

# Characteristics of Virulent ST5-SCCmec II Methicillin-Resistant *Staphylococcus aureus* Prevalent in a Surgery Ward

Lei Huang<sup>1,2</sup>, Chengcheng Liu<sup>1,2</sup>, Zhanjie Li<sup>3</sup>, Xu Huang<sup>1,4</sup>, Ruiying Zheng<sup>1,2</sup>, Zhixin Shi<sup>1,2</sup>, Xin Hong<sup>1,2</sup>, Yufeng Qin<sup>5</sup>, Genyan Liu<sup>1,2</sup>

<sup>1</sup>Department of Laboratory Medicine, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, People's Republic of China; <sup>2</sup>Branch of National Clinical Research Center for Laboratory Medicine, the First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, People's Republic of China; <sup>3</sup>Department of Infection Control, the First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, People's Republic of China; <sup>4</sup>Department of Laboratory Medicine, the Children's Hospital of Nanjing Medical University, Nanjing, Jiangsu, People's Republic of China; <sup>5</sup>School of Public Health, Nanjing Medical University, Nanjing, Jiangsu, People's Republic of China

Correspondence: Genyan Liu, Department of Laboratory Medicine, The First Affiliated Hospital with Nanjing Medical University, Nanjing, 210029, People's Republic of China, Tel +86 25 68308025, Fax +86 25 83724440, Email liugenyan@njmu.edu.cn

**Objective:** To investigate the transmission pathway of a MRSA prevalence in a pancreatic surgery ward in a Chinese teaching hospital.

**Methods:** Molecular epidemiology investigations were carried out combined PFGE, MLST, SCCmec typing and whole-genome sequencing for 20 successive MRSA isolates (2 isolates from the ward environment). Resistance and virulence genes were detected using specific PCR. Bacterial identification and AST were performed using the Vitek 2 Compact System. Clinical data of enrolled cases were retrieved from electronic case records.

**Results:** From January 2020 to May 2020, successive isolated 20 MRSA strains were clarified to 2 PFGE patterns (A = 19, B = 1) in the ward. Both isolates from environment and patients belonged to sequence type ST5-SCCmec II-*spa* type t311. MRSA-related resistance genes *mecA*, *blaZ*, *ermA*, *ant(4')-Ia* and *norA* were found in each clone. All 20 isolates carried *tst*, *hlg*, *hla*, *eta*, *eap*, *fnbA* and *seo* virulence genes, other virulence genes such as *sea*, *sec*, *seb*, *seg*, *sei*, *sem*, *sen*, *ebpS* and *fnbB* were also found in partial stains. All patients had fever symptom, 27.8% were accompanied by diarrhea, 88.9% had undergone surgery or invasive procedures within 30 days. Finally, 94.4% of these patients recovered.

**Conclusion:** This study confirmed a prevalence of ST5-MRSA-II-t311 clone in a surgery ward, indicated MRSA is a risk factor for post-surgery nosocomial infection and hand hygiene and environmental surveillance should not be ignored.

**Keywords:** methicillin-resistant *Staphylococcus aureus*, ST5, SCCmec II, *spa* type t311, diarrhea, virulence genes

## Introduction

*Staphylococcus aureus* (*S. aureus*) as one of the most common pathogens in hospitals and the community has been implicated in a variety of clinically important infections ranging from skin infections to invasive infections. The pathogenicity of *S. aureus* is related to various virulence factors. Hemolysin, enterotoxin, leukotoxin and toxic-shock syndrome toxin-1 (TSST-1) are common toxins that can induce severe infection symptoms.<sup>1,2</sup> Additionally, enzymes produced by *S. aureus* destroy host cells and tissues, incapacitating host immune defenses, thereby accelerating the growth and spread of bacteria in host cells. Immune evasion cluster (IEC) genes, such as *sak*, *chp*, *scn* and *sea*, are important for isolates to escape the immune response of the human host.<sup>2,3</sup>

*S. aureus* infections are particularly hard to treat for frequently occurring antibiotic resistance in clinical isolates, among which methicillin-resistant *S. aureus* (MRSA) are the most important clinically.<sup>4</sup> Moreover, MRSA has become one of the most popular healthcare associated pathogens in hospitals worldwide.<sup>5</sup> Nosocomial MRSA infections can lead to increased hospitalization costs and prolonged hospital stay.<sup>6</sup> The popular mode of MRSA transmission is through

direct contact, usually skin-to-skin contact with the hosts or infected persons, although contact with objects and surfaces may also have effects.<sup>7,8</sup> Nosocomial infections and outbreaks of MRSA are also closely related to hospital environmental contamination.<sup>9</sup> An important means to control the prevalence of MRSA is to identify the epidemic link and cut off the transmission route. Therefore, detecting the molecular typing of MRSA in patients and the environment is of great significance for identifying the correlation between strains and controlling their transmission and outbreaks.

Here, we report a MRSA prevalence identified as ST5-MRSA-II-t311 clone within a surgery ward, two homologous MRSA strains from the ward environment were isolated and further infection prevention and control (IPC) measures were carried out. Meanwhile, the characteristics of MRSA resistance, virulence and clinical symptoms of patients were also investigated.

## Materials and Methods

### Clinical Information and Strains

The pancreatic surgery ward is located in a large medical center with more than 3000 beds in Jiangsu Province, China. It has more than 30 beds, and most of the patients admitted had undergone surgery. Due to the continuous reporting of MRSA infection cases in this surgical ward, 27 MRSA strains were cultured from 18 patients from January 2020 to May 2020. Among which, 19 strains were aggregated isolated in April 2020 and aroused the attention of hospital infection prevention and control (IPC). During the IPC investigation, totally of 41 swabs from the ward environment, the nasal cavities and hands of medical staff were collected. Only 2 MRSA isolates with same resistant phenotype were found from the environment, one from the bed bar and the other from the caller of 2 patients, respectively. Clinical data were collected, including patient gender, age, invasive medical therapies and treatment outcomes.

### Antimicrobial Susceptibility Testing

All these MRSA strains were identified with antimicrobial resistance characteristic by the VITEK-2 compact system (bioMérieux, France) with the operating instructions. Fifteen routine antibiotics were tested. According to the results provided by VITEK-2, strains with positive cefoxitin screening test and oxacillin resistance (oxacillin minimum inhibitory concentration  $\geq 4$   $\mu\text{g/mL}$ ) were identified as MRSA. ATCC 29213 was used as the quality control strain, and the results were interpreted according to the Clinical and Laboratory Standards Institute guidelines (CLSI M100-S30).

### PFGE Analysis

The diversity of MRSA strains was analyzed using the PFGE methodology, as previously described<sup>10</sup>. We used *Salmonella enterica* serotype H9812 as the molecular size marker. Following digestion of chromosomal DNA with *Sma*I (TaRaKa, Dalian, China), the isolates were subjected to agarose gel electrophoresis on a Bio-Rad CHEF Mapper Pulsed Field Electrophoresis System at 14°C for 18 h with switch times of 4s and 40s at 6 V/cm. Computer-assisted analysis was performed using BioNumerics software (Applied Maths, Kortrijk, Belgium) and banding patterns were compared by the Unweighted Pair Group Method using the Arithmetic Mean (UPGMA). Clustering was defined as a group of isolates sharing >90% of pattern similarity.

### MLST Typing

The MRSA genomic DNA of the strains was extracted by TIANamp Bacteria DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. Primers for housekeeping genes were designed as described on the *Staphylococcus aureus* multilocus sequence typing website (<https://pubmlst.org/organisms/staphylococcus-aureus/primers>) and were synthesized by Nanjing Kingsley Company. PCR was carried out with reference to the protocol, and the amplified products were sent to Nanjing Kingsley Company for sequencing. Chromas software was used to read the sequencing results and observe peak values, and the DNA Star software package was used for sequence editing and correction followed by submission to the website ([https://pubmlst.org/bigdb?Db=pubmlst\\_Saureus\\_Seqdef&page=sequenceQuery](https://pubmlst.org/bigdb?Db=pubmlst_Saureus_Seqdef&page=sequenceQuery)) for comparison. Then, a number was given to each allele. Sequence types (STs) were assigned according to the combination of 7 allele numbers listed in the online database.

## SCCmec Typing

The SCCmec type was determined using multiplex PCR as previously described,<sup>11</sup> which generated specific amplification patterns for each SCCmec structure type (see [Supplementary Table 1](#) for primers). The PCR reaction mixtures contained 12.5 µL Premix Taq<sup>TM</sup> (Ex Taq<sup>TM</sup> Version 2.0) (TaRaKa, Dalian, China), 5 µL chromosomal DNA, 4.95 µL oligonucleotide primers (10 mM) and 2.55 µL Ranse-free water (TaRaKa, Dalian, China) in a final volume of 25 µL. PCR conditions were as follows: incubation at 94°C for 5 min, followed by 25 cycles of 94°C for 30s, 56°C for 30s and 72°C for 1 min; 25 cycles of 94°C for 30s, 52°C for 30s and 72°C for 1 min and a final extension step at 72°C for 10 min. Then the PCR products were analyzed by gel electrophoresis.

## Resistance and Virulence Genes Assays

PCR was used to detect resistance genes, including: *blaZ*, *mecA*, *ermA*, *ermB*, *ermC*, *ermT*, *msrA*, *tetK*, *tetM*, *tetL*, *tetO*, *aac(6')/aph(2'')*, *ant(4')-Ia*, *aph(3')-III* and *norA*. Virulence genes as follows: cytolytins (*hla*, *hlg*, *pvl*), SAgS (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sem*, *sen*, *seo*, *tst*), exfoliative toxins (*eta*, *etb*), adhesins (*bbp*, *cna*, *ebpS*, *fmbA*, *fmbB*, *eap*) and genes related to biofilm production (*icaA* and *sasG*) were also detected. Reaction mixtures contained 12.5 µL Premix Taq<sup>TM</sup> (Ex Taq<sup>TM</sup> Version 2.0) (TaRaKa, Dalian, China), 2 µL chromosomal DNA, oligonucleotide primers (10 mM) and 8.5 µL Ranse-free water (TaRaKa, Dalian, China) in a final volume of 25 µL. The PCR primers, reaction conditions and references are shown in [Supplementary Table 2](#)<sup>12–14</sup> and [Supplementary Table 3](#).<sup>15</sup> PCR products were analyzed by agarose gel electrophoresis.

## Whole Genome Sequencing

Three strains (two strains were isolated from patients, one strain was isolated from ward environment) among the MRSA clusters were selected for further analysis through whole genome sequencing (WGS). The cluster generation and sequencing were performed on Novaseq 6000 S4 platform, using NovaSeq 6000 S4 Reagent kit V1.5. The sequencing data was preliminarily assembled using software Newbler (version 2.8), and the assembled data was corrected by blasR comparison of single molecule sequencing data. The assembled sequence is used for molecular typing such as *spa*, MLST, SCCmec typing and analysis of resistance and virulence genes according to the methods previously reported.<sup>16</sup>

## Results

### Clinical Characteristics

A total of 27 MRSA strains from 18 patients were isolated in the general surgery ward from January 2020 to May 2020, and 1 to 3 samples were obtained from each patient. Different strains from the same patient showed identical resistance phenotypes and belonged to the same PFGE type. Finally, 18 nonrepetitive MRSA isolates recovered from 18 patients along with 2 environmental MRSA strains were selected for further molecular typing, resistance genes analysis and virulence genes detection. The specimens of selected 18 strains included ascites (11/18, 61%), pus (4/18, 22.2%), blood (1/18, 5.6%), sputum (1/18, 5.6%), and feces (1/18, 5.6%). Among the 18 patients, 22.2% (4/18) were female, 77.8% (14/18) were male, and 66.7% (12/18) were ≥60 years old; All patients had fever symptom, 27.8% (5/18) were accompanied with diarrhea, 88.9% (16/18) had undergone surgery or invasive procedures within 30 days. Finally, 94.4% (17/18) of these hospitalized patients finally recovered, and only 1 died ([Table 1](#)).

### Antimicrobial Susceptibility Testing

Antimicrobial susceptibility tests were performed on all MRSA strains, and their susceptibility profiles are listed in [Table 2](#). All MRSA strains exhibited 100% resistance to cefoxitin, penicillin, oxacillin, ciprofloxacin, levofloxacin, moxifloxacin, erythromycin and clindamycin. In addition, the resistance rate of tetracycline was 95%, and only 1 of these 20 MRSA strains was sensitive to tetracycline. Furthermore, these 20 isolates were 100% sensitive to gentamicin, quinupristin/dafopristin, linezolid, vancomycin, tigecycline, rifampicin and trimethoprim-sulfamethoxazole.

**Table 1** Characteristics of the 18 MRSA Isolates from Hospitalized Patients

Item	Group	Number	Ratio
Specimen Origin	Ascites	11	61.0%
	Pus	4	22.2%
	Blood	1	5.6%
	Sputum	1	5.6%
	Faeces	1	5.6%
Gender	Female	4	22.2%
	Male	14	77.8%
Age	≤60 years old	6	33.3%
	>60 years old	12	66.7%
Symptoms	Fever	18	100%
	Diarrhea	5	27.8%
Risk factor	Surgical/invasive procedures	16	88.9%
Outcomes	Recovered	17	94.4%
	Death	1	5.6%
No. of total	Total	18	

**Table 2** Antimicrobial Susceptibilities Among the 20 MRSA Isolates

Antibiotics	R	S	MIC Range (μg/mL)	Resistance Rate (%)
Penicillin	20	0	≥0.5	100
Oxacillin	20	0	≥4	100
Gentamicin	0	20	≤4	0
Ciprofloxacin	20	0	≥8	100
Levofloxacin	20	0	≥8	100
Moxifloxacin	20	0	≥4	100
Erythromycin	20	0	≥8	100
Clindamycin	20	0	≥8	100
Quinupristine/dafopristine	0	20	≤0.5	0
Linezolid	0	20	1–4	0
Vancomycin	0	20	≤2	0
Tetracycline	19	1	≥8 (R), ≤1 (S)	95
Tigecycline	0	20	≤0.25	0
Rifampicin	0	20	≤0.5	0
Trimethoprim-sulfamethoxazole	0	20	≤0.5	0

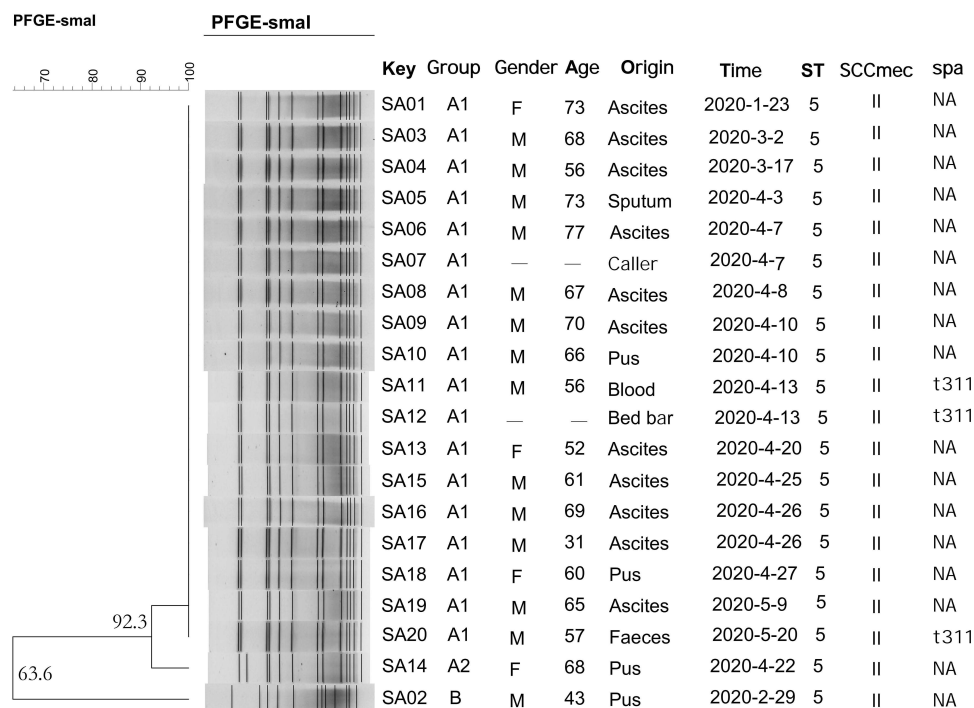
**Abbreviations:** R, resistant; S, susceptible; MIC, minimal inhibitory concentration.

## Molecular Genotyping

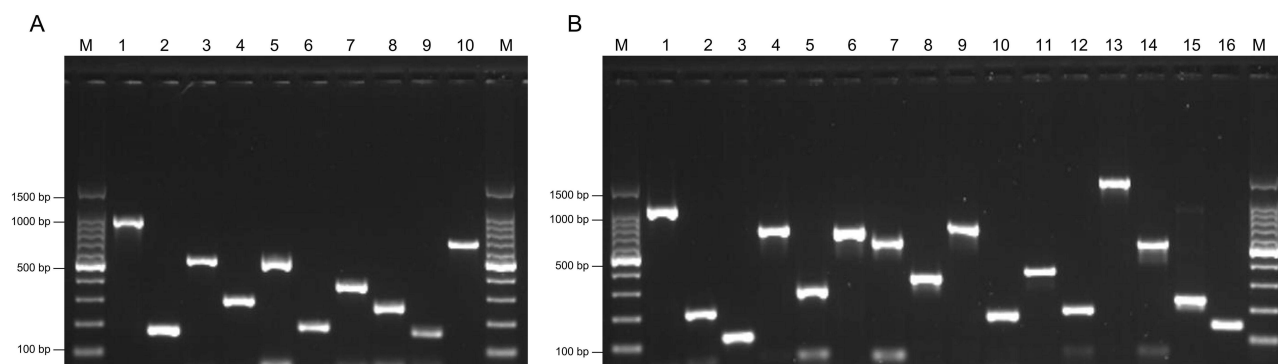
Dendrogram of PFGE patterns and molecular genotyping results of each strain were summarized and shown in [Figure 1](#). PFGE results showed that 18 clinical strains and 2 environmental strains were divided into 2 types, 19 strains belong to type A (18 strains of type A1, including 2 environmental strains, and 1 strain of type A2), and 1 strain belong to type B. All of these MRSA strains were identified as ST5 (1,4,1,4,12,1,10), CC5, SCCmec II. Results of selected 3 strains (PFGE type A1) WGS analysis belong to ST5-MRSA-II-t311 (*spa* type repeats succession: 26–23–17–34–20–17–12–17–16).

## Resistance Genes Detection

Most resistance genes of each MRSA isolates were detected. Among which, 100% isolates contain *blaZ*, *mecA*, *ermA*, *ant(4')-Ia* and *norA* genes, positive rate of *tetK* and *aac(6')/aph(2'')* was 95%, *aph(3')-III*, *msrA* and *tetL* was 5%. Representative PCR results are shown in [Figure 2A](#). These 20 strains showed 4 different resistance gene



**Figure 1** Dendrogram of PFGE patterns from 20 MRSA strains.



**Figure 2** Representative PCR results for resistance genes and virulence genes of MRSA isolates. (A). M, marker, lane 1 to lane 10 represent the PCR positive results of resistance genes *mecA*, *blaZ*, *aph(3')-III*, *ant(4')-Ia*, *aac(6')/aph(2'')*, *ermA*, *tetK*, *tetL*, *msrA* and *norA* respectively; (B). M, marker, lane 1 to lane 16 represent the PCR positive results of virulence genes *hlg*, *hla*, *sea*, *seb*, *sec*, *seg*, *sei*, *sem*, *sen*, *seo*, *tst*, *ebpS*, *fnbA*, *fnbB*, *eap* and *eta* respectively.

profiles, and the dominant resistance gene profile (17/20, 85%) contained 7 resistance genes, including *mecA*, *blaZ*, *aac(6')-aph(2'')*, *ant(4')-Ia*, *ermA*, *tet(K)*, *norA*. Among these strains, the largest number of resistance genes was 9 (Table 3). More resistance genes were found in 3 selected stains after WGS analysis, such as *aadD* and *ant(9)-Ia*.

## Virulence Genes Detection

The frequencies of virulence genes were *hlg*, *hla*, *tst*, *seo*, *eta*, *eap* and *fnbA* (100%), *sea* (95%), *ebpS* (90%), *sec* (85%), *seb* and *seg* (75%), *sei* (45%), *sem* (35%), *sen* (25%) and *fnbB* (5%), respectively, none of which contained *sed*, *see*, *seh*, *pvl*, *etb*, *bbp*, *cna*, *icaA* and *sasG* genes. Representative PCR results are shown in Figure 2B. The 20 strains exhibited 11 different virulence gene profiles, and 85% of the 20 strains had more than 10 virulence genes (Table 4).

**Table 3** Resistance Gene Profiles Among 20 MRSA Isolates

Resistance Genes	Resistance Gene Profiles	Patients	Ratio
5	<i>mecA</i> , <i>blaZ</i> , <i>ant(4')-Ia</i> , <i>erm(A)</i> , <i>norA</i>	1	0.05
7	<i>mecA</i> , <i>blaZ</i> , <i>aac(6')-aph(2'')</i> , <i>ant(4')-Ia</i> , <i>erm(A)</i> , <i>tet(K)</i> , <i>norA</i>	17	0.85
8	<i>mecA</i> , <i>blaZ</i> , <i>aac(6')-aph(2'')</i> , <i>ant(4')-Ia</i> , <i>erm(A)</i> , <i>msr(A)</i> , <i>tet(K)</i> , <i>norA</i>	1	0.05
9	<i>mecA</i> , <i>blaZ</i> , <i>aac(6')-aph(2'')</i> , <i>aph(3')-III</i> , <i>ant(4')-Ia</i> , <i>erm(A)</i> , <i>tet(K)</i> , <i>tet(L)</i> , <i>norA</i>	1	0.05

**Table 4** Virulence Gene Profiles Among 20 MRSA Isolates

Virulence Genes	Virulence Gene Profiles	Patients	Ratio
9	<i>hlg</i> , <i>hla</i> , <i>tst</i> , <i>eta</i> , <i>sea</i> , <i>seo</i> , <i>eap</i> , <i>ebpS</i> , <i>fnbA</i>	3	0.15
10	<i>hlg</i> , <i>hla</i> , <i>tst</i> , <i>eta</i> , <i>sea</i> , <i>sec</i> , <i>seg</i> , <i>seo</i> , <i>eap</i> , <i>fnbA</i>	1	0.05
10	<i>hlg</i> , <i>hla</i> , <i>tst</i> , <i>eta</i> , <i>sea</i> , <i>sec</i> , <i>seo</i> , <i>eap</i> , <i>ebpS</i> , <i>fnbA</i>	1	0.05
11	<i>hlg</i> , <i>hla</i> , <i>tst</i> , <i>eta</i> , <i>sea</i> , <i>seb</i> , <i>sec</i> , <i>seo</i> , <i>eap</i> , <i>ebpS</i> , <i>fnbA</i>	2	0.10
11	<i>hlg</i> , <i>hla</i> , <i>tst</i> , <i>eta</i> , <i>sea</i> , <i>seb</i> , <i>sec</i> , <i>seg</i> , <i>seo</i> , <i>eap</i> , <i>fnbA</i>	1	0.05
11	<i>hlg</i> , <i>hla</i> , <i>tst</i> , <i>eta</i> , <i>sea</i> , <i>seb</i> , <i>seg</i> , <i>seo</i> , <i>eap</i> , <i>ebpS</i> , <i>fnbA</i>	1	0.05
12	<i>hlg</i> , <i>hla</i> , <i>tst</i> , <i>eta</i> , <i>sea</i> , <i>seb</i> , <i>sec</i> , <i>seg</i> , <i>seo</i> , <i>eap</i> , <i>ebpS</i> , <i>fnbA</i>	3	0.15
13	<i>hlg</i> , <i>hla</i> , <i>tst</i> , <i>eta</i> , <i>sea</i> , <i>seb</i> , <i>sec</i> , <i>seg</i> , <i>sei</i> , <i>seo</i> , <i>eap</i> , <i>ebpS</i> , <i>fnbA</i>	2	0.10
14	<i>hlg</i> , <i>hla</i> , <i>tst</i> , <i>eta</i> , <i>sea</i> , <i>seb</i> , <i>sec</i> , <i>seg</i> , <i>sei</i> , <i>sem</i> , <i>seo</i> , <i>eap</i> , <i>ebpS</i> , <i>fnbA</i>	2	0.10
15	<i>hlg</i> , <i>hla</i> , <i>tst</i> , <i>eta</i> , <i>sea</i> , <i>seb</i> , <i>sec</i> , <i>seg</i> , <i>sei</i> , <i>sem</i> , <i>sen</i> , <i>seo</i> , <i>eap</i> , <i>ebpS</i> , <i>fnbA</i>	3	0.15
15	<i>hlg</i> , <i>hla</i> , <i>tst</i> , <i>eta</i> , <i>seb</i> , <i>sec</i> , <i>seg</i> , <i>sei</i> , <i>sem</i> , <i>sen</i> , <i>seo</i> , <i>eap</i> , <i>ebpS</i> , <i>fnbA</i> , <i>fnbB</i>	1	0.05

## Discussion

*S. aureus* is a key pathogen of nosocomial infection, especially MRSA, which poses a great challenge to health due to its multidrug resistance, prevalence and refractoriness.<sup>17–23</sup> *Staphylococcus aureus* is the most common pathogen causing surgical site infections (SSIs).<sup>24</sup> MRSA can survive for a long time in nature, increasing the risk of nosocomial infection of patients undergoing complex surgical procedures.<sup>25–27</sup> Previous reports unfold that methicillin-resistant *S. aureus* (MRSA) accounts for a high proportion of isolates in SSIs,<sup>24,28–30</sup> the possible cause is related to MRSA colonization.<sup>31–35</sup> Furthermore, advanced age and invasive operations have been confirmed as important risk factors for MRSA colonization.<sup>36–41</sup> From our investigation, 66.7% (12/18) of patients were  $\geq 60$  years old and 88.9% (16/18) had undergone surgery or invasive procedures within 30 days, all these may increase the risk of MRSA colonization and subsequent infection. As a pancreatic surgery ward, most patients underwent abdominal surgery or invasive operation. Of the 18 patients, most infections were surgical-site-related infections, in which, 11 infection samples were ascites and 4 were pus. Otherwise, health-care staff hand hygiene and ward environmental maintenance are important for infection control. In this study, two homologous MRSA strains from the ward environment were isolated, the transmission routes of MRSA in relation to environment should not be ignored. Indeed, the MRSA prevalence was successfully controlled after comprehensive infection control measures were applied in the ward.

The predominant sequence type of MRSA in this study is ST5-II-t311, which belongs to clonal complex 5 (CC5). ST5-MRSA-II are also known as New York/Japan clone, UKEMRSA-3, USA100, Rhine-Hesse Epidemic Strain, Irish AR7.3, AR7.4, AR7.11 and Canadian MRSA-2 clone.<sup>42</sup> Previous reports point out that ST5-SCC*mec* II is one of the most common types of hospital-associated MRSA isolate, widely prevalent in Asia, Europe, Africa, North and South America.<sup>43–45</sup> Furthermore, ST5-SCC*mec* II-t311 has also been reported as the predominant clone and spread between different hospitals in some areas of China,<sup>46,47</sup> to our knowledge, here is the first report of ST5-SCC*mec* II-t311 prevalence in Jiangsu Province.

A multicentre longitudinal study in China reported that CC5 harbored more adhesion-associated genes (*fnbA* and *sdr*) and superantigenic toxin gene *tst*, while ST5 often had *sec-sell*, exfoliative toxin encoding genes *eta/etb* were extremely rare and only detected in CC121.<sup>48</sup> ST5-MRSA-II-t311 strain reported by Wu et al contained *tst* and many enterotoxin genes including *sec*, *seg*, *sei*, *sel*, *sem*, *sen*, *seo* and *seu*. From our results, 20 MRSA strains in our investigation also



found virulence genes *tst*, *sec*, *seg*, *sei*, *seo* in different frequencies, but the *sel* and *seu* genes were not detected. While exfoliative toxin A gene (*eta*) was contained in each strain never reported in ST5-MRSA-II-t311 previously. In addition, this cluster of ST5-MRSA-II-t311 strains had high rate of *sea* gene (95%), which is more common in ST239; and high rate of *seb* gene (75%), which is more common in CC59.<sup>48</sup>

MRSA isolates were characterized by multiple drug resistance; there are slight differences in the resistance characteristics of MRSA ST5 isolates among different regions in China. MRSA ST5 isolates in Wuhan showed high resistance rate to erythromycin, clindamycin, ciprofloxacin, levofloxacin, moxifloxacin, tetracycline and gentamicin.<sup>49</sup> In Shanghai, MRSA ST5 strains show high rate of resistance to penicillin, cefazolin, cefoxitin, erythromycin, fosfomicin, gentamicin, levofloxacin.<sup>50</sup> In Zhejiang, MRSA ST5 strains show high rate of resistance to erythromycin, clindamycin, ciprofloxacin, levofloxacin, tetracycline, and but exhibit low rate of resistance to linezolid, vancomycin, trimethoprim-sulfamethoxazole, rifampicin, tigecycline and gentamicin.<sup>51</sup> Similar to MRSA ST5 isolates in Zhejiang, all MRSA isolates in this study exhibited extremely high resistance rate of cefoxitin, penicillin, oxacillin, erythromycin, clindamycin, ciprofloxacin, levofloxacin, moxifloxacin and tetracycline, but all showed sensitivity to gentamicin, quinupristin/dafopristin, linezolid, vancomycin, tigecycline, rifampicin and trimethoprim-sulfamethoxazole, which suggested their potential use for fighting infections with MRSA strains. In this study, most prevalent ST5-MRSA-II-t311 strains contained *blaZ*, *mecA*, *ermA ant(4')-Ia*, *norA*, *tetK* and *aac(6')/aph(2')*, little difference to previously reported ST5-MRSA-II-t311 in resistance genotype.<sup>47</sup>

## Conclusion

There is a prevalence of ST5-MRSA-II-t311 in a pancreatic surgery ward, and environmental pollution is an important transmission factor. Patients post abdominal surgery or invasive operation should enhance MRSA infection prevention and control, especially for elder patients.

## Data Sharing Statement

The clean data of whole-genome sequence of 3 selected MRSA strains can be obtained from the corresponding author.

## Institutional Review Board Statement

This study was approved by the Ethics Review Committee of the First Affiliated Hospital of Nanjing Medical University (Jiangsu Province Hospital) (Approval number.2022-SR-286).

## Informed Consent Statement

The study received hospital IRB approval (Approval number.2022-SR-286), and the requirement for written informed consent was waived because of the retrospective study design and the patients' original identification was anonymized.

## Acknowledgments

This study was supported by the Project of the Key Laboratory for Laboratory Medicine of Jiangsu Province (No. XK201749), the Science and Technology Development Fund of Nanjing Medical University (No. NMUB2018082) and the Key Project of Jiangsu Provincial Health Commission (No. ZDA2020011). We would like to thank professor Huimin Qian from the Jiangsu Provincial Center for Disease Control and Prevention for kindly providing reference strains and technical support for PFGE practice.

## Disclosure

All authors report no conflicts of interest relevant to this article.

## References

1. Ahmad-Mansour N, Loubet P, Pouget C, et al. Staphylococcus aureus toxins: an update on their pathogenic properties and potential treatments. *Toxins*. 2021;13(10):677. doi:10.3390/toxins13100677

2. Howden B, Giulieri S, Wong Fok Lung T, et al. Staphylococcus aureus host interactions and adaptation. *Nat Rev Microbiol*. 2023;1–16. doi:10.1038/s41579-023-00852-y
3. Peyrussou F, Nguyen TK, Najdovski T, Van Bambeke F. Host cell oxidative stress induces dormant staphylococcus aureus persisters. *Microbiol Spectr*. 2022;10(1):e0231321. doi:10.1128/spectrum.02313-21
4. Turner NA, Sharma-Kuinkel BK, Maskarinec SA, et al. Methicillin-resistant Staphylococcus aureus an overview of basic and clinical research. *Nat Rev Microbiol*. 2019;17(4):203–218. doi:10.1038/s41579-018-0147-4
5. Wozniak J, Mills R, Olson J, et al. Mortality risk profiling of staphylococcus aureus bacteremia by multi-omic serum analysis reveals early predictive and pathogenic signatures. *Cell*. 2020;182(5):1311–1327.e14. doi:10.1016/j.cell.2020.07.040
6. Lesens O, Hansmann Y, Brannigan E, et al. Healthcare-associated staphylococcus aureus bacteremia and the risk for methicillin resistance: is the centers for disease control and prevention definition for community-acquired bacteremia still appropriate? *Infect Control Hosp Epidemiol*. 2005;26(2):204–209. doi:10.1086/502527
7. Hamdan-Partida A, Gonzalez-Garcia S, Martinez-Ruiz FJ, Zavala-Sanchez MA, Bustos-Hamdan A, Bustos-Martinez J. Molecular characterization of staphylococcus aureus strains isolated from mobile phones. *Microorganisms*. 2022;10(3):669. doi:10.3390/microorganisms10030669
8. Raji MA, Suaifan G, Shibl A, et al. Aptasensor for the detection of Methicillin resistant Staphylococcus aureus on contaminated surfaces. *Biosens Bioelectron*. 2021;176:112910. doi:10.1016/j.bios.2020.112910
9. Wolfensberger A, Mang N, Gibson KE, et al. Understanding short-term transmission dynamics of methicillin-resistant Staphylococcus aureus in the patient room. *Infect Control Hosp Epidemiol*. 2022;43(9):1147–1154. doi:10.1017/ice.2021.350
10. Bannerman TL, Hancock GA, Tenover FC, Miller JM. Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of Staphylococcus aureus. *J Clin Microbiol*. 1995;33(3):551–555. doi:10.1128/jcm.33.3.551-555.1995
11. Zhang K, McClure JA, Conly JM. Enhanced multiplex PCR assay for typing of staphylococcal cassette chromosome mec types I to V in methicillin-resistant Staphylococcus aureus. *Mol Cell Probes*. 2012;26(5):218–221. doi:10.1016/j.mcp.2012.04.002
12. Koosha RZ, Fooladi AA, Hosseini HM, Aghdam EM. Prevalence of exfoliative toxin A and B genes in Staphylococcus aureus isolated from clinical specimens. *J Infect Public Health*. 2014;7(3):177–185. doi:10.1016/j.jiph.2013.11.003
13. Zhang P, Liu X, Zhang M, et al. Prevalence, antimicrobial resistance, and molecular characteristics of staphylococcus aureus and methicillin-resistant staphylococcus aureus from retail ice cream in Shaanxi Province, China. *Foodborne Pathog Dis*. 2022;19(3):217–225. doi:10.1089/fpd.2021.0069
14. Pourmand MR, Yousefi M, Salami SA, Amini M. Evaluation of expression of NorA efflux pump in ciprofloxacin resistant Staphylococcus aureus against hexahydroquinoline derivative by real-time PCR. *Acta Med Iran*. 2014;52(6):424–429.
15. Cavalcante FS, Saintive S, Carvalho Ferreira D, et al. Methicillin-resistant Staphylococcus aureus from infected skin lesions present several virulence genes and are associated with the CC30 in Brazilian children with atopic dermatitis. *Virulence*. 2021;12(1):260–269. doi:10.1080/21505594.2020.1869484
16. Campbell AJ, Mowlaboccus S, Coombs GW, et al. Whole genome sequencing and molecular epidemiology of pediatric Staphylococcus aureus bacteremia. *J Glob Antimicrob Resist*. 2022;29:197–206. doi:10.1016/j.jgar.2022.03.012
17. Ogura K, Furuya H, Takahashi N, et al. Interspecies regulation between staphylococcus caprae and staphylococcus aureus colonized on healed skin after injury. *Front Microbiol*. 2022;13:818398. doi:10.3389/fmicb.2022.818398
18. Hosaka Y, Yahara K, Clark A, et al. Surveillance of multi-drug resistance phenotypes in Staphylococcus aureus in Japan and correlation with whole-genome sequence findings. *J Hosp Infect*. 2022;123:34–42. doi:10.1016/j.jhin.2022.02.011
19. Gungor S, Karagoz A, Kocak N, Arslantas T. Methicillin-resistant Staphylococcus aureus in a Turkish hospital: characterization of clonal types and antibiotic susceptibility. *J Infect Dev Ctries*. 2021;15(12):1854–1860. doi:10.3855/jidc.14963
20. Konstantinovski MM, Veldkamp KE, Lavrijsen APM, et al. Hospital transmission of borderline oxacillin-resistant Staphylococcus aureus evaluated by whole-genome sequencing. *J Med Microbiol*. 2021;70(7). doi:10.1099/jmm.0.001384
21. Maharjan M, Sah AK, Pyakurel S, et al. Molecular confirmation of vancomycin-resistant staphylococcus aureus with vana gene from a hospital in Kathmandu. *Int J Microbiol*. 2021;2021:3847347. doi:10.1155/2021/3847347
22. Katoziyan A, Imani Fooladi AA, Taheri RA, Vatanpour S. Multi-drug resistance of staphylococcus aureus strains in baqiyatallah hospital: a primary step towards digital health biomonitoring systems. *Iran J Pharm Res*. 2020;19(3):321–328. doi:10.22037/ijpr.2020.112966.14042
23. Sapkota J, Sharma M, Jha B, Bhatt CP. Prevalence of staphylococcus aureus isolated from clinical samples in a tertiary care hospital: a descriptive cross-sectional study. *JNMA J Nepal Med Assoc*. 2019;57(220):398–402. doi:10.31729/jnma.4673
24. Zarb P, Coignard B, Griskeviciene J, et al. The European Centre for Disease Prevention and Control (ECDC) pilot point prevalence survey of healthcare-associated infections and antimicrobial use. *Euro Surveill*. 2012;17(46). doi:10.2807/ese.17.46.20316-en
25. Kaiki Y, Kitagawa H, Hara T, et al. Methicillin-resistant Staphylococcus aureus contamination of hospital-use-only mobile phones and efficacy of 222-nm ultraviolet disinfection. *Am J Infect Control*. 2021;49(6):800–803. doi:10.1016/j.ajic.2020.11.011
26. Kitagawa H, Mori M, Kashiya S, et al. Effect of pulsed xenon ultraviolet disinfection on methicillin-resistant Staphylococcus aureus contamination of high-touch surfaces in a Japanese hospital. *Am J Infect Control*. 2020;48(2):139–142. doi:10.1016/j.ajic.2019.08.033
27. Khan F, Ahmad B, Ahmed J, Yoo HS, Bashir S. Investigation of Staphylococcus aureus, prevailing in the environment of Khyber Teaching Hospital, Peshawar, Pakistan. *Pak J Pharm Sci*. 2018;31(2):429–437.
28. Bhattacharya S, Pal K, Jain S, Chatterjee SS, Konar J. Surgical site infection by methicillin resistant staphylococcus aureus- on decline? *J Clin Diagn Res*. 2016;10(9):DC32–DC36. doi:10.7860/JCDR/2016/21664.8587
29. Sporer SM, Rogers T, Abella L. Methicillin-resistant and methicillin-sensitive staphylococcus aureus screening and decolonization to reduce surgical site infection in elective total joint arthroplasty. *J Arthroplasty*. 2016;31(9 Suppl):144–147. doi:10.1016/j.arth.2016.05.019
30. Sganga G, Tascini C, Sozio E, et al. Focus on the prophylaxis, epidemiology and therapy of methicillin-resistant Staphylococcus aureus surgical site infections and a position paper on associated risk factors: the perspective of an Italian group of surgeons. *World J Emerg Surg*. 2016;11:26. doi:10.1186/s13017-016-0086-1
31. Al-Kharabsheh R, Ahmad M. Skin and mucous membranes colonisation with Staphylococcus aureus or MRSA as a risk factor for surgical site infections in elective caesarean section. *J Obstet Gynaecol*. 2022;42(5):888–893. doi:10.1080/01443615.2021.1954147
32. Karanika S, Kinamon T, Grigoras C, Mylonakis E. Colonization with methicillin-resistant staphylococcus aureus and risk for infection among asymptomatic athletes: a systematic review and meta analysis. *Clin Infect Dis*. 2016;63(2):195–204. doi:10.1093/cid/ciw240



33. Davis MF, Peterson AE, Julian KG, et al. Household risk factors for colonization with multidrug-resistant *Staphylococcus aureus* isolates. *PLoS One*. 2013;8(1):e54733. doi:10.1371/journal.pone.0054733
34. Safdar N, Bradley EA. The risk of infection after nasal colonization with *Staphylococcus aureus*. *Am J Med*. 2008;121(4):310–315. doi:10.1016/j.amjmed.2007.07.034
35. Haley CC, Mittal D, Laviolette A, Jannapureddy S, Parvez N, Haley RW. Methicillin-resistant *Staphylococcus aureus* infection or colonization present at hospital admission: multivariable risk factor screening to increase efficiency of surveillance culturing. *J Clin Microbiol*. 2007;45(9):3031–3038. doi:10.1128/JCM.00315-07
36. Khalili H, Najari-Peerayeh S, Mahrooghi M, Mansouri P, Bakhshi B. Methicillin-resistant *Staphylococcus aureus* colonization of infectious and non-infectious skin and soft tissue lesions in patients in Tehran. *BMC Microbiol*. 2021;21(1):282. doi:10.1186/s12866-021-02340-w
37. He W, Gu F, Zhang J, et al. Molecular characteristics and risk factor analysis of *Staphylococcus aureus* colonization put insight into CC1 colonization in three nursing homes in Shanghai. *PLoS One*. 2021;16(10):e0253858. doi:10.1371/journal.pone.0253858
38. Baroja I, Guerra S, Coral-Almeida M, et al. *Staphylococcus aureus* Methicillin-resistant nasal colonization among health care workers of a tertiary hospital in Ecuador and associated risk factors. *Infect Drug Resist*. 2021;14:3433–3440. doi:10.2147/idr.s326148
39. Nelwan E, Sinto R, Subekti D, et al. Screening of methicillin-resistant *Staphylococcus aureus* nasal colonization among elective surgery patients in referral hospital in Indonesia. *BMC Res Notes*. 2018;11(1):56. doi:10.1186/s13104-018-3150-y
40. Crowcroft N, Maguire H, Fleming M, Peacock J, Thomas J. Methicillin-resistant *Staphylococcus aureus*: investigation of a hospital outbreak using a case-control study. *J Hosp Infect*. 1996;34(4):301–309. doi:10.1016/s0195-6701(96)90110-3
41. Manzur A, Gudiol F. Methicillin-resistant *Staphylococcus aureus* in long-term-care facilities. *Clin Microbiol Infect*. 2009;15(7):26–30. doi:10.1111/j.1469-0691.2009.03093.x
42. Monecke S, Ehrlich R, Slickers P, Wiese N, Jonas D. Intra-strain variability of methicillin-resistant *Staphylococcus aureus* strains ST228-MRSA-I and ST5-MRSA-II. *Eur J Clin Microbiol Infect Dis*. 2009;28(11):1383–1390. doi:10.1007/s10096-009-0796-3
43. Zhao R, Wang X, Wang X, et al. Molecular characterization and virulence gene profiling of methicillin-resistant *Staphylococcus aureus* associated with bloodstream infections in southern China. *Front Microbiol*. 2022;13:1008052. doi:10.3389/fmicb.2022.1008052
44. Machado TS, Pinheiro FR, Andre LSP, et al. virulence factors found in nasal colonization and infection of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates and their ability to form a biofilm. *Toxins*. 2020;13(1):14. doi:10.3390/toxins13010014
45. Kong H, Yu F, Zhang W, Li X, Wang H. Molecular epidemiology and antibiotic resistance profiles of methicillin-resistant *Staphylococcus aureus* strains in a tertiary hospital in China. *Front Microbiol*. 2017;8:838. doi:10.3389/fmicb.2017.00838
46. Wu DD, Wang ZG, Wang HP, et al. Predominance of ST5-II-t311 clone among healthcare-associated methicillin-resistant *Staphylococcus aureus* isolates recovered from Zhejiang, China. *Int J Infect Dis*. 2018;71:107–112. doi:10.1016/j.ijid.2018.04.798
47. Wu D, Wang H, Zhu F, et al. Characterization of an ST5-SCCmec II-t311 methicillin-resistant *Staphylococcus aureus* strain with a widespread *cfr*-positive plasmid. *J Infect Chemother*. 2020;26(7):699–705. doi:10.1016/j.jiac.2020.02.018
48. Wang B, Xu Y, Zhao H, et al. Methicillin-resistant *Staphylococcus aureus* in China: a multicenter longitudinal study and whole-genome sequencing. *Emerg Microbes Infect*. 2022;11:1. doi:10.1080/22221751.2022.2032373
49. Li X, Zhang J, Zhang Y, et al. Methicillin-resistant *Staphylococcus aureus* of the clonal lineage ST5-SCCmecII-t2460 was associated with high mortality in a Wuhan hospital. *Braz J Microbiol*. 2021;52(4):1929–1936. doi:10.1007/s42770-021-00557-5
50. Chen T, Zhao L, Liu Y, et al. Mechanisms of high-level fosfomycin resistance in *Staphylococcus aureus* epidemic lineage ST5. *J Antimicrob Chemother*. 2022;77(10):2816–2826. doi:10.1093/jac/dkac236
51. Chen Y, Sun L, Wu D, Wang H, Ji S, Yu Y. Using core-genome multilocus sequence typing to monitor the changing epidemiology of methicillin-resistant *Staphylococcus aureus* in a teaching hospital. *Clin Infect Dis*. 2018;67(suppl\_2):S241–S248. doi:10.1093/cid/ciy644

## Infection and Drug Resistance

Dovepress

### Publish your work in this journal

Infection and Drug Resistance is an international, peer-reviewed open-access journal that focuses on the optimal treatment of infection (bacterial, fungal and viral) and the development and institution of preventive strategies to minimize the development and spread of resistance. The journal is specifically concerned with the epidemiology of antibiotic resistance and the mechanisms of resistance development and diffusion in both hospitals and the community. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/infection-and-drug-resistance-journal>