Molecular characteristics and virulence gene profiles of Staphylococcus aureus isolates in Hainan, China

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Abstract

Background: There have been no reports regarding the molecular characteristics, virulence features, and antibiotic resistance profiles of Staphylococcus aureus (S. aureus) from Hainan, the southernmost province of China. Methods: 227 S. aureus isolates, consisting of 76 methicillin-resistant S. aureus (MRSA) and 151 methicillin-susceptible S. aureus (MSSA), were collected in 2013-2014 and 2018-2019 in Hainan, and investigated for their molecular characteristics, virulence genes, and antibiotic resistance profiles. Results: Thirty-four sequence types (STs) and 79 spa types were identified based on multilocus sequence typing (MLST) and spa typing, respectively. ST398 (14.1%, 32/227) was found to be the most prevalent, and moreover, the prevalence of ST398-MSSA increased significantly from 2013-2014 (5.5%, 5/91) to 2018-2019 (18.4%, 25/136). Seventy-six MRSA isolates were subject to staphylococcus chromosomal cassette mec (SCCmec) typing. SCCmec-IVa was the predominant SCCmec type, and specifically ST45-SCCmec IVa, an infrequent type in mainland China, was predominant in S. aureus from Hainan. Eleven virulence genes, including the Panton-Valentine leukocidin (pvl) and eta, were determined, and the positive rates of eta and pvl were found to be 57.3% and 47.6%. Such high prevalence has never been seen in mainland China before.

Background

Staphylococcus aureus (S. aureus) is an important gram-positive pathogen causing various infectious diseases including pneumoniae and bacteremia. One study found that compared with matched uninfected patients, patients with healthcare-associated S. aureus infections had an excess one-year mortality of 20.2%[1]. The genotype of S. aureus has been reported to influence the complications, severity, and mortality of infection. One study showed that the strains clonal complex 5 (CC5) and CC30 exhibited a significant trend toward increasing levels of hematogenous complications[2]. Another study found that patients with S. aureus sequence type 121 (ST121) infections often needed longer hospitalization and prolonged antimicrobial therapy[3], whereas bloodstream infections by CC398, a methicillin-susceptible Staphylococcus aureus (MSSA), are associated with high mortality[4]. Therefore, analysis of the molecular characteristics and virulence gene profiles of S. aureus is important for prognosis of infection.
The molecular characteristics of *S. aureus* vary with region. In many Asian countries including China and Thailand, ST239 has been found to be the most prevalent type[5-8], whereas in the United States, ST8 (USA300) and ST121 are the most frequently observed[3, 9]. Even within China, the molecular characteristics of *S. aureus* isolates differ among cities; the predominant types in Wenzhou are ST188 and ST7[10], the major type in Dalian and Shenyang is ST5[11], whereas in Chengdu, ST59 is prevalent[12]. The molecular characteristics of *S. aureus* are also reported to have varied over time. Since 2000, ST239-t030-SCCmeIII has rapidly replaced ST239-t037-SCCmeIII, becoming the major clone of *S. aureus* isolates in Chinese tertiary hospital care[2], whereas ST239-t030-MRSA, which in 2013 was the predominant genotype among all methicillin-resistant *S. aureus* (MRSA) strains in China, had been replaced by ST59-t437-MRSA by 2016[13]. Therefore, when monitoring the molecular characteristics of *S. aureus* isolates, it is preferable to focus on a specific region of interest at a particular time.

Hainan, the southernmost province of China, is surrounded by the South China Sea, and has a uniquely tropical monsoon and marine climate that is significantly different from that in the mainland. The island has been called a “natural large greenhouse,” and the hot and humid climate is conducive to bacterial growth. Studies of the molecular characteristics and antibiotic resistance profiles of *S. aureus* isolates from China have been carried out in various provinces in the last 10 years, such as Zhejiang, Guangdong, and Guangxi[14-17]. To date, however, no study has focused on the molecular characteristics and virulence gene profiles of *S. aureus* isolates in Hainan, and no hospital in Hainan has been included in any multi-center studies concerned with those characteristics of *S. aureus* in China[13, 18, 19]. Not even the CHINET surveillance system includes any hospital from Hainan. Although the total area of Hainan is relatively small, its population has now reached 10 million, and moreover, its tropical monsoon and marine climate is unique in China. These are important motivations to investigate the molecular characteristics, virulence genes, and antibiotic resistance profiles of *S. aureus* isolates from the Hainan province.

**Methods**

*S. aureus isolates and primers*
A total of 227 non-duplicate *S. aureus* isolates were collected from Hainan General Hospital, Haikou People’s Hospital and First Hospital Affiliated to Hainan Medical college in 2013-2014 (n=91) and 2018-2019 (n=136), respectively. These isolates were derived from diverse clinical specimens, including cutaneous abscess and wound secretion (n=110, 48.5%), sputum and pharynx swabs (n=48, 21.1%), blood (n=42, 18.5%), and others (catheter tip, marrow, pleural fluid, cerebrospinal fluid, cystic cavity fluid, drainage liquid, ascites, joint fluid, biopsy, and urine) (n=27, 11.9%). These isolates were identified by conventional microbiological methods including Gram staining, catalase, and coagulase tests, and confirmed with a VITEK 2 Compact system and a VITEK 2 AST-GP67 Test Kit (bioMerieux, Inc., Durham, NC, USA). All isolates were stored at -80°C for further experiments. All primers used in this study were synthesized by Tianyihuiyuan (China) (Table 1). This study was approved by the Ethics Committee of Hainan General Hospital. This was a retrospective study without any collection of clinical and personal information from patients, so informed consent was not required.

*Antimicrobial susceptibility testing*

A VITEK 2 Compact system and a VITEK 2 AST-GP67 Test Kit (bioMerieux, Inc., Durham, NC, USA) were used to carry out an antimicrobial susceptibility test. Twelve antibiotics were tested, including cefoxitin (FOX), clindamycin (CLI), erythromycin (ERY), gentamicin (GEN), levofloxacin (LEV), linezolid (LZD), oxacillin (OXA), penicillin (PEN), rifampicin (RIF), trimethoprim/sulfamethoxazole (SXT), tetracycline (TET), and vancomycin (VAN). *S. aureus* ATCC 25923 and ATCC25913 were used as the quality control strains. In addition, MRSAs were further identified using PCR for amplification of *mecA* as described previously, and MRSA N315 was used as the positive control strain.

*Staphylococcal protein A (spa) typing*

Chromosomal DNAs were extracted from *S. aureus* isolates as described previously[20]. The extracted chromosomal DNAs were stored at -20°C for *spa*, *Staphylococcus* chromosomal cassette *mec* (SCCmec), and multilocus sequence typing, and detection of virulence genes. For *spa* typing, the variable repeat region of *spa* was amplified using oligonucleotide primers[20, 21](see Table 1) followed by sequencing. The PCR mixture and conditions were similar to those described
previously[20]. The resulting amplicons were purified and subjected to Sanger dideoxy DNA sequencing (Tianyihuiyuan, China) followed by analysis using the Ridom web server (http://spaserver.ridom.de). *S. aureus* isolates that could not be classified as any known *spa* type were defined as nontypable (NT).

**Multilocus sequence typing (MLST)**

MLST was carried out according to the protocol described previously[20, 22]. Seven housekeeping genes of *S. aureus*, namely *arcC, aroE, glpF, gmk, pta, tpi*, and *yqil*, were adopted for MLST. Seven respective PCR assays were conducted to amplify these 7 housekeeping genes. These amplicons were sequenced using Sanger dideoxy DNA sequencing (Tianyihuiyuan, China). The resulting sequences were compared with the known alleles in the MLST database (http://saureus.mlst.net), which was used to determine ST. *S. aureus* isolates that could not be assigned to any known ST were defined as NT. The clustering of related STs, which were defined as CCs, was determined using eBURST.

**Staphylococcus chromosomal cassette mec (SCCmec) typing**

The MRSA isolates were subjected to SCCmec typing as previously described[23]. MRSA isolates with suspected SCCmecIV were recharacterized by additional multiplex PCR as subtypes IVa, IVb, IVc, and IVd as described by Zhang et al.[24]. MRSA isolates that could not be assigned to any above type were defined as NT. All primers are listed in Table 1.

**Detection of virulence genes**

Eleven virulence genes, including the Panton-Valentine leukocidin (*pvl*), the staphylococcal enterotoxin genes (*sea, seb, sec*), the exfoliative toxin genes (*eta, etb*), the hemolysin gene (*hla, hlb*), and the adhesion factor genes (*fnbA, fnbB, clfA*) were detected using PCR assays. The PCR mixture and conditions were similar to those described previously[20].

**Statistical analysis**

Statistical analyses were performed using SPSS Statistics 24.0 for Windows. Data were analyzed using the chi-square or Fisher’s exact tests. All statistical tests were two-tailed, and *p*<0.05 or *p*<0.01 (Fisher’s exact tests among three groups) was considered to be statistically significant.

**Results**
Antimicrobial susceptibility test

The antimicrobial resistance profiles of the 227 S. aureus isolates are listed in Table 2. No S. aureus isolate was resistant to VAN or LZD, while a minority were resistant to GEN (14.1%), LEV (10.6%), and RIF (19.8%). Less than 50% were resistant to the remaining antibiotics, except for PEN, to which 92.5% had resistance (Table 2). Seventy-six (33.5%) S. aureus isolates, including an oxacillin susceptible-MRSA (OS-MRSA) belonging to ST120-t2613, were found to be MRSA. The resistance rates of these MRSA isolates to FOX, PEN, OXA, ERY, GEN, CLI, RIF, and LEV were 100.0%, 100.0%, 98.7%, 75.0%, 64.5%, 18.4%, 17.1%, 15.8%, and 9.7%, respectively, which were significantly higher than those of the MSSA isolates (Table 2). However, there was no significant difference in the resistance rates to VAN, LZD, SXT, and TET between the MRSA and MSSA isolates. The rates of multidrug resistance (MDR) of the MRSA and MSSA isolates were 73.7% and 37.7%, respectively. The chi-square test showed that the prevalence of MDR was significantly higher in the MRSA isolates than in the MSSA isolates (Table 2). In addition, when comparing the S. aureus isolates collected in 2013-2014 with those from 2018-2019, the resistance rates to all antibiotics except SXT were broadly similar. Compared with those collected in 2018-2019, the S. aureus isolates from 2013-2014 had a higher resistance rate to SXT (64.8% vs. 5.9%, p<0.05) and a greater prevalence of MDR (61.5% vs. 41.9%, p<0.05) (Table 2).

MLST, spa, and SCCmec typing

Thirty-four STs belonging to 18 clonal complexes (CCs) and 3 singletons were identified by eBURST. As shown in Table 3 and Figure 1. ST398 (14.1%) was the most prevalent followed by ST188 (13.2%) and ST45 (10.1%). In addition, 7 isolates were determined to be NT (Table 3). By spa typing, 79 spa types were found. The most prevalent was t189 (12.3%) followed by t437 (7.9%), t116 (7.5%), and t011 (6.6%). Meanwhile, 8 isolates were NT (Table 3). When the STs and spa typing were combined, the predominant combinations were ST188-t189 (12.3%, 28/227), ST45-t116 (7.5%, 17/227), ST59-t437 (7.0%, 16/227), ST398-t011 (6.6%, 15/227), ST398-t034 (4.8%, 11/227), and ST7-t091 (4.8%, 11/227) (Table 3). A strong association was observed between certain STs and spa types: ST188 was primarily associated with t189 (93.3%, 28/30); ST45 was associated mainly with t116 (73.9%, 17/23);
and ST59 was associated mainly with t437 (72.7%, 16/22) (Table 3).

The major types of *S. aureus* collected in 2013-2014 were ST188 (14.3%), ST45 (14.3%), ST59 (8.8%), and ST88 (8.8%), whereas in 2018-2019, ST398 (19.9%), ST188 (12.5%), ST59 (10.3%), ST45 (7.4%), and ST7 (7.4%) were the top five types (Table 3). Among the STs that exhibited OXA sensitivity, the two predominant types in 2013-2014 were ST188-MSSA (14.3%) and ST45-MRSA (12.1%), whereas in 2018-2019 they were ST398-MSSA (18.4%) and ST59-MRSA (8.1%). The prevalence of ST398-MSSA markedly increased from 2013-2014 (5.5%) to 2018-2019 (18.4%), and this increase was significant (*p*<0.05) (Table 3).

Among the 76 MRSA isolates, 6 SCCmec types or subtypes, namely types I, II, III, IVa, IVc, and V, were found. The most common SCCmec type was IVa, which was found in 43 isolates (56.6%, 43/76), while type I, II, III, IVc, and V were found in 1, 3, 6, 5, and 9 isolates, respectively. Nine isolates, including OS-MRSA, were classified as NT for SCCmec typing. When the STs and SCCmec typing were combined, the predominant combination was ST45-SCCmec IVa (8.8%, 20/227), and there was no significant difference in the positive rate of ST45-SCCmec IVa between the *S. aureus* isolates collected in 2013-2014 and 2018-2019 (12.1% vs. 6.6%, *p*>0.05) (Table 3).

**Virulence gene profiles**

The frequencies of the virulence genes identified in the 227 *S. aureus* isolates are listed in Table 4. *ClfA* was present in all *S. aureus* isolates, *hla*, *hlb*, and *eta* were detected in 98.7%, 70.9%, and 57.3% of these isolates, respectively, whereas the remainder were found in less than 50.0%. One hundred and twenty (52.9%) *S. aureus* isolates harbored 6 or more virulence genes. Of those 120 isolates, 11 contained 9 virulence genes, 31 had 8 such genes, 38 carried 7, and 40 carried 6. Compared with those in the MSSA isolates, the positive rates of *fnbA*, *sea*, and *sec* were significantly higher in the MRSA isolates, but there was no significant difference in the rate of harboring 6 or more virulence genes between the MRSA and MSSA isolates (56.6% vs. 51.0%, *p*>0.05). Compared with those collected in 2013-2014, the *S. aureus* isolates from 2018-2019 had higher positive rates of *pvl*, *fnbB*, *hla*, *seb*, *eta*, and *etb* and higher rates of harboring 6 or more virulence genes (Table 4).
The most abundant sequence type found in this study was ST398 (32/227, 14.1%) followed by ST188 (30/227, 13.2%) and ST45 (23/227, 10.1%). Majorities of ST398 (30/32, 93.8%) and ST188 (29/30, 96.7%) isolates were MSSA, whereas the majority of ST45 (20/23, 87.0%) isolates were MRSA, and all ST45-MRSA isolates belonged to the SCCmec IVa type (Table 3). ST45 isolates had higher resistance rates to OXA and FOX than ST398 ($c^2=36.318, p<0.01$) and ST188 isolates ($c^2=38.055, p<0.01$), whereas ST398 ($c^2=17.685, p<0.01$) and ST188 isolates ($p<0.01$) had higher resistance rates to TET than did ST45 isolates (Table 2). In addition, there was no significant difference in resistance rate to any antibiotics between ST398 and ST188 isolates (Table 2).

Of the 11 tested virulence genes, pvl and fnbB were found to be more frequent in ST398 isolates than in ST45 ($c^2=22.010$ and $c^2=30.457$, respectively, $p<0.01$) and ST188 isolates ($c^2=12.790$ and $c^2=38.027$, respectively, $p<0.01$). The prevalence of sec in ST45 isolates was higher than that of ST398 ($c^2=43.487, p<0.01$) and ST188 isolates ($c^2=32.500, p<0.01$), while the prevalence of eta in ST45 isolates was higher than in ST188 isolates ($c^2=14.339, p<0.01$). However, the positive rate of hlb in ST45 isolates was lower than that of ST398 ($c^2=7.118, p<0.01$) and ST188 isolates ($c^2=7.248, p<0.01$). There was no significant difference in the positive rate of any other virulence genes between any two of the three STs (Table 4).

Discussion
A total of 227 S. aureus isolates were collected in 2013-2014 and 2018-2019 from three hospitals in Hainan province for investigation of their antimicrobial resistance, virulence gene profiles, and molecular characteristics. The results showed that all isolates were susceptible to VAN and LZD, in agreement with the majority of previous studies carried out in mainland China[25-27]. In addition, when comparing the S. aureus isolates collected in 2013-2014 and 2018-2019, no significant difference was found in the resistance rates to the remaining antibiotics except that to SXT. Therefore, both sets of isolates were combined for analysis, and the average resistance rates to PEN, ERY, CLI, TET, FOX, OXA, GEN, LEV, and RIF were found to be 92.5%, 48.9%, 41.4%, 37.4%, 33.5%, 33.0%, 11.9%, 9.7%, and 8.8%. For comparison, in mainland China in the first half of 2018, the
corresponding average rates were reported to be 92.7%, 64.5%, 38.4%, unreported, 34.4%, 34.4%, 18.7%, 22.4%, and 5.2% (http://www.chinets.com). The resistance rate to SXT was 5.9% in the S. aureus isolates collected in 2018-2019, which was significantly lower than for those collected in 2013-2014 (64.8%), whereas the resistance rate to SXT in mainland China was 14.3% in the first half of 2018 (http://www.chinets.com). The S. aureus isolates from Hainan had similar resistance rates against the majority of antibiotics to those from mainland China, but lower resistance rates to ERY and LEV. Therefore, the antibiotic resistance profiles of the Hainan isolates were to a certain extent geographically distinct.

MLST typing, spa typing, and SCCmec typing were performed to analyze the molecular characteristics of the S. aureus isolates. ST398, ST188, and ST45 were the predominant STs among the S. aureus isolates in this study, among which ST398 and ST45 were the predominant clones in the MSSA and MRSA isolates, respectively. In addition, the most common SCCmec type was IVa, and ST45-SCCmec IVa was the most prevalent combination of ST and SCCmec typing in the MRSA isolates. ST188 and ST239 were previously reported as the predominant STs in MSSA and MRSA isolates, respectively[11, 18, 19, 28, 29]. Among these studies, two were multiple-center studies that showed that ST239-SCCmec III was the predominant MRSA genotype, but observed no ST45 clones at all[11, 19]. A recent study in Shanghai showed that ST239-t030 and ST239-t037 were being driven out by the continual growth of the ST5-t2460 clone[30]. Therefore, it can be concluded that the molecular characteristics of S. aureus isolates in Hainan province are significantly different from those in mainland China. It is reasonable to speculate that this difference is associated with the unique climate of coastal regions.

Supporting our speculation, similar molecular characteristics to those of the Hainan isolates have been reported in several coastal countries and cities. For instance, ST45-SCCmec IV was the major type in Belgium and Slovenia, accounting for up to 51.2% and 21.1% of MRSA isolates, respectively[31, 32]. In addition, ST45-SCCmec IV was the second most prevalent clone in Poland and Hong Kong, accounting for 15.3% and 10.0% of MRSA isolates, respectively[33, 34]. ST398 was found to be the most prevalent in Hainan province, and moreover, in the short span of five years, the prevalence of ST398-MSSA increased from 5.5% to 18.4%. In cohorts of patients in France,
ST398 MSSA was shown to increase from zero cases in 1999 to 4.6% of cases in 2010, including 13.8% of cases with *S. aureus* bloodstream infections[4, 35]. Another retrospective study in France found that only 1.9% of bone and joint infection (BJI) MSSA strains were screened to be ST398 in 2008, whereas in 2010-2012, 14.0% of BJI MSSA strains belonged to ST398[36]. Therefore, ST398 MSSA has emerged as an invasive pathogen causing bloodstream infections, BJIs, and potentially other conditions. Although ST398 MSSA has not been well studied, it is more closely linked with human infections than ST398 MRSA. Moreover, it was found that 30-day all-cause mortality was higher for patients with ST398 MSSA bloodstream infection than for a control group with non-ST398 MSSA infection[4]. Considering that ST398 MSSA has become the most prevalent ST in *S. aureus* isolates from Hainan province and may be linked to higher mortality, it is necessary to monitor the changes in the molecular characteristics of *S. aureus* to prevent the wider dissemination of that strain.

The virulence factors of *S. aureus* play an important role during pathogenesis[37, 38]. Similar to the majority of studies in mainland China[8, 39], almost all strains in our study were positive for *clfA* and *hla*, confirming that these were the most common virulence factors in *S. aureus*, and there was no regional difference in their distribution. Notably, the positive rates of *eta* and *pvl* were 57.3% and 47.6%, much higher than those in mainland China[10, 15, 18]. ST45, a common type of *S. aureus* isolate in Hainan province, was found to have an *eta* prevalence of 95.7% in our results. Meanwhile, ST398, a clone with a low prevalence of *pvl* in previous studies[35, 40], was found to have a frequency of 81.3% in this study. Together, these findings indicate that *S. aureus* isolates in Hainan province have somewhat higher positive rates of *eta* and *pvl*. This implies that the molecular characteristics of *S. aureus* isolates affect their virulence gene profiles, leading us to conclude that *S. aureus* isolates collected in Hainan have distinct virulence gene profiles compared with those collected in mainland China. In addition, compared with the 2013-2014 isolates, the *S. aureus* isolates collected in 2018-2019 carried more virulence genes, but their rate of MDR was lower. This trend may be related to the balance of energetic requirements[41].

Conclusions
S. aureus isolates in Hainan province have unique molecular characteristics and virulence gene profiles. ST398-MSSA was the most common type of MSSA isolate and ST45-SCCmec IVa was the predominant type of MRSA isolate, neither of which had been reported in China before. Differences were also found between the antibiotic resistance and virulence gene profiles of the ST398 and ST45 isolates. ST398-MSSA showed a clear growth trend from 2013-2014 to 2018-2019, which deserves attention from public health services.

Abbreviations

S. aureus Staphylococcus aureus
MRSA Methicillin-resistant Staphylococcus aureus
MSSA Methicillin-susceptible Staphylococcus aureus
MLST Multilocus sequence typing
SCCmec Staphylococcus chromosomal cassette mec
PVL Panton-Valentine leukocidin
CC Clonal complex
OS-MRSA Oxacillin susceptible-MRSA
MDR Multidrug resistance

Declarations

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Availability of data and materials

All data supporting the conclusions of this article are included within the article.

Authors’ contributions

YL designed the studies and obtained funding; XL performed the experiments; XL and CL performed
the statistical analysis; XL wrote the manuscript; YL contributed to manuscript revision; KX and TH contributed the materials. All authors read and approved the submitted version.

**Competing interests**

The authors declare no conflicts of interest.

**Consent for publication**

Written informed consent for publication was obtained from all participants.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Hainan General Hospital. This was a retrospective study without any collection of clinical and personal information from patients, so informed consent was not required.

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### Tables

**Table 1** Primers used in MLST, *spa*, SCCmeC typing, and the results of SCCmeC types I–V

| Primer  | Nucleotide sequence (5'-3') | Target | Amplicon |
|---------|----------------------------|--------|----------|
| β       | ATTGCCTTGATAATAGCCYTCT      | ccrA2-B|          |
| a3      | TAAAGGCATCAATGCACAAACACT    |        |          |
| ccrCF   | CGTCTATTACAAGATGTTAAGGATAAT | ccrC   |          |
| ccrCR   | CCTTTATAGACTGGATTATTCAAAATAT |    |          |
| 1272F1  | GCCACTCATAACATATGGAA        | IS1272 |          |
| 1272R1  | CATCCGAGTGAAACCCAAA         |        |          |
| 5RmeC   | TATACAAACCGAACAACCTAC       | mecA–IS431 |        |
| 5R431   | CGGCTACGATAACATCC           |        |          |
| Type IVa-F | GCCTTATTCAAGAAACCG       |        |          |
| Type IVa-R | CTACTCTTCTGAAAGCGTCG     |        |          |
| Type IVb-F | TCTGGAATTACTTCAGCTGC     |        |          |
| Type IVb-R | AAACAATTTGCTCTCCCTC     |        |          |
| Type IVc-F | ACAATATTGTATATCGGAGAGC   |        |          |
| Type IVc-R | TTGGATGAGGATTGCTGG      |        |          |
| Type IVd-F | CTCAAAATACGGAACCCCAATACA |        |          |
| Type IVd-R | TGCTCCAGTAATTGCTAAAG  |        |          |
| Spa-1113f | TAAAGACGATCCTTCGGTGAGC   | spa    |          |
| Spa-1514r | CAGCAGTAGGGCTTTGCTT     |        |          |
| arcC-F   | TGGATTCCACGCGCGGTTTGGTC   | arcC   |          |
| arcC-R   | AGG TATCTGCTCAAATCAGCG   |        |          |
| Gene | Primer 1 | Primer 2 |
|------|----------|----------|
| aroE | ATCGGAAATCCTATTTACATTC | aroE |
| aroE-R | GGTGTGTATTAAATAACGATATC |  |
| glpF-F | CTAGGAACCTGAACTTTAATCC | glpF |
| glpF-R | TGGTAAAATCGATGTGTTTACATT |  |
| gmk-F | ATCGTTTATCGGAGGACATTC | gmk |
| gmk-R | TCATTAACTACAACGTAATCGTA |  |
| pta-F | GTTAATAACGTATCTACCTGAAGG | pta |
| pta-R | GACCTTTTTTGTGAAAAGCTTA |  |
| tpi-F | TCGTTCATTCTGAACGTCGTTAA | tpi |
| tpi-R | TTTGCACCTTCTAAACATTGATC |  |
| yqiL-F | CAGCATACAGGACACCTTTTAC | yqiL |
| yqiL-R | CGTTGAGGAAATCGACTGGAAC |  |
| PVL-F | ATCATTAGGTAAAATGCTGGGAGCATGCTACCA | PVL |
| PVL-R | GCATCAAATGTATTGGGATAGCAAAGGC |  |
| FnbA-F | GTGAAAGTTTTAGAGGTTGAAGATTAG | fnbA |
| FnbA-R | GCTCTTGAAGACCATTTCCTTCAC |  |
| FnbB-F | GTACAGGCTGAAATGCTGAATTGATACT | fnbB |
| FnbB-R | CAAGTTCGATAGGAGTACTATGTTC |  |
| Hla-F | CTGATCTATCAGGAAGATTTGATTC | hla |
| Hla-R | CTTTCCAGCTACTTTTTATCTGT |  |
| Hlb-F | GTGCACCTTACTGACAAATAGTGC | hlb |
| Hlb-R | GTTGGATAGTAGCTACCTTCAGT |  |
| Sea-F | GAAAAAGTCTGAATTGGGAAACA | sea |
| Sea-R | CAAATAAATCGTAATTAACCGGAAGTTC |  |
| Seb-F | ATTCTATTAAGGACACTAAGTGAGGGA | seb |
| Seb-R | ATCCCGTTTCCTATAAGGGGACT |  |
| Sec-F | GTAAAGTTACGGTGGGAAAACCTTG | sec |
| Sec-R | CATATCATACAAAAAGATTTGCCGT |  |
| eta-F | CGCTGGGAGCATTCTCCTAATGG | eta |
| eta-R | TACATGGCCGCACTTGCTTGT |  |
| etb-F | CAGATAAAGAGCTTTATACACACATTAC | etb |
| etb-R | AGTGAACCTTATCTTTCTATTGAAAAACTC |  |
| clfA-F | ATGGCGTTGGCATTTGCTTGC | clfA |
| clfA-R | CGTTTCTCCGATGCTTTTG |  |

Table 2  Antimicrobial resistance profiles of main types of *S. aureus* isolates.
| isolates   | CLI n(%) | ERY n(%) | OXA n(%) | PEN n(%) | FOX n(%) | SXT n(%) | RIF n(%) |
|-----------|----------|----------|----------|----------|----------|----------|----------|
| 2013-2014 (91) | 44(48.4) | 50(54.9) | 33(36.3) | 82(90.1) | 33(36.3) | 59(64.8) | 9(9.9)   |
| 2018-2019 (136) | 50(36.8) | 61(44.9) | 42(30.9) | 128(94.1) | 43(31.6) | 8(5.9)   | 11(8.1)  |
| ST398 (32) | 9(28.1)  | 13(40.6) | 2(6.3)   | 31(96.9)  | 2(6.3)   | 5(15.6)  | 2(6.3)   |
| ST188 (30) | 6(20.0)  | 8(26.7)  | 1(3.3)   | 26(86.7)  | 1(3.3)   | 12(40.0) | 1(3.3)   |
| ST45 (23)  | 9(39.1)  | 12(52.2) | 20(87.0) | 23(100.0) | 20(87.0) | 10(43.5) | 2(8.7)   |
| MRSA (76)  | 49(64.5) | 57(75.0) | 75(98.7) | 76(100.0) | 76(100.0)| 26(34.2) | 13(17.1) |
| MSSA (151) | 45(29.8) | 54(35.8) | 0(0.0)   | 134(88.7) | 0(0.0)   | 41(27.2) | 7(4.6)   |
| Total (227)| 94(41.4) | 111(48.9)| 75(33.0) | 210(92.5) | 76(33.5) | 67(29.5) | 20(8.8)  |

**P value**

\( p_{value}^a \) 0.082 0.136 0.398 0.261 0.398 <0.01 0.639

\( p_{value}^b \) <0.01 <0.01 <0.01 0.002 <0.01 0.271 0.002

**Table 3 Molecular characteristics of S. aureus isolates collected in this study**

| CC (no.) | 2013-2014 (91 isolates) |
|----------|-------------------------|
|          | MLST(no.) | spa(no.) | MRSA(no.) | MSSA(no.) | SCCmec(no.) | MLST(no.) |
| CC398(32)| ST398(5)   | t011(3)  | 3          |           |             | ST398(27) |
|          |           |         | t034(2)   | 2          |             |           |

CLI clindamycin, ERY erythromycin, OXA oxacillin, PEN penicillin, FOX cefoxitin, SXT trimethoprim/sulfamethoxazole, RIF rifampicin, VAN vancomycin, LZD linezolid, LEV levofloxacin, GEN gentamicin, TET tetracycline. MDR multidrug-resistant.

\(^a\) The resistance rate of S. aureus isolates to antibiotics in 2013-2014 were compared with those in 2018-2019.

\(^b\) The resistance rate to antibiotics in MRSA isolates were compared with those in MSSA isolates.
|   |   |   |   |   |   |
|---|---|---|---|---|---|
| CC59(30) | ST59(8) | t437(4) | 1 | 3 | IVa(1) |
|   |   | t411(1) | 1 |   | V(1) |
|   |   | t1212(1) | 1 |   |   |
|   |   | t2356(1) | 1 | IVa(1) | ST338(3) |
|   |   | t3592(1) | 1 |   | V(1) |
| ST338(2) | t1751(2) | 2 |   |   |   |
| ST1778(2) | t437(1) | 1 |   | ST2041(1) |   |
|   | t2365(1) | 1 | IVa(1) |   |   |
| CC188(30) | ST188(13) | t189(12) | 12 |   | ST188(17) |
|   | t4950(1) | 1 |   |   |   |
| CC45(25) | ST45(13) | t116(10) | 8 | 2 | IVa(8) |
|   | t015(1) | 1 | IVa(1) | ST45(10) |   |
|   | t2131(1) | 1 | IVa(1) |   |   |
|   | NT(1) | 1 | IVa(1) |   |   |
|   | ST965(1) | t062(1) | 1 | IVa(1) |   |
|   | ST508(2) |   |   |   |   |
| CC5(17) | ST5(6) | t002(3) | 3 |   | ST5(8) |
|   | t954(1) | 1 |   |   |   |
|   | t6212(1) | 1 |   |   |   |
|   | t2358(1) | 1 | IVa(1) |   |   |
|   | ST965(1) | t062(1) | 1 | IVa(1) |   |
|   | ST764(1) |   |   |   |   |
|   | ST2633(1) |   |   |   |   |
| CC88(16) | ST88(8) | t1376(4) | 1 | 3 | II(1) |
|   | t2592(1) | 1 | IVa(1) | ST88(8) |   |
|   | t3622(1) | 1 |   |   |   |
|   | t15796(1) | 1 |   |   |   |
|   | NT(1) | 1 |   |   |   |
| CC7(15) | ST7(4) | t091(4) | 4 |   | ST7(10) |

21
| CC1(14) | ST1(4) | t127(1) | 1 | ST789(1) |
| ST610(1) | t2207(1) | 1 | II(1) | ST2583(1) |
| CC8(8) | ST239(3) | t030(2) | 2 | III(2) | ST239(3) |
| t037(1) | 1 | III(1) | ST630(2) |
| CC2580(6) | ST2580(5) | t3351(4) | 4 | IVa(1), IVc(3) | ST2580(1) |
| t4875(1) | 1 | IVC(1) | ST72(4) |
| CC72(6) | ST72(2) | t148(2) | 2 | ST120(1) |
| CC121(5) | ST121(4) | t269(1) | 1 | ST97(1) |
| t162(2) | 2 | ST464(1) |
| t159(1) | 1 | ST9(1) |
| CC97(3) | ST464(1) | t3992(1) | 1 | ST15(1) |
| CC9(2) | ST9(1) | t899(1) | 1 | ST509(2) |
| CC15(2) | ST15(1) | t1492(1) | 1 | ST1281(2) |
| CC509(2) | ST1281(2) | | | ST2196(2) |
| CC1281(2) | ST1281(2) | | | |
| CC2196(2) | ST1281(2) | | | |
| Singletons(3) | ST6(1) | t304(1) | 1 | IVa(1) |
| ST25(1) |
| ST944(1) | t616(1) | 1 | NT(1) |
| NT(7) | NT(6) | t084(1) | 1 | NT1 |
| t796(1) | 1 | t084(1) | 1 |
| t037(1) | 1 | IVa(1) |
| t091(1) | 1 | t12584(1) | 1 |
Table 4  The frequencies of virulence genes among main types of *S. aureus* isolates

| Virulence genes | *S. aureus* (n=227)n(%) | MRSA (n=76)n(%) | MSSA (n=151)n(%) | ST398n=32)n(%) | ST188n=30)n(%) | ST45 (n=23)n(%) |
|-----------------|--------------------------|-----------------|------------------|----------------|----------------|-----------------|
| *pvl*           | 108(47.6)                | 31(40.8)        | 77(51.0)         | 26(81.3)       | 11(36.7)       | 4(17.4)         |
| *fnbA*          | 87(35.7)                 | 36(47.4)        | 51(33.8)         | 7(21.9)        | 7(23.3)        | 10(43.5)        |
| *fnbB*          | 113(49.8)                | 31(40.8)        | 82(54.3)         | 31(96.9)       | 6(20.0)        | 6(26.1)         |
| *hla*           | 224(98.7)                | 74(97.4)        | 150(99.3)        | 32(100.0)      | 29(96.7)       | 23(100.0)       |
| *hla*           | 161(70.9)                | 51(67.1)        | 110(72.8)        | 20(62.5)       | 19(63.3)       | 6(26.1)         |
| *sea*           | 35(15.4)                 | 18(23.7)        | 17(11.3)         | 5(15.6)        | 2(6.7)         | 1(4.3)          |
| *seb*           | 108(47.6)                | 38(50.0)        | 70(46.4)         | 11(34.4)       | 18(60.0)       | 8(34.8)         |
| *sec*           | 63(27.8)                 | 38(50.0)        | 25(16.6)         | 2(6.3)         | 5(16.7)        | 22(95.7)        |
| *eta*           | 130(57.3)                | 47(61.8)        | 83(55.0)         | 24(75.0)       | 14(46.7)       | 22(95.7)        |
| *etb*           | 43(18.9)                 | 15(19.7)        | 28(18.5)         | 10(31.3)       | 6(20.0)        | 3(13.0)         |
| *clfA*          | 227(100.0)               | 76(100.0)       | 151(100.0)       | 32(100.0)      | 30(100.0)      | 23(100.0)       |

^a^The positive rates of virulence genes in MRSA isolates were compared with those in MSSA isolates.

^b^The positive rates of virulence genes of *S. aureus* isolates in 2013-2014 were compared with those in 2018-2019.

Figures
Distribution of STs in the clonal complexes. The diagram generated by eBURST based on the MLST data of this study, representing the relationships of 220 S. aureus isolates identified by MLST typing. Each number implies an MLST ST, STs that are linked by a line belong to the same cluster and the dot area indicates the prevalence of the ST in the MLST data of this study.