# 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, antibiotic resistance profiles and main antibiotic resistance genes. Results: Forty sequence types (STs) including three new STs (ST5489, ST5492 and ST5493), and 79 Staphylococcal protein A ( 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 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 (SCC mec ) typing. SCC mec- IVa was the predominant SCC mec type, and specifically, ST45-SCC mec IVa, an infrequent type in mainland China, was predominant in S. aureus from Hainan. The antibiotic resistance profiles and antibiotic resistance genes of S . aureus show distinctive features in Hainan. The resistant rates of the MRSA isolates to a variety of antibiotics were significantly higher than those of the MSSA isolates. The predominant erythromycin and tetracycline resistance genes were ermC (90.1%, 100/111) and tetK (91.8%, 78/85), respectively. Eleven virulence genes, including the Panton-Valentine leukocidin ( pvl ) and eta , were determined, and the frequency of eta and pvl were found to be 57.3% and 47.6%. Such high prevalence has never been seen in mainland China before. Conclusion: S. aureus isolates in Hainan have unique molecular characteristics, virulence gene and antibiotic resistance profiles, and main antibiotic resistance genes which may be associated with the special geographical location of Hainan and local trends in antibiotic use.


## Background

*Staphylococcus aureus* (*S. aureus*) is an important gram-positive pathogen causing various infectious diseases including pneumoniae and bacteremia. A previous study showed that patients with *S. aureus* infections had an excess one-year mortality of 20.2% compared with matched uninfected inpatients[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-SCC*mec*III has rapidly replaced ST239-t037-SCC*mec*III, 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]. In addition, a study reported that the prevalence of predominant clones, ST239-t030 and ST239-t037 were replaced by the continually growing ST5-t2460 clone in 2017 in Shanghai[14]. 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[15-18]. 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, 19, 20]. 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.

## Materials And Methods

*S. aureus isolates and primers*

A total of 227 consecutive and non-duplicate *S. aureus* isolates were collected from three hospitals in 2013-2014 (n=91) and 2018-2019 (n=136), respectively. Of the three hospitals, Hainan General Hospital is a large teaching hospital with over 100,000 admissions per year in Xiuying district of Haikou city; Haikou People’s Hospital is a medium-sized teaching hospital with about 50,000 admissions per year in Meinan district of Haikou city; and First Hospital Affiliated to Hainan Medical college is a medium-sized teaching hospital with 50,000 admissions per year in Longhua district of Haikou city. These isolates were collected from inpatients who had cough, fever and other clinical symptoms related to infection, and moreover, peripheral white blood cell and/or neutrophil counts were elevated at least. 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%). Only the first positive culture in the course of infection was included for further analysis. 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) .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, and results were interpreted in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI M100-S29)[21]. In addition, *S. aureus* isolates were further identified using PCR for amplification of *mecA* as described previously[22], and MRSA N315 was used as the positive control strain. The *mecA*-positive and cefoxitin-resistant isolates (cefoxitin minimum inhibitory concentration ≥8 μg/mL) were identified as MRSA. Isolates resistant to three or more different antimicrobial classes were defined as multidrug-resistant (MDR) isolates.

*Staphylococcal protein A (spa) typing*

Chromosomal DNAs were extracted from *S. aureus* isolates as described previously[23]. The extracted chromosomal DNAs were stored at -20°C for *spa*, *Staphylococcus* chromosomal cassette *mec* (SCC*mec*), and multilocus sequence typing, and detection of virulence genes. For *spa* typing, the variable repeat region of *spa* was amplified using oligonucleotide primers[23, 24] (see **Table 1**) followed by sequencing. The PCR mixture and conditions were similar to those described previously[23]. 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[23, 25]. 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 submitted to the MLST database and assigned to new STs. 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 SCC*mec* typing as previously described[26]. MRSA isolates with suspected SCC*mec*IV were recharacterized by additional multiplex PCR as subtypes IVa, IVb, IVc, and IVd as described by Zhang et al.[22]. 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 and antibiotic resistance 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[23]. The common ERY resistance genes (*ermA, ermB, ermC*), and the TET resistance genes (*tetL, tetK, tetM, tetO*) were examined using PCR assays as previously described[27, 28].

*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 and antibiotic resistance genes testing*

A total of 227 *S.aureus* were performed for antimicrobial susceptibility testing. The antimicrobial resistance profiles of the *S. aureus*，MRSA，MSSA and multidrug-resistant (MDR) isolates were showed in **Figure 1**. 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% of isolates were resistant to the remaining antibiotics, except for PEN, to which 92.5% had resistance. All of 76 FOX-resistant isolates, including an OXA susceptible-MRSA (OS-MRSA), were found to be *mecA*-positive, then classified to be MRSA isolates. Statistical analysis showed the resistant rates of the MRSA isolates to PEN (100.0% vs. 88.7%, $p=0.002$), ERY (75.0% vs. 35.8%, $p<0.001$), CLI (64.5% vs. 29.8%, $p<0.001$), GEN (18.4% vs. 8.6%, $p=0.031$), RIF (17.1% vs. 4.6%, $p=0.002$) and LEV (15.8% vs. 6.6%, $p=0.028$) were significantly higher than those of the MSSA isolates ,respectively.

A total of 111 ERY-resistant *S. aureus* isolates were found and used to examine the presence of e*rm*. The most prevalent *erm* was *ermC (*90.1%,100/111), followed by *ermB* (38.7%, 43/111) and *ermA* (21.6%, 24/111). ERY-resistant MRSA isolates had higher frequencies of *ermA* than ERY-resistant MSSA isolates (c2=6.855, p<0.05). All of 85 TET-resistant isolates carried TET-resistant gene *tet*. The prevalences of *tetK, tetM, tetL* and *tetO* were 91.8% (78/85), 67.1% (57/85), 23.5% (20/85) and 0.0% (0/85), respectively. TET-resistant MRSA isolates had higher frequencies *tetM* than TET-resistant MSSA isolates(c2=5.227, p<0.05).

One hundred thirteen (49.8%,) *S. aureus* isolates were found to multidrug-resistant (MDR) because of resistance to > 3 classes of antibiotics (**Table 2**). The chi-square test showed that the prevalence of MDR was significantly higher in the MRSA isolates than in the MSSA isolates (**Table 2**)(c2=26.115, p<0.05). 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%, $p<0.05$).

*MLST, spa, and SCCmec typing*

Forty STs belonging to 19 clonal complexes (CCs) and 2 singletons were identified by eBURST. As shown in **Table 3** and **Figure 2.** ST398 (14.1%, 32/227) was the most prevalent followed by ST188 (13.2%, 30/227) and ST45 (10.1%, 23/227). In addition, 3 isolates could not be assigned to any known ST, so these novel alleles were submitted to the MLST database and 3 new STs including ST5489, ST5492 and ST5493, were assigned. By *spa* typing, 79 *spa* types were found. The most prevalent was t189 (12.3%, 28/227) followed by t437 (7.9%, 18/227), t116 (7.5%, 17/227), and t011 (6.6%, 15/227). 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). 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).

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. 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 ($p<0.05$).

Among the 76 MRSA isolates, 6 SCC*mec* types or subtypes, namely types I, II, III, IVa, IVc, and V, were found. The most common SCC*mec* 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 SCC*mec* typing. When the STs and SCC*mec* typing were combined, the predominant combination was ST45-SCC*mec* IVa (8.8%, 20/227), and there was no significant difference in the positive rate of ST45-SCC*mec* 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 remaining ones were found in less than 50%. 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 frequency 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 frequency of *pvl*, *fnbB*, *hlb*, *seb, eta*, and *etb* and higher rates of harboring 6 or more virulence genes.

*Characteristics of the major clones ST398, ST188, and ST45*

The most abundant sequence type found in this study was ST398 (14.1%, 32/227) followed by ST188 (13.2%, 30/227) and ST45 (10.1%, 23/227). Majorities of ST398 (93.8%, 30/32) and ST188 (96.7%, 29/30) isolates were MSSA, whereas the majority of ST45 (87.0%, 20/23) isolates were MRSA, and all ST45-MRSA isolates belonged to the SCC*mec* IVa type (**Table 2**, **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. In addition, there was no significant difference in resistance rate to any antibiotics between ST398, ST188 and ST45 isolates.

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[29-31]. 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% (www.chinets.com), in Turkey, America, Russia and Australia in 2017, the average rates to OXA were 23.0%, 45.0%, 16.0 and 19.0%, and to RIF were 14.0%, 1.0%, 2.0%, 1.0% , respectively (resistancemap.cddep.org/ AntibioticResistance.php). The *S. aureus* isolates from Hainan had similar resistance rates against part of antibiotics to those from mainland China and other countries, but differences of resistance rates to

ERY and LEV existed. In addition, 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 difference of resistance to SXT may be due to the reduced usage frequency of SXT in recent years.

ERY and TET resistance is always attributed to the presence of resistance genes *erm* and *tet,* respectively. It was found that the predominant resistance gene in ERY-resistant isolates was *ermC*, which differed from previous studies that most ERY-resistant strains harboured *ermA*[27, 32]. In present study, *ermB* was present in 38.7% of ERY-resistant isolates, while in most previous studies, *ermB* was rare or even not detected[27, 32]. Therefore it is concluded that *S. aureus* isolates in Hainan have characteristic resistance genes causing erythromycin resistance. Most of TET-resistanct isolates harbored *tetM* and *tetK,* indicating *tetM* and *tetK* were resistance determinants being responsible for resistance to TET, which was consistent with results of previous studies[28, 32, 33]. Our study showed that the frequency of *tetM* was higher in TET resistant MRSA than that in TET resistant MSSA, which was consistent with the previous study that the resistance mechanism mediated by *tetM* is predominant among TET resistant MRSA[34].

MLST typing, *spa* typing, and SCC*mec* 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 SCC*mec* type was IVa, and ST45-SCC*mec* IVa was the most prevalent combination of ST and SCC*mec* typing in the MRSA isolates. ST188 and ST239 were previously reported as the predominant STs in MSSA and MRSA isolates, respectively[11, 19, 20, 35, 36]. Among these studies, two were multiple-center studies that showed that ST239-SCC*mec* III was the predominant MRSA genotype, but observed no ST45 clones at all[11, 20]. 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[14]. Therefore, it can be concluded that the molecular characteristics of *S. aureus* isolates in Hainan province are significantly different from those in mainland China. Combined with the above mentioned resistance status of *S. aureus* in Hainan and the special geographical location of Hainan. It is reasonable to speculate that the divergent molecular characteristics of *S. aureus* isolates in Hainan province is associated with the difference of antibiotics usage.

ST398 MSSA was found to be the most prevalent in Hainan province, and the patients with the ST398 MSSA isolates have no history of contact with livestock, therefore, the ST398 MSSA isolates we collected are of human origin. In addition, in the short span of five years, the prevalence of ST398 MSSA increased from 5.5% to 18.4% in Hainan province. Similar to the epidemic situation in Hainan province, ST398 MSSA have been increasingly reported as a cause of invasive infections in patients without livestock contact[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, 37]. 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[38]. Therefore, ST398 MSSA has emerged as an invasive pathogen causing bloodstream infections, BJIs, and potentially other conditions. Evidence suggests ST398 MRSA and ST398 MSSA belong to distinct lineages[39]. It is well known that ST398 MRSA lineage, associated with livestock, has been a worldwide threat within the last decade[4]. However, ST398 MSSA is a frequent source of *S. aureus* infections between individuals in households. This contrasts with the limited transmissibility of livestock-associated ST398 MRSA strains between humans[40]. ST398 MSSA has enhanced adhesion to human skin keratinocytes and keratin and it is more closely linked with human infections than ST398 MRSA, it was found that the 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 of patients, 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[41, 42]. Similar to the majority of studies in mainland China[8, 43], 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 frequency of *eta* and *pvl* were 57.3% and 47.6%, much higher than those in mainland China[10, 16, 19]. 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[37, 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*. Previous studies reported some virulence genes are linked to specific molecular types[43, 44]. For example, ST8 (USA300) was linked to the acquisition of the enterotoxin Q and K genes. ST36 (USA200) was associated with the acquisition of the enterotoxin A gene, and the toxic shock syndrome toxin 1 gene[44]. Therefore, it is rational to speculate that that the higher rates could be associated with the different distribution of STs. 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. The opposite variation trend between antibiotic resistance and virulence may be related to balance the energetic requirements for expressing resistance and producing toxins, which suggests that antibiotic resistance and virulence of pathogen are opposite during evolution[45, 46]. This study has some limitations. First, the small sample size limited the broad representativeness of the study. Secondly, we had no information about the relationship between clinical data (e.g. mortality, severity) and molecular characteristics of isolates, further research will focus on these fields.

## 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-SCC*mec* 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

SCC*mec* - *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 this study, and the results of SCC*mec* types I–V

| Primer | Nucleotide sequence(5'-3') | Target gene | Amplicon size(*bp*) | SCC*mec* type | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | I | II | III | IV | IVa | IVb | IVc | IVd | V |
| β | ATTGCCTTGATAATAGCCYTCT | *ccrA2-B* | 937 | | X | | X | | | | | |
| a3 | TAAAGGCATCAATGCACAAACACT | | | | | | | | | | | |
| ccrCF | CGTCTATTACAAGATGTTAAGGATAAT | *ccrC* | 518 | | | X | | | | | | X |
| ccrCR | CCTTTATAGACTGGATTATTCAAAATAT | | | | | | | | | | | |
| 1272F1 | GCCACTCATAACATATGGAA | *IS1272* | 415 | X | | | X | | | | | |
| 1272R1 | CATCCGAGTGAAACCCAAA | | | | | | | | | | | |
| 5R*mecA* | TATACCAAACCCGACAACTAC | *mecA–IS431* | 359 | | | | | | | | | X |
| 5R431 | CGGCTACAGTGATAACATCC | | | | | | | | | | | |
| Type IVa-F | GCCTTATTCGAAGAAACCG | □ | 776 | | | | | X | | | | |
| Type IVa-R | CTACTCTTCTGAAAAGCGTCG | | | | | | | | | | | |
| Type IVb-F | TCTGGAATTACTTCAGCTGC | □ | 493 | | | | | | X | | | |
| Type IVb-R | AAACAATATTGCTCTCCCTC | | | | | | | | | | | |
| Type IVc-F | ACAATATTTGTATTATCGGAGAGC | □ | 200 | | | | | | | X | | |
| Type IVc-R | TTGGTATGAGGTATTGCTGG | | | | | | | | | | | |
| Type IVd-F | CTCAAAATACGGACCCCAATACA | □ | 881 | | | | | | | | X | |
| Type IVd-R | TGCTCCAGTAATTGCTAAAG | | | | | | | | | | | |
| Spa-1113f | TAAAGACGATCCTTCGGTGAGC | *spa* | □ | | | | | | | | | |
| Spa-1514r | CAGCAGTAGTGCCGTTTGCTT | | | | | | | | | | | |
| arcC-F | TTGATTCACCAGCGCGTATTGTC | *arcC* | 456 | | | | | | | | | |
| arcC-R | AGG TATCTGCTTCAATCAGCG | | | | | | | | | | | |
| aroE-F | ATCGGAAATCCTATTTCACATTC | *aroE* | 456 | | | | | | | | | |
| aroE-R | GGTGTTGTATTAATAACGATATC | | | | | | | | | | | |
| glpF-F | CTAGGAACTGCAATCTTAATCC | *glpF* | 465 | | | | | | | | | |
| glpF-R | TGGTAAAATCGCATGTCCAATTC | | | | | | | | | | | |
| gmk-F | ATCGTTTTATCGGGACCATC | *gmk* | 417 | | | | | | | | | |
| gmk-R | TCATTAACTACAACGTAATCGTA | | | | | | | | | | | |
| pta-F | GTTAAAATCGTATTACCTGAAGG | *pta* | 474 | | | | | | | | | |
| pta-R | GACCCTTTTGTTGAAAAGCTTAA | | | | | | | | | | | |
| tpi-F | TCGTTCATTCTGAACGTCGTGAA | *tpi* | 402 | | | | | | | | | |
| tpi-R | TTTGCACCTTCTAACAATTGTAC | | | | | | | | | | | |
| yqiL-F | CAGCATACAGGACACCTATTGGC | *yqiL* | 516 | | | | | | | | | |
| yqiL-R | CGTTGAGGAATCGATACTGGAAC | | | | | | | | | | | |
| PVL-F | ATCATTAGGTAAAATGTCTGGACATGATCCA | *pvl* | 433 | | | | | | | | | |
| PVL-R | GCATCAASTGTATTGGATAGCAAAAGC | | | | | | | | | | | |
| FnbA-F | GTGAAGTTTTAGAAGGTGGAAAGATTAG | *fnbA* | 643 | | | | | | | | | |
| FnbA-R | GCTCTTGTAAGACCATTTTTCTTCAC | | | | | | | | | | | |
| FnbB-F | GTAACAGCTAATGGTCGAATTGATACT | *fnbB* | 524 | | | | | | | | | |
| FnbB-R | CAAGTTCGATAGGAGTACTATGTTC | | | | | | | | | | | |
| Hla-F | CTGATTACTATCCAAGAAATTCGATTG | *hla* | 209 | | | | | | | | | |
| Hla-R | CTTTCCAGCCTACTTTTTTATCAGT | | | | | | | | | | | |
| Hlb-F | GTGCACTTACTGACAATAGTGC | *hlb* | 309 | | | | | | | | | |
| Hlb-R | GTTGATGAGTAGCTACCTTCAGT | | | | | | | | | | | |
| Sea-F | GAAAAAAGTCTGAATTGCAGGGAACA | *sea* | 560 | | | | | | | | | |
| Sea-R | CAAATAAATCGTAATTAACCGAAGGTTC | | | | | | | | | | | |
| Seb-F | ATTCTATTAAGGACACTAAGTTAGGGA | *seb* | 404 | | | | | | | | | |
| Seb-R | ATCCCGTTTCATAAGGCGAGT | | | | | | | | | | | |
| Sec-F | GTAAAGTTACAGGTGGCAAAACTTG | *sec* | 297 | | | | | | | | | |
| Sec-R | CATATCATACCAAAAAGTATTGCCGT | | | | | | | | | | | |
| eta-F | CGCTGCGGACATTCCTACATGG | *eta* | 676 | | | | | | | | | |
| eta-R | TACATGCCCGCCACTTGCTTGT | | | | | | | | | | | |
| etb-F | CAGATAAAGAGCTTTATACACACATTAC | *etb* | 612 | | | | | | | | | |
| etb-R | AGTGAACTTATCTTTCTATTGAAAAACACTC | | | | | | | | | | | |
| clfA-F | ATTGGCGTGGCTTCAGTGCT | *clfa* | 292 | | | | | | | | | |
| clfA-R | CGTTTCTTCCGTAGTTGCATTTG | | | | | | | | | | | |
| ermA-F | GTTCAAGAAC AATCAATACA GAG | *ermA* | 421 | | | | | | | | | |
| ermA-R | GGATCAGGAA AAGGACATTT TAC | | | | | | | | | | | |
| ermB-F | CCGTTTACGA AATTGGAACA GGTAAAGGGC | *ermB* | 359 | | | | | | | | | |
| ermB-R | GAATCGAGAC TTGAGTGTGC | | | | | | | | | | | |
| ermC-F | GCTAATATTG TTTAAATCGT CAATTCC | *ermC* | 572 | | | | | | | | | |
| ermC-R | GGATCAGGAA AAGGACATTT TAC | | | | | | | | | | | |
| tetM-F | AGTGGAGCGATTACAGAA | *tetM* | 158 | | | | | | | | | |
| tetM-R | CATATGTCCTGGCGTGTCTA | | | | | | | | | | | |
| tetK-F | GTAGCGACAATAGGTAATAGT | *tetK* | 360 | | | | | | | | | |
| tetK-R | GTAGTGACAATAAACCTCCTA | | | | | | | | | | | |
| tetL-F | ATAAATTGTTTCGGGTCGGTAAT | *tetL* | 1077 | | | | | | | | | |
| tetL-R | AACCAGCCAACTAATGACAATGAT | | | | | | | | | | | |
| tetO-F | AACTTAGGCATTCTGGCTCAC | *tetO* | 514 | | | | | | | | | |
| tetO-R | TCCCACTGTTCCATATCGTCA | | | | | | | | | | | |

**Table 2** The frequency of MDRs, main STs, and virulence genes among MRSA and MSSA.

| isolates(n) | MDRs | Main STs | | | Virulence genes | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MDRs | ST398 | ST188 | ST45 | *pvl* | *fnbA* | *fnbB* | *hla* | *hlb* | *sea* | *seb* | *sec* | *eta* | *etb* | *clfA* |
| | (n,%) | (n,%) | (n,%) | (n,%) | (n,%) | (n,%) | (n,%) | (n,%) | (n,%) | (n,%) | (n,%) | (n,%) | (n,%) | (n,%) | (n,%) |
| MRSA(76) | 56 | 2 | 1 | 20 | 31 | 36 | 31 | 74 | 51 | 18 | 38 | 38 | 47 | 15 | 76 |
| | (73.7) | (2.6) | (1.3) | (26.3) | (40.8) | (47.4) | (40.8) | (97.4) | (67.1) | (23.7) | (50.0) | (50.0) | (61.8) | (19.7) | (100.0) |
| MSSA(151) | 57 | 30 | 29 | 3 | 77 | 51 | 82 | 150 | 110 | 17 | 70 | 25 | 83 | 28 | 151 |
| | (37.7) | (19.9) | (19.2) | (2.0) | (51.0) | (33.8) | (54.3) | (99.3) | (72.8) | (11.3) | (46.4) | (16.6) | (55.0) | (18.5) | (100.0) |
| *S.aureus*(227) | 113 | 32 | 30 | 23 | 108 | 87 | 113 | 224 | 161 | 35 | 108 | 63 | 130 | 43 | 227 |
| | (49.8) | (14.1) | (13.2) | (10.1) | (47.6) | (35.7) | (49.8) | (98.7) | (70.9) | (15.4) | (47.6) | (27.8) | (57.3) | (18.9) | (100.0) |
| *p* value* | <0.01 | <0.01 | <0.01 | <0.01 | 0.146 | 0.047 | 0.055 | 0.542 | 0.369 | 0.014 | 0.604 | <0.01 | 0.323 | 0.829 | |

* The frequency of MDRs, main STs, and virulence genes in MRSA isolates were compared with those in MSSA isolates.

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

| CC (no.) | 2013-2014 (91 isolates) | | | | | 2018-2019 (136 isolates) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | MLST(no.) | *spa*(no.) | MRSA(no.) | MSSA(no.) | SCC*mec*(no.) | MLST(no.) | *spa*(no.) | MRSA(no.) | MSSA(no.) | SCC*mec*(no.) |
| CC398(32) | ST398(5) | t011(3) | | 3 | | ST398(27) | t011(12) | | 12 | |
| | | t034(2) | | 2 | | | t034(9) | 2 | 7 | V(2) |
| | | | | | | | t1451(3) | | 3 | |
| | | | | | | | t571(1) | | 1 | |
| | | | | | | | t1580 (1) | | 1 | |
| | | | | | | | NT(1) | | 1 | |
| CC59(30) | ST59(8) | t437(4) | 1 | 3 | IVa(1) | ST59(14) | t437(12) | 9 | | IVa(5), V(4) |
| | | t441(1) | 1 | | V(1) | | t3385(1) | 1 | | IVa(1) |
| | | t1212(1) | | 1 | | | t5795(1) | 1 | | IVa(1) |
| | | t2356(1) | 1 | | IVa(1) | ST338(3) | t437(1) | | 1 | |
| | | t3592(1) | 1 | | V(1) | | t1751(2) | 2 | | V(1),NT(1) |
| | ST338(2) | t1751(2) | | 2 | | | | | | |
| | ST1778(2) | t437(1) | | 1 | | ST2041(1) | t13874(1) | | 1 | |
| | | t2365(1) | 1 | | IVa(1) | | | | | |
| CC188(30) | ST188(13) | t189(12) | | 12 | | ST188(17) | t189(16) | 1 | 15 | IVa(1) |
| | | t4950(1) | | 1 | | | t2174(1) | | 1 | |
| CC45(25) | ST45(13) | t116(10) | 8 | 2 | IVa(8) | ST45(10) | t116(7) | 6 | 1 | IVa(6) |
| | | t015(1) | 1 | | IVa(1) | | t026(1) | 1 | | IVa(1) |
| | | t2131(1) | 1 | | IVa(1) | | t157(1) | 1 | | IVa(1) |
| | | NT(1) | 1 | | IVa(1) | | t3349(1) | 1 | | IVa(1) |
| | | | | | | ST508(2) | t1203(1) | 1 | | NT(1) |
| | | | | | | | t908(1) | 1 | | IVa(1) |
| CC5(17) | ST5(6) | t002(3) | | 3 | | ST5(8) | t2358(2) | 2 | | IVa(2) |
| | | t954(1) | | 1 | | | t548(1) | | 1 | |
| | | t6212(1) | | 1 | | | t777(1) | | 1 | |
| | | t2358(1) | 1 | | IVa(1) | | t1265(1) | | 1 | |
| | ST965(1) | t062(1) | 1 | | IVa(1) | | t179(1) | | 1 | |
| | | | | | | | t2980(1) | | 1 | |
| | | | | | | | t9987(1) | | 1 | |
| | | | | | | ST764(1) | t1084(1) | 1 | | II(1) |
| | | | | | | ST2633(1) | t010(1) | | 1 | |
| CC7(17) | ST7(4) | t091(4) | | 4 | | ST7(10) | t091(7) | | 7 | |
| | | | | | | | t867(1) | | 1 | |
| | | | | | | | t2874(1) | | 1 | |
| | | | | | | | t3932(1) | | 1 | |
| | ST5489(1) | t091(1) | | 1 | | ST789(1) | t2453(1) | | 1 | |
| | ST4457(1) | t796(1) | | 1 | | | | | | |
| CC88(16) | ST88(8) | t1376(4) | 1 | 3 | II(1) | ST88(8) | t1376(3) | 1 | 2 | IVa(1) |
| | | t2592(1) | 1 | | IVa(1) | | t4333(2) | | 2 | |
| | | t3622(1) | | 1 | | | NT(3) | | 3 | |
| | | t15796(1) | | 1 | | | | | | |
| | | NT(1) | | 1 | | | | | | |
| CC1(14) | ST1(4) | t127(1) | | 1 | | ST1(8) | t127(5) | 1 | 4 | NT(1) |
| | | t2207(3) | 3 | | NT(3) | | t2207(2) | 2 | | NT(2) |
| | ST610(1) | t2207(1) | 1 | | II(1) | | t114(1) | | 1 | |
| | | | | | | ST2583(1) | t1381(1) | 1 | | IVa(1) |
| CC8(9) | ST239(3) | t030(2) | 2 | | III(2) | ST239(3) | t030(2) | 2 | | III(2) |
| | | t037(1) | 1 | | III(1) | | t037(1) | 1 | | III(1) |
| | | | | | | ST630(2) | t377(1) | | 1 | |
| | | | | | | | t4549(1) | | 1 | |
| | | | | | | ST5492(1) | t1987(1) | | 1 | |
| CC2580(6) | ST2580(5) | t3351(4) | 4 | | IVa(1), IVc(3) | ST2580(1) | t3351(1) | 1 | | IVc(1) |
| | | t4875(1) | 1 | | IVc(1) | | | | | |
| CC72(6) | ST72(2) | t148(2) | | 2 | | ST72(4) | t148(3) | | 3 | |
| | | | | | | | t3092(1) | | 1 | |
| CC121(5) | ST121(4) | t269(1) | | 1 | | ST120(1) | t2613(1) | 1 | | NT(1) |
| | | t162(2) | | 2 | | | | | | |
| | | t159(1) | | 1 | | | | | | |
| CC15(4) | ST15(1) | t1492(1) | | 1 | | ST15(1) | t085(1) | | 1 | |
| | ST4438(2) | t084(2) | | 2 | | | | | | |
| CC97(3) | ST464(1) | t3992(1) | | 1 | | ST97(1) | t267(1) | | 1 | |
| | | | | | | ST464(1) | t3904(1) | | 1 | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| CC2196(3) | ST4435(1) | t037(1) | 1 | | IVa(1) | ST2196(2) | NT(2) | | 2 | |
| CC9(2) | ST9(1) | t899(1) | | 1 | | ST9(1) | t899(1) | 1 | | I(1) |
| CC509(2) | | | | | | ST509(2) | t375(2) | 1 | 1 | IVa(1) |
| CC1281(2) | | | | | | ST1281(2) | t164(2) | | 2 | |
| CC25(2) | ST5493(1) | t12584(1) | | 1 | | ST25(1) | t280(1) | | 1 | |
| Singletons(2) | ST6(1) | t304(1) | 1 | | IVa(1) | | | | | |
| | ST944(1) | t616(1) | | 1 | | | | | | |

NT: non-typeable

**Table 4** The frequency of virulence genes among main types of *S. aureus* isolates and the comparison of two time periods

| Virulence genes | *S. aureus* (n=227)n(%) | ST398（n=32)n(%) | ST188（n=30)n(%) | ST45 (n=23)n(%) | 2013-2014 (n=91)n(%) | 2018-2019 (n=136)n(%) | *P* value* |
|---|---|---|---|---|---|---|---|
| *pvl* | 108(47.6) | 26(81.3) | 11(36.7) | 4(17.4) | 25(27.5) | 83(61.0) | <0.01 |
| *fnbA* | 87(35.7) | 7(21.9) | 7(23.3) | 10(43.5) | 33(36.3) | 54(39.7) | 0.601 |
| *fnbB* | 113(49.8) | 31(96.9) | 6(20.0) | 6(26.1) | 19(20.9) | 94(69.1) | <0.01 |
| *hla* | 224(98.7) | 32(100.0) | 29(96.7) | 23(100.0) | 91(100.0) | 133(97.8) | 0.405 |
| *hlb* | 161(70.9) | 20(62.5) | 19(63.3) | 6(26.1) | 44(48.4) | 117(86.0) | <0.01 |
| *sea* | 35(15.4) | 5(15.6) | 2(6.7) | 1(4.3) | 13(14.3) | 22(16.2) | 0.699 |
| *seb* | 108(47.6) | 11(34.4) | 18(60.0) | 8(34.8) | 35(38.5) | 73(53.7) | 0.024 |
| *sec* | 63(27.8) | 2(6.3) | 5(16.7) | 22(95.7) | 28(30.8) | 35(25.7) | 0.406 |
| *eta* | 130(57.3) | 24(75.0) | 14(46.7) | 22(95.7) | 21(23.1) | 109(80.1) | <0.01 |
| *etb* | 43(18.9) | 10(31.3) | 6(20.0) | 3(13.0) | 0(0.0) | 43(31.6) | <0.01 |
| *clfA* | 227(100.0) | 32(100.0) | 30(100.0) | 23(100.0) | 91(100.0) | 136(100.0) | – |

*The frequency of virulence genes of *S. aureus* isolates in 2013-2014 were compared with those in 2018-2019.

## Figures

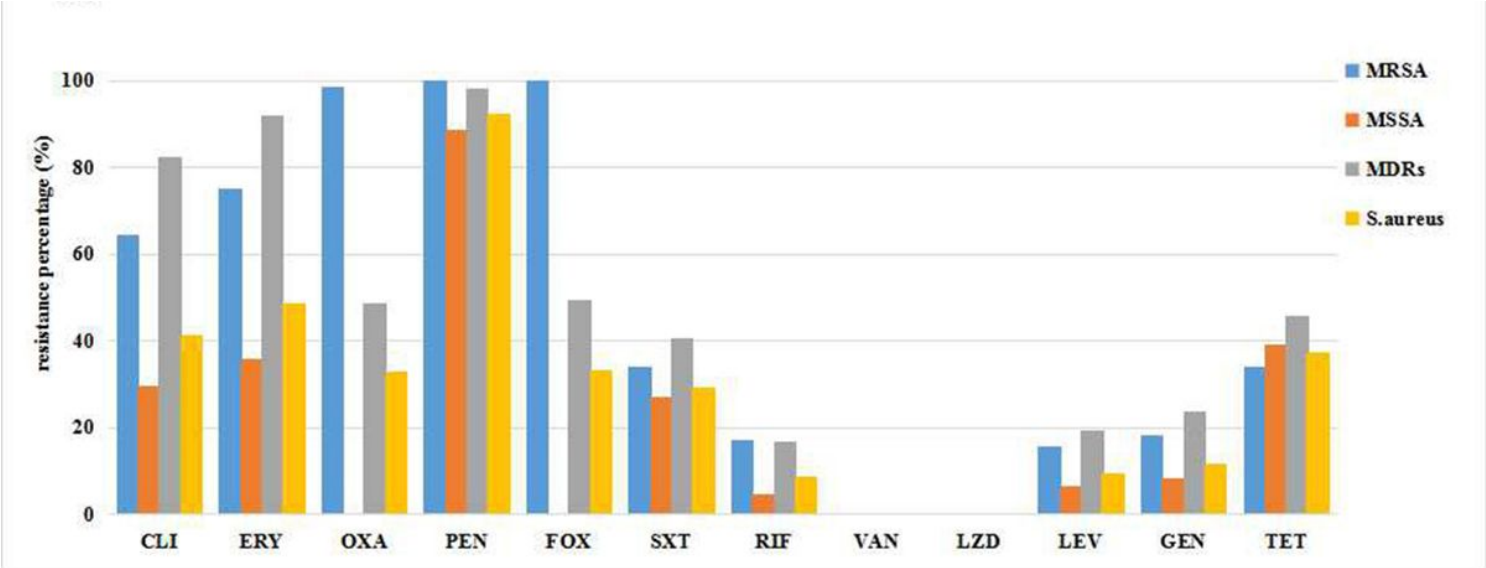

Figure 1

Antimicrobial resistance profiles of MRSA, MSSA, MDRs and S. aureus isolates.

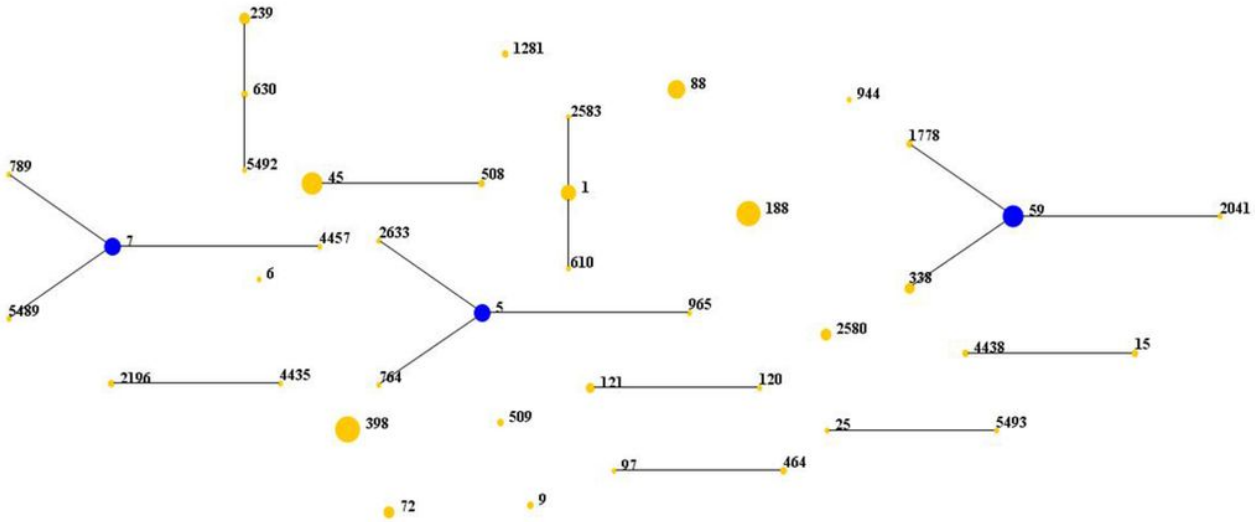


Figure 2

Distribution of STs in the clonal complexes. The diagram generated by eBURST based on the MLST data of this study, representing the relationships of 227 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.