.7
fnbB	20	9.6
icaA	209	100.0
icaD	184	88.0
cna	50	23.9
bap	9	4.3

Notes: [a]Names of different virulent genes. [b]The number of virulence genes carried by 209 MRSA isolate strains. [c]Analysis of virulence genes of 209 MRSA isolate strains.

Abbreviations: *pvl(lukS-PV/lukF-PV)*, the panton-valentine leukocidin gene; *sea*, staphylococcal enterotoxins a gene; *seb*, staphylococcal enterotoxins b gene; *hla*, the hemolysin a gene; *hlb*, the hemolysin b gene; *tsst*, the toxic shock syndrome toxin gene; *fnbA*, the fibrinogen-binding protein A gene; *fnbB*, the fibrinogen-binding protein B gene; *icaA*, the intracellular adhesion molecule A gene; *icaD*, the intracellular adhesion molecule D gene; *cna*, the collagen adhesion gene; *bap*, the biofilm-associated protein gene.

CC398 were the most common. CC8-ST8 (USA300) is common in the United States.[40] CA-MRSA comprises CC22-ST22 and CC30-ST36 in Britain,[41] CC30-ST30 in Australia,[42] and CC59-ST59 in East Asia.[39] CC59-ST59 is also common in Taiwan, Hong Kong, Vietnam, and Sri Lanka.[43] However, the main types of inland China differ. For example, predominant types are CC398-ST398 and CC5-ST5 in Shanghai (eastern China), CC59-ST338 and CC8-ST239 in Guangdong (southern China), CC8-ST239 in Wuhan (central China), CC5-ST5, CC8-ST239, and CC59-ST59 in Zhejiang (south-eastern China), and CC45-ST45 in Hainan (southern China). Thus, the prevalence of the dominant MRSA type varies across the world and are associated with specific geographical regions.

According to previous reports, most HA-MRSA isolates carry SCC*mec* I, II, or III elements, and CA-MRSA isolates carry SCC*mec* IV or V elements.[44,45] In this study, HA-MRSA (I+II+III) accounted for 52 strains (24.88%) and CA-

Table 5 The Frequency Analysis of Antimicrobial Resistance Between ST59 and Non-ST59

Isolates (N)	MDRs N (%)	Resistant Isolates N (%)															
	MDRs	PENG	ERY	CM	TET	CMP	GEN	NOR	CIP	LEV	RIF	SXT	FT	QDA	VAN	TEC	LZD
ST59(n=104)	88(84.6)	104 (100.0)	85 (81.7)	64 (61.5)	42 (40.4)	43 (41.3)	21 (20.2)	17 (16.3)	14 (13.5)	3 (2.9)	4 (3.8)	2 (1.9)	1 (1.0)	2 (1.9)	0	0	0
non-ST59(n=105)[b]	77(73.3)	105 (100.0)	78 (75.1)	45 (42.9)	35 (33.3)	29 (27.6)	24 (22.9)	28 (26.7)	27 (25.7)	23 (21.9)	10 (9.5)	8 (7.6)	2 (1.9)	0 (0)	0	0	0
Total(n=209)[c]	165(78.9)	209 (100.0)	163 (78.0)	109 (52.2)	77 (36.8)	72 (34.4)	45 (21.5)	45 (21.5)	41 (19.6)	26 (12.4)	14 (6.7)	10 (4.8)	3 (1.4)	2 (1.0)	0	0	0
χ^2	4.001	-	1.687	7.307	1.116	4.36	0.22	3.294	4.974	15.65	1.863	2.576	0	0.495	-	-	-
***P*-value[a]**	0.045	-	0.194	<0.01	0.291	0.037	0.639	0.07	0.026	<0.01	0.172	0.109	1	0.498	-	-	-

Notes: [a]The frequency of MDRs and antimicrobial resistance were compared between ST59 and non-ST59. [b]Other MLST types were detected, except ST59. [c]The total quantities of MDRs and antimicrobial resistance, respectively.
Abbreviations: PENG, penicillin G; ERY, erythromycin; CM, clindamycin; TET, tetracycline; CMP, chloramphenicol; GEN, gentamicin; NOR, norfloxacin; CIP, ciprofloxacin; LEV, levofloxacin; RIF, rifampicin; SXT, trimethoprim/sulfamethoxazole; FT, nitrofurantoin; QDA, quinupristin-dalfopristin; VAN, vancomycin; TEC, teicoplanin; LZD, linezolid; MDR, multidrug resistance.

Table 6 The Frequency Analysis of Virulence Gene Between ST59 and Non-ST59

Isolates (N)	Virulence Genes N (%)											
	pvl (lukS-PV/lukF-PV)	***sea***	***seb***	***hla***	***hlb***	***tsst***	***fnbA***	***fnbB***	***icaA***	***icaD***	***cna***	***bap***
ST59(n=104)	36 (34.6)	13 (12.5)	83 (79.8)	101 (97.1)	88 (84.6)	0 (0)	66 (63.5)	0 (0)	104 (100.0)	91 (87.5)	1 (1.0)	0 (0)
non-ST59(n=105)[b]	64 (61.0)	7 (6.7)	21 (20.0)	85 (81.0)	38 (36.2)	5 (4.8)	63 (60.0)	20 (19.0)	105 (100.0)	93 (88.6)	49 (46.7)	9 (8.6)
Total(n=209)[c]	100 (47.8)	20 (9.6)	104 (49.8)	186 (89.0)	126 (60.3)	5 (2.4)	129 (61.7)	20 (9.6)	209 (100.0)	184 (88.0)	50 (23.9)	9 (4.3)
χ^2	14.52	2.055	74.76	12.34	51.18	3.239	0.265	19.76	-	0.057	57.49	7.351
***P*-value[a]**	<0.01	0.152	<0.01	<0.01	<0.01	0.06	0.607	<0.01	-	0.811	<0.01	<0.01

Notes: [a]The frequency of virulence genes were compared between ST59 and non-ST59. [b]Other MLST types were detected, except ST59. [c]The total quantities of virulence genes.

Abbreviations: *pvl(lukS-PV/lukF-PV)*, the panton-valentine leukocidin gene; *sea*, staphylococcal enterotoxins a gene; *seb*, staphylococcal enterotoxins b gene; *hla*, the hemolysin a gene; *hlb*, the hemolysin b gene; *tsst*, the toxic shock syndrome toxin gene; *fnbA*, the fibrinogen-binding protein A gene; *fnbB*, the fibrinogen-binding protein B gene; *icaA*, the intracellular adhesion molecule A gene; *icaD*, the intracellular adhesion molecule D gene; *cna*, the collagen adhesion gene; *bap*, the biofilm-associated protein gene.

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MRSA (IV+V+XII) for 129 (61.72%). The remaining 28 non-type strains could not be discriminated by the method used (Table 2). Therefore, the main MRSA isolate in the study area was CA-MRSA. It is therefore reasonable that most people become infected with MRSA before they visit the hospital. Notably, 15 strains of CC59-ST338, which belongs to CA-MRSA, were detected,[4] though it was found to be SCC*mec* II. Hence, it is controversial to identify HA-MRSA or CA-MRSA by the ST method with the SCC*mec* method. More accurate identification methods need to be explored in the future. In addition, 4 new uncommon MLST types were identified in the study region.

Antimicrobial sensitivity tests showed that all 209 MRSA isolates were susceptible to VAN, TEC, and LZD, but none of the MRSA isolates were susceptible to PENG and CFX. The findings suggest that VAN, TEC, and LZD remain the most effective antimicrobial agents for MRSA isolates in this area. The high resistance rates to ERY, CM, and GEN are consistent with the results of CHINET in 2020. However, the resistance rate to LEV, RIF and SXT in this study was lower than that to CIP and NOR and 20.2% lower than data according to CHINET in 2020. This might be due to different antibiotic usages and health care plans in different regional hospitals.[46] In general, the ability of MRSA to adapt changes under the bacteriostatic pressure of antibiotics, which might lead to certain differences in LEV, RIF, and SXT resistance rates (Table 3).

Here, the study found the multidrug resistance rate to be 79.0% (165/209). Among them, CC8-t030 and CC1-t899 exhibited high-intensity multidrug resistance against 6–8 classes of antibiotics. Such a situation has been reported to be related to the misuse of antibiotics.[47,48] Additionally, ST59 shows a wide spectrum of antimicrobial resistance, though only 2 ST59 isolates were resistant to QDA. Interestingly, there are few reports about resistance of MRSA strains to QDA.[35] It was also found that ST45, ST72, and ST630 isolates were completely resistant to fluoroquinolones; ST22, ST45, and ST630 isolates were completely sensitive to TET. These results suggest a certain correlation between MLST strain type and antimicrobial susceptibility.[49] Furthermore, antibiotic resistance rates to CM and CMP were higher and to CIP and LEV lower in non-ST59 isolates than in ST59 isolates (Table 5). This indicates that ST59 has a high antimicrobial resistance rate, especially to CM and CMP, in Guiyang. According to the above results, it was concluded that the antimicrobial resistance rate of MRSA strains was higher; ST59 isolates are the main MRSA type and exhibit unique characteristics in Guiyang.

Virulence factors are the main factors involved in the colonization and pathogenicity of pathogenic bacteria and facilitate invasive infection,[36,50,51] causing a strong inflammatory response and promoting infection syndrome.[4,38,51] In addition, virulence factors help bacteria form biofilm for stable attachment, ensuring pathogen survival and infection. This study examined 12 virulence genes, with different detection rates. Among them, the detection rates of the *icaA, hla, icaD, fnbA* and *hlb* genes were 100.0%, 89.0%, 88.0%, 61.7% and 60.3%, respectively (Table 4). It has been suggested that MRSA strains might have strong haemolytic ability and cell adhesion ability in the study area.[52]

In our virulence gene assay, rates of *cna, fnbB*, and *bap* gene detection were lower than those of other virulence genes, but they were particularly associated with certain types. All ST22, ST398, ST45, and ST239 isolates carried the *cna* gene. The *bap* gene was detected only in four CC8-t4549 isolates. CC59 isolates did not carry the *fnbB* gene. This information provides an important basis to further study the mechanism of bacterial adhesion and biofilm formation. The cytotoxin *pvl* (lukS-PV/lukF-PV) gene was detected in multiple STs. PVL protein can cause tissue necrosis and leukocyte destruction.[53] Among these isolates, ST22 81.5% (22/27) and ST338 91.3% (21/23) had higher carriage rates. This was the same as reported for *pvl* (lukS-PV/lukF-PV)-positive CC22-MRSA isolates in Kuwaiti hospitals[54] but different from *pvl* (lukS-PV/lukF-PV)-negative ST22-MRSA isolates reported in Urumqi (Figure 1).[55] This indicates some differences in the *pvl* (lukS-PV/lukF-PV) gene -carrying rate of ST22-MRSA isolates from different regions and possibly differences in their invasive abilities.

The *seb* gene was mainly detected in CC59 isolates. According to reports, SEB also promotes systemic *S. aureus* infection.[56] Therefore, the systemic infection rate caused by ST59 might be higher. Although the detection rate of the *tsst* gene was lower, the ST5-t2460 isolates had a higher rate of the *tsst* gene positivity (Figure 1). Toxic shock syndrome (TSS) is an acute systemic disease affecting different organ systems of the body, resulting in severe disease.[27,28] If a patient is infected with the ST5-t2460 isolate, clinicians should be highly vigilant about systemic infection and implement emergency measures in advance. Analysis of ST59 and non-ST59 isolates showed that the *seb, hla*, and *hlb* genes were more frequent in ST59 isolates than in non-ST59 isolates (Table 6). This suggested that ST59 might have

strong haemolytic toxicity and systemic infection capacity. According to the above detection of virulence genes carried by STs, we speculate the existence of a relationship between virulence gene profile and MLST type.

Conclusion

This study revealed the molecular characteristics, antimicrobial resistance, and virulence gene-carrying status of MRSA isolates in Guiyang. Due to the special geographical environment and the habit of antibiotic use, ST59-SCC*mec* IV-t437 was found to be the main epidemic clone type, with a wide spectrum of antimicrobial resistance, many virulence genes, and strong adaptability and pathogenicity. Public health departments should be aware of and monitor this strain.

Abbreviations

CHINET, China Antimicrobial Surveillance Network; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; HA-MRSA, hospital-acquired methicillin-resistant *Staphylococcus aureus*; CA-MRSA, community-acquired methicillin-resistant *Staphylococcus aureus*; MLST, multilocus sequence typing; CCs, clonal complexes; *spa, Staphylococcal* protein A; SCC*mec, Staphylococcal* chromosomal cassette *mec*; NT, non-type; STs, sequence types; MDR, multidrug resistance; MSCRAMMs, microbial surface components recognizing adhesive matrix molecules; CLSI, Clinical and Laboratory Standard Institute; FT, nitrofurantoin; SXT, trimethoprim/sulfamethoxazole; CIP, ciprofloxacin; CM, clindamycin; QDA, quinupristin-dalfopristin; CMP, chloramphenicol; NOR, norfloxacin; GEN, gentamicin; LEV, levofloxacin; ERY, erythromycin; RIF, rifampicin; LZD, linezolid; TET, tetracycline; TEC, teicoplanin; VAN, vancomycin; PENG, penicillin G; CFX, cefoxitin; *sea, seb, Staphylococcal* enterotoxin genes; *pvl* (lukS-PV/lukF-PV), Panton-Valentine leukocidin gene; *tsst*, toxic shock syndrome toxin gene; *hla, hlb*, haemolysin genes; *fbA, fnbB*, fibrinogen-binding protein genes; *icaA, icaD*, intracellular adhesion molecules genes; *cna*, collagen adhesion gene; *bap*, biofilm-associated protein gene.

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Author Contributions

Su-Wen Yang designed and implemented the experiments and drafted the manuscript. Bing Wang designed, analysed and interpreted the data, aided in the manuscript preparation, and assisted in the research design. Jing Li, Xue Zhao, Yan Zhu, and Qian Sun collected, identified, and performed the antibiotic testing and analysed the data. Xiao-Jun Wen and Hong-Mei Liu analysed the data, assisted in the design, and provided funding. All authors assisted in editing and approved the final manuscript. All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no competing interests in this work.

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